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# Mapping the Gene for Autosomal Dominant Restless Legs Syndrome in an Irish family 

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## $\underline{\text { Abstract }}$

Restless Legs Syndrome (RLS) is a common neurological disorder affecting nearly $15 \%$ of the general population. Ironically, RLS can be described as the most common condition one has never heard of. It is usually characterised by uncomfortable, unpleasant sensations in the lower limbs inducing an uncontrollable desire to move the legs. RLS exhibits a circadian pattern with symptoms present predominantly in the evening or at night, thus leading to sleep disruption and daytime somnolence.

RLS is generally classified into primary (idiopathic) and secondary (symptomatic) forms. Primary RLS includes sporadic and familial cases of which the age of onset is usually less than 45 years and progresses slowly with a female to male ratio of 2:1. Secondary forms often occur as a complication of another health condition, such as iron deficiency or thyroid dysfunction. The age of onset is usually over 45 years, with an equal male to female ratio and more rapid progression.

Ekbom described the familial component of the disorder in 1945 and since then many studies have been published on the familial forms of the disorder. Molecular genetic studies have so far identified ten loci (5q, 12q, 14p, 9p, 20p, 16p, 19p, 4q, 17p). No specific gene within these loci has been identified thus far. Association mapping has highlighted a further five areas of interest. RLS6 has been found to be associated with SNPs in the BTBD9 gene. Four other variants were found within intronic and intergenic regions of MEIS1, MAP2K5/LBXCOR1, PTPRD and NOS1.

The pathophysiology of RLS is complex and remains to be fully elucidated. Conditions associated with secondary RLS, such as pregnancy or end-stage renal disease, are characterised by iron deficiency, which suggests that disturbed iron homeostasis plays a role. Dopaminergic dysfunction in subcortical systems also appears to play a central role.

An ongoing study within the Department of Pathology (University College Cork) is investigating the genetic characteristics of RLS in Irish families. A three generation RLS pedigree RLS3002 consisting of 11 affected and 7 unaffected living family members was recruited. The family had been examined for four of the known loci ( $5 \mathrm{q}, 12 \mathrm{q}, 14 \mathrm{p}$ and 9 p ) (Abdulrahim 2008). The aim of this study was to continue examining this Irish RLS pedigree for possible linkage to the previously described loci and associated regions. Using informative microsatellite markers linkage was excluded to the loci on $5 \mathrm{q}, 12 \mathrm{q}, 14 \mathrm{p}, 9 \mathrm{p}, 20 \mathrm{p}, 16 \mathrm{p}, 19 \mathrm{p}, 4 \mathrm{q}, 17 \mathrm{p}$ and also within the regions reported to be associated with RLS. This suggested the presence of a new unidentified locus. A genome-wide scan was performed using two microsatellite marker screening sets (Research Genetics Inc. Mapping set and the Applied Biosystems Linkage mapping set version 2.5). Linkage analysis was conducted under an autosomal dominant model with a penetrance of $95 \%$ and an allele frequency of 0.01 . A maximum LOD score of 3.59 at $\theta=0.00$ for marker D19S878 indicated significant linkage on chromosome 19p. Haplotype analysis defined a genetic region of 6.57 cM on chromosome 19 p 13.3 , corresponding to 2.5 Mb . There are approximately 100 genes annotated within the critical region. Sequencing of two candidate genes, KLF16 and GAMT, selected on the assumed pathophysiology of RLS, did not identify any sequence variant.

This study provides evidence of a novel RLS locus in an Irish pedigree, thus supporting the picture of RLS as a genetically heterogeneous trait.

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## Abbreviations

| ${ }^{\circ} \mathrm{C}$ | Degree Celsius |
| :---: | :---: |
| $\Theta$ | Recombination fraction |
| AD | Antidepressant |
| AGE | Agarose gel electrophoresis |
| bp | base pair |
| CEPH | Centre d'Etude du Polymorphime Humain |
| Chr | Chromosome |
| cM | Centi Morgan |
| CNS | Central Nervous System |
| COMT | Catechol-O-methyltransferase |
| CSF | Cerebrospinal Fluid |
| DA | Dopamine |
| DAT | Dopamine Transporter |
| ddNTP | Dideoxynucleotide triphosphate |
| DNA | Deoxyribonucleic acid |
| DOPA | 2,3-dihydroxyphenylalanine |
| DOPAC | Dihydroxyphenylacetic Acid |
| EDTA | Ethylene diamine tetra acetic acid |
| ECG | Electroencephalogram |
| EMG | Electromyography |
| GWAS | Genome-Wide Association Study |
| ICD | Impulse Control Disorder |
| IRLS | International RLS Study Group Severity Scale |
| IRLSSG | International Restless Legs Syndrome Study Group |
| JHSS | John Hopkins Severity Scale |
| Kb | Kilobase |
| LOD | Logarithm of the Odds |
| MAO | Monoamine Oxidase |
| Mb | Mega bases |
| MgCl | Magnesium Chloride |


| MRI | Magnetic resonance imaging |
| :--- | :--- |
| NCBI | National Center for Biotechnology Information |
| NOS1 | Nitric Oxide Synthase |
| PC | Personalised Computer |
| PCR | Polymerase Chain Reaction |
| PD | Parkinson's Disease |
| PET | Position Electron Tomography |
| PLMI | Periodic Limb Movement Index |
| PLMS | Periodic Limb Movements during Sleep |
| PNS | Peripheral Nervous System |
| POP | Performance Optimized Polymer |
| PSG | Polysomnography |
| PTPRD | protein tyrosine phosphatase receptor type delta |
| QoL | Quality of Life |
| REST | RLS epidemiology, symptoms and treatment |
| RLS | Restless Legs Syndrome |
| RLS-DI | RLS diagnostic index |
| RPM | Revolutions per minute |
| SIT | Suggested immobilization test |
| SN | Substantia Nigra |
| SPECT | Singe Photon Emission Tomographic |
| TENS | Transcutaneous Electric Nerve Stimulation |
| TH | Tyrosine Hydroxylase |
| T | Melting Temperature |
| UCC | University College Cork |
| UV | Ultraviolet |
| VMAT | Vesicular Monamine Transporter |
| VTA | Ventral Tegmental Area |
| YKS | Yokukansan |

## Chapter 1

## Restless Legs Syndrome

### 1.1 Introduction

Restless legs syndrome (RLS) is a neurological condition characterised by a distressing need or urge to move the legs (an akathisia focused on the legs), usually accompanied by an uncomfortable sensation in the legs (a paresthesia) described as a crawling, muscle ache or tension (Earley 2003). It is usually brought on by rest, worse in the evening or night and partially or totally relieved by movement. This scenario leads to nocturnal sleep disruption and consequently to daytime somnolence. Although the entity was mentioned in English literature by Thomas Willis in 1965, it was not until the Swedish neurologist Karl Axel Ekbom’s lucid description in 1945, under the heading "Restless legs: a clinical study", that the scientific world became aware of this condition. Therefore, this disorder is often referred to as Ekbom's syndrome (Hening 2004).

RLS is a heterogenous disorder, the common feature of which is a circadian occurrence of disabling sensory symptoms at rest. However, in addition to sensorial symptoms such as dyesthesias, patients experience motor symptoms. These rhythmic or semirhythmic movements of the legs are otherwise known as periodic limb movements during sleep (PLMS) that occur every 5-90 seconds. PLMS, which occur in various sleep disorders as well as other neurological diseases, are observed in at least 80\% of RLS patients (Montplaisir, Boucher et al. 1997). When present, PLMS support the diagnosis, and provide a measure of the clinical severity of this disorder.

RLS can be classified into two broad groups, according to the age of onset and the presence or absence of a familial history of RLS. Primary RLS is also known as idiopathic RLS. The name is applied to those cases of RLS where there is a positive family history of RLS and the age of onset is less than 45 . The positive family
history and early age of onset indicate that genetic factors play a role in this RLS phenotype (Allen and Earley 2000). RLS familial aggregation accounts for up to $65 \%$ of reported cases (primary), with inheritance following an autosomal dominant pattern in at least one-third of familial cases (Montplaisir, Boucher et al. 1997; Ondo, Vuong et al. 2000). Secondary RLS is also known as "symptomatic RLS" or "sporadic RLS". The name "secondary RLS", represents cases of RLS where age of onset is usually greater than 45 years and there is no familial link with the syndrome. This indicates that environmental factors play a role in this phenotype. The sudden and late onset of the disease usually occurs secondary to other conditions, particularly iron-deficiency anemia and end-stage renal disease. Familial (primary) and sporadic (secondary) RLS cases present similar signs, symptoms and clinical course. However the symptoms of secondary RLS are usually resolved when the underlying conditions are corrected.

Epidemiological studies indicate that the symptoms of RLS are present in about 5$10 \%$ of the general population. Despite this high frequency of affected individuals, experience suggests the disease is often misdiagnosed, under-diagnosed or undiagnosed. It follows that there is a need to understand more about RLS and increase awareness of how it is most likely to present in a medical practice.

### 1.2 Diagnosing RLS

### 1.2.1 Clinical features of RLS

In 1995, the International Restless Legs Syndrome Study Group (IRLSSG) developed standardized criteria for the diagnosis of restless legs syndrome. Since that time, additional scientific scrutiny and clinical experience led to a better understanding of the condition. Subsequently a consensus conference held at the NIH in conjunction with the IRLSSG, resulted in revised diagnostic criteria for RLS to replace the previously proposed criteria. The primary revision from the previously proposed criteria include four criteria that are essential for the diagnosis of RLS in cognitively intact adult, three supportive criteria which may support the diagnosis in uncertain clinical cases, and three additional features of the disorder which deserve consideration when evaluating the patient with a potential diagnosis of RLS (Allen, Picchietti et al. 2003). These criteria are outlined in Table 1.1 below.

## Table 1.1: The clinical features and diagnostic criteria for RLS

## Essential Diagnostic Criteria for RLS

An urge to move the lower limbs, usually accompanied or caused by uncomfortable and unpleasant sensations in the lower limbs (sometimes the urge to move is present without the uncomfortable sensations and sometimes the arms or other body parts are involved in addition to the legs).

The urge to move or unpleasant sensations begin to worsen during periods of rest or inactivity such as lying or sitting.

The urge to move or unpleasant sensations are partially or totally relieved by movement, such as walking or stretching, at least as long as the activity continues.

The urge to move or unpleasant sensations are worse in the evening or at night than during the day or only occur in the evening or night (When symptoms are very severe, the worsening at night may not be noticeable but must have been previously present).

Table 1.1: The clinical features and diagnostic criteria for RLS (continued)

## Supportive Clinical Features of RLS

- Periodic Limb Movements during Sleep (PLMS)

Although the presence of PLMS is not specific to RLS an elevated PLM index (i.e. $>5$ movements/hr of sleep) is supportive of the diagnosis of RLS. Approximately $80 \%$ of patients with RLS have associated PLMS, which are usually present in the legs and less often in the arms.

- Response to Dopaminergic Therapy

Nearly all people with RLS show at least an initial positive therapeutic response to either L-dopa dopamine-receptor agonist at doses considered to be very low in relation to the traditional doses of these medications used for the treatment of Parkinson's disease. This initial response is not, however, universally maintained.

- Family History

The presence of RLS among first degree relatives is 3 to 5 times greater than in people without family history of RLS.
Associated Features of RLS

- Natural Clinical Course

The clinical course of the disorder varies considerably, but certain patterns have been identified that may be helpful to the experienced clinician. When the age of onset of RLS symptoms is less than 50 years, the onset is often more insidious; when the age of onset is more than 50 years, the symptoms often occur more abruptly and more severely. In some patients, RLS can be intermittent and may spontaneously remit for many years.

- Sleep Disturbance

Disturbed sleep is a common major morbidity for RLS and deserves special consideration in planning treatment. This symptom is often the primary reason the patient seeks medical attention.

- Medical Evaluation/ Physical evaluation

The physical examination is generally normal and does not contribute to the diagnosis except for the identification of comorbid or secondary causes of RLS. Iron status, in particular, should be evaluated because decreased iron stores are significant potential risk factors that can be treated. The presence of peripheral neuropathy and radiculopathy should also be determined because the conditions have a possible, although uncertain, association with RLS and may require different treatment.

The uncomfortable sensations occur only when the limbs are at rest for a length of time, and they are typically relieved by movement. Individuals declare that they experience an almost irresistible urge to move the limbs. Symptoms of RLS can be very severe e.g. during flights, train or car journeys, at the cinema or the theatre. Symptoms can be especially troublesome in the late evening when patients are preparing for or attempting to sleep. Sensations may last for hours and persist, in some unfortunate suffers, into the early hours of the morning. Individuals often have to get up and walk around a few times per night to get relief. This form of coping behaviour has been named 'Night-walker's syndrome'. Loss of sleep is a grim consequence both to patients and their partners (Ekbom and Ulfberg 2009).

In addition to the standard criteria, the IRLSSG also outlined diagnostic criteria for RLS in special populations including children, adolescents and the cognitively impaired elderly (Allen, Picchietti et al. 2003). If a clear verbal description cannot be given, the diagnosis in children can be supported by a positive family history or a PLM index >5 per hour in overnight polysomnography. On the contrary, in elderly patients with cognitive impairment, diagnosis may be difficult when memory or language problems prevent a proper history taking. In this patient group, the consensus paper advises referral for observation, e.g., leg kicking, holding the legs and rubbing them etc.

### 1.2.2 Assessment Scales

RLS is said to be a subjective disorder, therefore it may be difficult for examiners to evaluate the exact severity of symptoms in individuals. However, in 2003, the IRLSSG rating scale was established. This scale is an easily administered instrument, which can be applied to all patients with RLS. It contains 10 items that are completed by patients themselves assessing the frequency and severity of symptoms over the preceding week. Responses are graded from 0 to 4 (e.g. $0=$ absence of symptoms, $4=$ very severe symptoms), with a maximum total score of 40. It can be used to accurately measure disease severity for clinical assessment, research, or therapeutic trials (Walters, LeBrocq et al. 2003). It is said to be the gold standard for measuring symptom severity in patients with RLS. A different scale, The John Hopkins Severity Scale (JHSS), predominantly assesses the time of daily onset of RLS symptoms. Other scales are used to examine quality of life in RLS. A list of assessment scales used in RLS are listed in Table 1.2.

Table 1.2: Scales for assessing RLS (Hogl, Garcia-Borreguero et al. 2009).

| Severity scales |
| :--- |
| IRLS ( International RLS Study Group Severity Scale) |
| John Hopkins Severity Scale (JHSS) |
| Quality of life scales |
| RLS Quality of life (QoL) questionnaire by Kohnen |
| RLS QoL questionnaire by Allen |
| RLS-QLI instrument |
| Other scales for RLS |
| Clinical Global Impression, Patient Global Impression |
| RLS diagnostic index (RLS-DI) |
| Augmentation Severity Rating Scale of the European RLS study group |

### 1.2.3 Diagnosis

According to the mentioned diagnostic criteria, the diagnosis of RLS is based primarily on the patient's history. Approximately $60-80 \%$ of all RLS cases are idiopathic (primary). However, it is necessary that secondary causes or RLSassociated conditions are considered, and addressed where possible. Neurological examinations and neurophysiological examinations along with laboratory tests may be carried out to identify signs or symptoms of secondary RLS (Odin, Mrowka et al. 2002). Such laboratory tests include: complete blood panel; renal parameters; iron, transferrin and ferritin levels.

The diagnosis of RLS is based primarily on clinical criteria. Nonetheless, some of the following instrumental tools may be useful in doubtful cases, to confirm the diagnosis, or to aid in differential diagnosis. Instrumental diagnosis however is not commonly used to diagnose RLS (Zucconi and Ferini-Strambi 2004).

Polysomnography (PSG) enables recording of PLMS and periodic/non-periodic leg movements during wakefulness, either before or after sleep onset. These movements may be detected by Electromyography (EMG) recordings, most commonly of the tibialis anterior muscle. It is advised that PLM scoring is carried out according to the American Sleep Disorder Association rules; per hour of sleep, a pathological value is defined as more than five PLM/hour of sleep (Chaudhuri 2003). In using the PSG for assessment it should be noted that not all patients with RLS have demonstrated a Periodic Limb Movement Index (PLMI) greater than five, PLMS are present in other sleep pathologies, and at least $30 \%$ of older people have a PLMS index greater than 5 (Zucconi and Ferini-Strambi 2004)

Suggested immobilization test (SIT) is a new tool to evaluate the effect of immobility on sensory and motor symptoms. The patient attempts to maintain a seated posture without moving their legs for a 60 minute period, while the anterior tibialis EMG is again used to detect PLM. A PLM measure, along with quantification of the sensory symptoms, allows discrimination between RLS patients and controls (Michaud, Chabli et al. 2000).

Actigraphy is a method used to monitor muscle activity during the night, using an ankle placed portable activity meter. It has certain advantages such as avoiding the need for laboratory studies, allowing monitoring in the patients home, and increased accuracy and sensitivity since recordings can be made over multiple nights. However, the majority of actigraphical devices cannot differentiate between PLM and other involuntary movements associated with apnoea (Hening, Walters et al. 1999).

Other methods used to evaluate RLS include: Singe Photon Emission Tomographic (SPECT), Position Emission Tomographic (PET), Magnetic Resonance Imaging (MRI) and functional MRI.

### 1.2.4 Co-Morbid conditions \& Differential diagnosis

The majority of RLS cases appear idiopathic, however a vast number of co-morbid conditions have been described in association with RLS. The majorities of these comorbid conditions, with a few exceptions, have an unknown relationship to RLS, and most likely represent coincidental occurrence.

In recent years, several studies identified a higher prevalence of RLS in patients with migraine (d'Onofrio, Bussone et al. 2008). Migraine has been reported to have an association with poor sleep quality. It is uncertain if RLS is also associated with other primary headache disorders, however, it was found that the frequencies of RLS in patients with migraine were higher in those with tension type headaches or cluster headaches (Chen, Fuh et al. 2010). This study also demonstrated co-morbid RLS in migraine patients worsened sleep quality. It is thought that a shared underlying mechanism, related to the dopaminergic system, may account for the association between migraine features and co-morbid RLS.

Contrary to co-morbidity issues, differential diagnosis should distinguish RLS from conditions related to movement as well as sleep disorders. Conditions that can often be confused with RLS include those with prominent sensory symptoms in the legs such as neuropathies (patients complaining of numbness, tingling or pain), nerve conditions due to compression (meralgia paraesthetica), and radiculopathies. Several other conditions relating to motor restlessness and /or sensory symptoms include: painful legs and moving toes with segmental and semi-continuous distal movements during wakefulness, continuing during sleep and not alleviated by movements; neuropathic pain syndromes not consistently associated with rest and sleep; akathisia (excessive movement) which does not correlate with rest or time of day, which may
result from medications such as neuroleptics or other dopamine blocking agents; perplexing restlessness associated with insomnia, anxiety and panic attacks; nocturnal leg cramps unrelated to sensory symptoms or the urge to move the legs during the evening or the day, and different from nocturnal myoclonus; and propiospinal myclonus which is associated with relaxed wakefulness and recognized by axial and diffuse myoclonic jerks (Chaudhuri 2003; Garcia-Borreguero, Odin et al. 2004; Zucconi and Ferini-Strambi 2004). Apart from PLMS, most forms of "myoclonus" are either shorter in duration, suppressed during sleep or have a very different periodicity (Montplaisir 2004). Vesper's Curse, which is the combination of mild underlying congestive heart failure and lumbar stenosis, often causes nocturnal pain in the lower limbs and should be regarded in the differential diagnosis (Stiasny, Oertel et al. 2002). The majority of these conditions may not present with an urge to move the legs, may be present throughout the day, and symptoms may not be consistently worse in the evening or at rest. Nonetheless satisfying the four RLS diagnostic criteria outlined above will typically distinguish RLS dysthesias from other types of leg discomfort.

### 1.3.5 Diagnostic rates

With regard to the rates of diagnosing, the Dortmund Health Study, which was a population-based survey in Germany ( $\mathrm{n}=1312$ ), conducted face-to-face interviews in randomly selected individuals aged 25-75 years (Happe, Vennemann et al. 2008). They reported that the overall prevalence of people with a known doctor diagnosis of RLS was $2.3 \%$; however, a further $6.5 \%$ fulfilled the four minimal criteria yet were not aware of their RLS. The overall prevalence was $8.8 \%$ in this sample, with a ratio of diagnosed:undiagnosed RLS of 1:3.

In the European context, the lowest diagnostic rate for RLS was reported from the UK. In a general practice research database involving 1,516,692 individuals, the prevalence of RLS with a registered diagnosis of RLS was only $0.25 \%$. Only $0.6 \%$ of these patients had received a prescription for levodopa or a dopamine agonists, while many were prescribed medications not effective in RLS (e.g. oxerutins and quinine)(Van De Vijver, Walley et al. 2004). These findings are further supported by the French epidemiological survey where $60 \%$ of the RLS affected individuals had previously received a vascular diagnosis (Tison, Crochard et al. 2005) and an Irish study reported that only $16.7 \%$ of RLS sufferers were diagnosed with RLS (O'Keeffe, Egan et al. 2007). Furthermore, these findings are supported by the RLS epidemiology, symptoms and treatment (REST) studies where patients with RLS had been given a diagnosis of lower back pain, depression, spinal problems, depression, anxiety, peripheral neuropathy and were transferred to psychiatrists, vascular surgeons and orthopaedics (Hening, Walters et al. 2004).

### 1.3 Epidemiology

At least in Western countries, prevalence rates of RLS reveal this disorder as one of the most common neurological movement disorders. From the initial work of Ekbom to the end of the 1980s, a prevalence rate between 1 and $5 \%$ was estimated in the general population (Garcia-Borreguero, Odin et al. 2004). However, these studies were very limited and comprised of imprecise diagnostic criteria. Diagnostic criteria for RLS has provided data that is more detailed and population-based studies have shown that RLS affects 5 to $15 \%$ of the general population (Table 1.3). It has been noted however that prevalence estimates in the general population do not overlap across certain studies. Such discrepancies may be due to the different targeted patient population, the fluctuating course of initial symptoms and the numerous methodological tactics used such as telephone interviews, questionnaire surveys, direct observations and face-to-face interviews. It was found that in certain studies in western countries that used only the International Restless Legs Syndrome Study Group (IRLSSG) criteria the RLS prevalence rates ranged from 7.2 to $11.5 \%$ (Allen, Walters et al. 2005; Bjorvatn, Leissner et al. 2005).

In Western countries there is a female preponderance ranging from 13-17\% (Lavigne and Montplaisir 1994) to $13.8 \%$ (Rothdach, Trenkwalder et al. 2000). A number of factors have been considered in explaining this sex difference. Firstly, patterns of inheritance may be a reason for female dominance. There will be a disproportionate number of women with RLS if the transmission of RLS is sex-linked and compromises females. A second contribution to the increased numbers of women in treatment studies may be a result of a gender bias in co-morbid medical disorders. Depression, anxiety and other common psychological disorders have a higher
prevalence in women. Such disorders may intensify the symptoms of RLS (Rothdach, Trenkwalder et al. 2000), or provide a secondary cause for insomnia, thus increasing the likelihood of RLS being reported. A third reason for the gender bias may be related to the impact of RLS on sleep. The gender bias in sleep complaints with RLS correlates with the gender bias found in other forms of insomnia. It was therefore proposed that female subjects with RLS are more likely to have increased sleep disturbances than their male counterparts (Bentley, Rosman et al. 2006). Furthermore it has been shown that female preponderance may be related to parity. Interestingly, nulliparous women had the same risk for RLS as age-matched men, whereas the risk for RLS increased gradually for women with one child (OR, 1.98; $95 \%$ CI, 1.25-3.13), two children (OR, 3.04; $95 \%$ CI;2.11-4.40), and three or more children (OR, 3.57;95\% CI, 2.30-5.55)(Berger, Luedemann et al. 2004).

While RLS can occur in all ethnic backgrounds, epidemiologic studies suggest ethnic variation. Asian studies reported a prevalence rate of $0.6 \%$ in a selected healthy general population 55 years of age and older (Tan, Seah et al. 2001). A similar low prevalence rate of $1.5 \%$ and $<1 \%$ was also reported in Japan (Mizuno, Miyaoka et al. 2005) and Singapore, respectively. Prevalence estimates also vary in Western countries; for example, prevalence is considerably higher in Norway (14.3\%) than in Denmark (8.8\%) (Bjorvatn, Leissner et al. 2005). Regional variations may be caused by environmental factors, awareness or the complex influence of variable genetic susceptibility.

Prevalence of RLS also increases with age (Lavigne and Montplaisir 1994; Allen, Walters et al. 2005). RLS is often associated with diseases of middle to old age. However, assessments indicate that the onset of the syndrome may occur before the
age of twenty years in up to $43 \%$ of adult cases (Chaudhuri, Appiah-Kubi et al. 2001). Thus far, no definite age of onset can be determined. Onset before 18 years of age has been reported (Walters, Hickey et al. 1996). However, children with RLS often describe symptoms that do not meet IRLSSG criteria but rather correspond to growing pains. One study, which used the newly established diagnostic criteria for RLS in children (Allen, Picchietti et al. 2003), concluded a prevalence rate of $5.9 \%$ in children (Kotagal and Silber 2004). Table 1.3 summarises the population prevalence studies which were carried out in recent years.

Table 1.3: Population prevalence of RLS (Mata, Bodkin et al. 2006).

| Reference | Population | $\mathrm{N} /$ age | Frequency | Comments |
| :--- | :--- | :--- | :--- | :--- |
| Ekbom (1960) | Sweden | $\mathrm{n}=500$ | $\sim 5 \%$ | Healthy controls |
| Strang (1967) | Australia | $\mathrm{n}=320$ | $2.5 \%$ | Relatives of P.D. |
| Lavigne (1994) | Canada | $\mathrm{n}=2019$ | $0-15 \%$ | Face to face interview |
| Kageyama (2000) | Japan | $\mathrm{n}=4612$ | $5-15 \%$ | Questionnaire survey |
| Rothdach (2000) | Germany | $\mathrm{n}=369$ | $9.8 \%$ | Face to face interview |
| Berger (2004) | NE | $\mathrm{n}=4310$ | $10.6 \%$ | Face to face interview |
|  | Germany |  |  |  |
| Henning (2004) | Europe \& N. | $\mathrm{n}=23,052$ | $11.1 \%$ | Questionnaire survey |
|  | America |  |  |  |
| Allen (2005) | Europe \& N. | $\mathrm{n}=15,391$ | $2.7 \%$ | Face to face interview |
| Hogl (2005) | America |  | $\mathrm{n}=701$ | $10.6 \%$ |
| Bjorvtan (2005) | Denmark | $\mathrm{n}=2005$ | $11.5 \%$ | Face to face interview |
| Hadjigeorgiou | Central | $\mathrm{n}=3033$ | $3.9 \%$ | Face to face interview |
| (2007) | Greece |  | $\mathrm{n}=1000$ | $5.0 \%$ |
| Ulfberg (2007) | Sweden | Germany | $\mathrm{n}=1312$ | $8.8 \%$ |

(Lavigne and Montplaisir 1994; Kageyama, Kabuto et al. 2000; Rothdach, Trenkwalder et al. 2000; Berger, Luedemann et al. 2004; Hening, Walters et al. 2004; Allen, Walters et al. 2005; Bjorvatn, Leissner et al. 2005; Hogl, Kiechl et al. 2005; Hadjigeorgiou, Stefanidis et al. 2007; Ulfberg, Bjorvatn et al. 2007; Happe, Vennemann et al. 2008).

### 1.4 Pathophysiology

The pathophysiologic basis of RLS has not been fully elucidated. However, multiple etiologies including abnormalities of the central and peripheral Nervous System (CNS and PNS), vascular, metabolic, and genetic components have been proposed (Chaudhuri, Appiah-Kubi et al. 2001). In determining the pathophysiology of RLS, the primary areas of interest may be divided into three main themes, CNS dysfunction, the dopaminergic system and iron insufficiency. The genetics of RLS is discussed separately (section 1.9).

### 1.4.1 Localisation of CNS dysfunction in RLS

One important cue to pathophysiology may be the immediate and almost complete therapeutic response of RLS to dopamine treatment (Hening, Allen et al. 1999; Hening, Walters et al. 2004). This indicates rather clearly that RLS results from abnormal functioning of the CNS rather than the PNS. Treatment with centrally active dopamine antagonists causes intensification of RLS symptoms and thus the reversal of the dramatic benefits from treatment. However, such a striking effect is not experienced when treated with a peripherally active dopamine antagonist (Allen and Earley 2001). Although it is clear that RLS dysfunction involves the CNS, the exact areas of the CNS involved are somewhat unclear. So far no structural abnormalities have been noted in MRI imaging. However, Bucher and associates localized for the first time cerebral generators associated with sensory leg discomfort and periodic limb movements in 19 patients with restless legs syndrome, using highresolution functional magnetic resonance imaging. It was therefore proposed that and thalamic activation may occur because of sensory leg discomfort and that the red nucleus and brainstem are involved in the generation of periodic limb movements in
patients with restless legs syndrome (Bucher, Seelos et al. 1997). These results correspond with the absence of a cortical prepotential in patients with RLS before they experience PLMS providing further confirmation of a subcortical site (Stiasny, Oertel et al. 2002). The subcortical dopamine systems, in particular, have been suggested as candidates given their involvement in sensory and motor regulation, including modulation of spinal activity.

Transcranial magnetic stimulation studies have also found that, compared with controls, RLS patients show reduced intracortical inhibition for both foot and hand, as well as an increased cortical silent period without other changes. These findings also suggest abnormal (i.e., subcortical) functioning of the CNS (Bucher, Trenkwalder et al. 1996).

### 1.5 Dopamine Dysfunction in RLS

Dopamine (DA) is a neurotransmitter that is involved in behaviours such as movement, motivaton, cognition, reward and feeding. Dopamine has various functions within the CNS, including regulation of sensory and motor functions. Dopaminergic systems have been connected with a number of movement disorders, such as Tourette's syndrome, Parkinson's disease and Huntington's corea. In addition, animal studies have supported suggestions that the dopaminergic system is involved in the regulation of sleep (Bagetta, De Sarro et al. 1988).

### 1.5.1 Dopamine pathways

Three dopamine pathways are seen in the central nervous system (figure 1.1). The first is the nigrostriatal DA pathway. It originates in the substantia nigra pars compacta neurons and targets dorsal striatum. It is thought to be involved in modulating movement. The second pathway is the mesocorticolimbic DA system, which originates in the midbrain ventral tegmental area (VTA) and targets the ventral striatum (nucleus accumbens), the limbic system, the hippocampus, the prefrontal cortex, and modulates cognition, emotion and reward. The third pathway is the tuberoinfundibular pathway which originates in the hypothalamic paraventricular and acuate nuclei and targets the anterior pituitary where is inhibits prolactin release.


Figure 1.1: Dopamine Pathways
The three dopaminergic pathways; the nigrostriatal pathway which originates from the substantia nigra, the mesocorticolimbic pathway which originates from ventral tegmental area, and the tuberoindundibular pathway which originates from the hypothalamus
(http://www.cnsforum.com/imagebank/item/Neuro_path_DA/default.aspx).

Tyrosine hydroxylase ( TH ) is the rate limiting enzyme in dopamine biosynthesis, which converts the amino acid tyrosine to 2,3-dihydroxyphenylalanine (DOPA). The conversion of DOPA to dopamine is catalysed by the enzyme DOPA decarboxylase (DDC) (figure 1.2).


L-Tyrosine





## Dopamine

Figure 1.2. Dopamine Biosynthesis
A diagram of dopamine biosynthesis from tyrosine (http://scienceblogs.com/neurotopia).

Dopamine is moved into vesicles by the vesicular monamine transporter (VMAT), after being synthesised. The vesicles fuse with synaptic membrane with the electrical firing of the neuron, thus releasing DA into the synaptic cleft. DA then acts on preand post-synaptic DA receptors. There are at least five subtypes of DA receptors; D1, D2, D3. D4 and D5. They are divided into D1-like (D1, D5) and D2-like (D2, D3, D4) classes. The D2-like receptors have a high affinity for DA and dominate while asleep when DA levels are low. On the contrary D1-like receptors have a low affinity for DA and function while awake (during the day). The dopamine transporter (DAT) is a membrane spanning protein which terminates dopamine action. This rapidly reuptakes DA into the pre-synaptic terminal. Catechol-O-methyltransferase (COMT) also terminates DA activity by degrading it into homovanillic acid. Similarly, monamine oxidase (MAO) has the ability to degrade dopamine to dihydroxyphenylacetic acid (DOPAC).

### 1.5.2 Evidence for the involvement of Dopamine in the pathophysiology of RLS

Akpinar first suggested the dopaminergic deficiency theory of RLS in 1982. Pharmacologic and neuroimaging studies have subsequently provided evidence of the involvement of the central dopaminergic system, the striatonigral system particularly implicated.

Parkinson's Disease (PD)

RLS and Parkinson's disease (PD) both respond positively to dopaminergic treatments, both show dopaminergic abnormalities on functional imaging and are both associated with PLMS. RLS significantly improves with dopaminergic medications, usually at doses lower than those required to treat Parkinson's disease. Owing to the manner in which RLS responds to these medications, and observed coexistence within the same patients, an etiologic link between RLS and PD has been proposed.

The phenomenon of rebound and augmentation in RLS patients is essentially similar to fluctuations in motor responses and dyskinesias that can be observed in PD following long-term levodopa treatment (Appiah-Kubi, Pal et al. 2002). Ondo and colleagues (2002) suggested that RLS is not a risk factor for developing PD, as they observed that PD symptoms preceded RLS symptoms in $68 \%$ of cases (Ondo, Vuong et al. 2002). The study found a high prevalence of RLS symptoms; however, it failed to address the matter of whether or not they occur more often in PD patients than in the general population. Another population-based study with a larger sample did not find parkinsonian symptoms among RLS patients (Rothdach, Trenkwalder et al.
2000). The prevalence of RLS in PD patients is similar to that of the general population (Appiah-Kubi, Pal et al. 2002). In PD, the olfactory function is abnormal whereas it is normal in patients with RLS, thus reflecting the integrity of the A16 dopaminergic group (Adler, Gwinn et al. 1998). In addition, in patients with PD, an increase in total iron concentration in the Substantia Nigra (SN) was demonstrated by both pathologic and MRI approaches, but the opposite was demonstrated in RLS (Barriere, Cazalets et al. 2005). For these reason it has been suggested that the two diseases do not share the same pathophysiological mechanisms. Nonetheless, the positive response of RLS patients to dopmainergic treatments presents a strong argument in favour of the dopaminergic system involvement in the pathogenesis of RLS.

### 1.5.3 Central Nervous System (CNS)

## Cerebrospinal fluid (CSF)

The dopmaine system has also been explored by measuring the metabolites of dopmaine synthesis and the degradation of CSF. It has been suggested that the dopamine system is largely involved in the circadian pattern of RLS (Section 1.5.1). Lower dopamine and homovanillic acid levels in nocturnal urinary excretion have been found in subjects with PLMS. Larger circadian changes in CSF dopamine metabolites have been reported in RLS patients compared to controls: in patients, all metabolites were higher in the morning (at 10am) compared to the evening values (at 10p.m.) values (Earley, Hyland et al. 2006).

Neuroimaging studies:

Some positron emission tomography (PET) and single-photon emission computed tomography (SPECT) studies have suggested mild but significant pre- and postsynaptic dopamine receptor abnormalities in the basal ganglia (Stiasny, Oertel et al. 2002), while other studies contradict these findings (Allen, Picchietti et al. 2003). Furthermore several studies have shown decreased striatal dopmaine uptake and dopaminergic (D2) striatal binding in RLS patients, while results of other studies have not demonstrated differences on functional imaging in RLS patients compared with controls (Trenkwalder, Walters et al. 1999; Turjanski, Lees et al. 1999). PET studies on 13 patients with idiopathic RLS showed significantly decreased D2 binding potentials in the caudate and putamen compared to controls (Michaud, Soucy et al. 2002). Similarly in a SPECT study the dopamine status was assessed in 10 RLS/PLMS patients, and significantly lower striatal D2 binding was observed in patients compared to controls (Ruottinen, Partinen et al. 2000). Furthermore, fluorodopa PET studies of 9 patients with RLS showed a statistically significant, $88 \%$ reduction in fluorodopa uptake in the caudate and an $89 \%$ reduction in the putamen compared to controls. These findings indicated striatal presynaptic and postsynaptic dopaminergic dysfunction in RLS (Trenkwalder, Walters et al. 1996). Differences in striatal dopamine transporter and dopamine D2-receptor binding on SPECT among 25 RLS drug-naive or L-dopa-treated patients and 10 controls could not be demonstrated in another study (Trenkwalder, Walters et al. 1999). Therefore brain-imaging studies of the dopmainergic system failed to reveal consistent abnormalities. Dysfunction of dopaminergic pathways other than the nigrostriatal axis, e.g the diencephalospinal system, may play an important role in the pathogenesis of RLS (Chahine and Chemali 2006).

### 1.5.4 Pharmocological evidence

The strongest argument for a dopaminergic role in RLS is the excellent response to low-dose dopaminergic agents (DA) (Hening, Allen et al. 1999). To date, all evaluated agents that enhance dopamine activity in the body have been found to reduce RLS symptoms in open-labelled uncontrolled trials. Double-blind, placebo controlled studies have demonstrated the treatment benefit for Levodopa (Brodeur, Montplaisir et al. 1988; Trenkwalder, Stiasny et al. 1995) and for the currently used major dopamine agonists, i.e. pergolide (Wetter, Stiasny et al. 1999; Trenkwalder, Hundemer et al. 2004), pramipexole (Montplaisir, Nicolas et al. 1999) and ropinirole (Bliwise, Freeman et al. 2005). These medications are effective at remarkably low doses relative to that given for Parkinson's disease, often at the minimum tablet size available to the patient e.g. carbidopa/Levodopa at $25 / 100 \mathrm{mg}$ or pergolide at 12.5 mg (Allen 2004). Furthermore, they are effective immediately after the first therapeutic dose for almost all RLS patients. The immediate, universal and almost complete relief of RLS symptoms by low doses of Das provides strong support for a possible dopaminergic abnormality in this disorder.

Reversal of beneficial effects of dopamine agonists by antagonists further support the role of dopamine. This was first reported in a study published in 1991, when Pimozide, a dopamine agonist was shown to reverse treatment benefits of the opioid Codeine in an RLS patient (Montplaisir, Lorrain et al. 1991). In a later study, Metoclopramide given intravenously produced RLS symptoms on a suggested immobilization test in five out of six subjects evaluated and produced an increase in prolactin release that was at least three times greater than that expected from agematched normals (Winkelmann, Schadrack et al. 2001).

### 1.6 Iron pathology in RLS

The connection between iron deficiency and RLS was first proposed in the 1950's when intravenous infusions of iron were used for the treatment of RLS. Norlander reported that the creeping sensations in the legs of his patients ceased with iron therapy (Norlander 1953). Two years later in 1955, K.A. Ekbom discussed the relationship between RLS and iron deficiency when he described two male patients with malignancies that were severely iron deficient and also suffered from RLS (Ekbom 1955). Ekbom also noted that one in four of his RLS patients were iron deficient and that the RLS was increased among blood donors. This hypothesis of the relationship between RLS and low iron levels has prompted numerous studies into the role of iron in RLS. Investigations have shown a correlation between low body stores of iron and symptoms of RLS.

### 1.6.1 Evidence for the involvement of iron in the pathophysiology of RLS

Pregnancy

Pregnant women have a two to three times higher risk of experiencing RLS than the general population (Manconi, Govoni et al. 2004). In 11-27\% of pregnant women in Western countries, RLS symptoms are reported, despite supplementation with iron and folate. In a Japanese survey, a similar prevalence rate of $19 \%$ was reported (Suzuki, Ohida et al. 2003). Those women affected by RLS were also found to have a lower haemoglobin level and lower average red blood cell size, or Mean Corpuscular Volume (MCV), than those who did not experience RLS symptoms. In 2007, Tunc et al agreed that low iron levels were a risk factor for RLS in pregnant women. First signs and symptoms of RLS may only appear during pregnancy. However, pre-existing RLS is considerably exacerbated during pregnancy, mostly during the third trimester (Goodman, Brodie et al. 1988; Suzuki, Ohida et al. 2003; Manconi, Govoni et al. 2004). In the study carried out by Manconi and associates (2004), $16.7 \%$ of the women never experienced RLS symptoms before the pregnancy, whereas $9.9 \%$ had already experienced symptoms (Manconi, Govoni et al. 2004). In 2009 Dzaja et al found RLS to occur in some pregnant women during the third trimester, however this study concluded that transiently elevated Estradiol levels were responsible for triggering the RLS among this patient group (Dzaja, Wehrle et al. 2009). Notably, this study was conducted on a particularly small number of subjects, therefore analysis could not have fully assessed all of the factors influencing RLS symptoms. In 2008, Ghorayeb et al also discussed female hormonal changes as a factor in RLS symptoms severity, but on a larger number of patients,
and concluded that in those female patients with RLS, hormonal changes during pregnancy could not account for the worsening of symptoms (Ghorayeb, Bioulac et al. 2008). This study agreed with the findings by Manconi and associates that approximately $23 \%$ of women with RLS admitted worsening of symptoms during pregnancy and concluded that these changes in iron status could best explain these changes in symptom severity. Pregnancy related RLS is usually mild and transitory, with a tendency to disappear after delivery. One of the largest studies on RLS during pregnancy reported complete resolution of RLS in $96 \%$ of women 4 weeks after delivery (Goodman, Brodie et al. 1988).

Uremia and End-stage renal disease (ESRD)

One of the best-studied secondary causes of RLS is end-stage renal disease (ESRD) with dialysis. Reports found $62 \%$ of individuals with ESRD presented with mild RLS. Furthermore, prevalence rates of $20-25 \%$ were observed in patients with more severe RLS (Winkelman, Chertow et al. 1996; Hui, Wong et al. 2000). RLS symptoms with ESRD have been associated with increased mortality, with kidney transplantation resolving the renal disease and, also leading to complete resolution of RLS within 1-21 days after transplant (Allen 2004). Uremic patients who also were diagnosed with RLS had lower hemoglobin than those who did not have RLS (Takaki, Nishi et al. 2003). Moreover, RLS symptoms improved in patients on dialysis by regulation of anemia with erythroprotein and intravenous iron (Benz, Pressman et al. 1999). Another study linked low parathormone levels in uremic patients with RLS (Rijsman and de Weerd 1999). These studies show that it is the iron insufficiency associated with ESRD that produces the RLS symptoms.

Iron Deficiency

Anaemic patients have been studied and oral iron suuplements has been found to improve RLS symptoms in some of these patients (O'Keeffe, Gavin et al. 1994). In 2004, Earley et al showed successful treatment of 7 out of 10 RLS patients with IV iron treatment (Earley, Heckler et al. 2004).

Other conditions which have been investigated include low-density lipoprotein apheresis (Tings, Schettler et al. 2004), and these studies agree with the hypothese put forward by Allen et al in 2007 that "all conditions that compromise iron availability will increase the risk of RLS leading to a higher than expected prevalence of RLS in these conditions" (Allen and Earley 2007).

The hypothesis that iron status correlated with RLS symptom occurrence led to further studies of iron and how it affected RLS patients.

### 1.6.3 Peripheral Iron Status

Ferritin generally provides a measure of iron storage, with low values indicating low iron storage. Serum ferritin reflects the levels of iron in the peripheral stores and CSF ferritin reflects the levels of iron in the brain stores. Similarly, transferrin provides a measure of the tissues need for iron ,where the serum transferring reflects the peripheral tissue's iron needs, and the CSF transferrin reflects the brain's iron needs. As the iron requirements of a tissue increase, the level of transferring found in the fluid associated with that tissue also increase.

The serum ferritin values of patients believed to be iron deficient was compared to the iron status of their bone marrow in a study carried out by Guyatt and colleagues (Guyatt, Patterson et al. 1990). It was discovered that a serum ferritin of below 45 $\mu \mathrm{g} / \mathrm{L}$ indicated that the patients peripeheral iron stores were depleted. Nonetheless it is important to keep in mind that ferritin has phase-reactive properties and can give falsely elevated results. Therefore measurements of percentage transferrin saturation and total iron binding capacity (TIBC) are more useful for clinically determining iron store status. TIBC is a measurement of the maximum amount of iron that the blood can carry. In 2006 a double-blind controlled study found that for those RLS patients with low ferritin values oral iron treatment was successful in treating their symptoms, thereby agreeing with the 1994 study by O' Keeffe et al (O'Keeffe, Gavin et al. 1994), but disagreeing with the findings of the 2000 study by Davis et al (Davis, Rajput et al. 2000). This study by Davis et al, found no improvement of RLS symptoms following administration of oral iron, however the study did not include a sufficient number of patients with low serum ferritin. The American National Heart, Lung and Blood institute suggest that in RLS patients with a serum ferritin of less
than $50 \mu \mathrm{~g} / \mathrm{L}$, iron deficiency can be implicated in the syndrome (National Heart 2000). Thereby in a 2004 study it was recommended that RLS patients with a serum ferritin of less than $50 \mu \mathrm{~g} / \mathrm{L}$ should be considered for oral iron treatment (Silber, Ehrenberg et al. 2004).

### 1.6.4 Central Nervous System (CNS)

## Autopsy Studies

Generally when cells are iron deficient they respond to this deficient state by increasing the quantity of Transferrin Receptors (TfR) on their surface, thereby increasing the chance that transferrin will bind to the cell and supply iron (Aisen, Enns et al. 2001). The ability to either increase or decrease the cells TfRs concentration is achieved by post-transcriptional regulation by two iron regulation proteins (IRPs); IRP1 and IRP2.

Autopsy studies which have been performed so far have focused primarily on the substantia nigra. This is due to its dopmaine producing activity. These autopsy studies have demonstrated the following:
a) Neuromelanin cells are the darkly pigmented neuronal cells present in the brain. These cells produce monoamine neurotransmitters e.g. dopamine. The neuromelanin cells of the substantia nigra are similar in density between the brains of RLS patients and those of controls (Connor, Boyer et al. 2003).
b) Compared to the brains of control subjects, the neuromelanin cells of the substantia nigra of RLS patients have an increased concentration of Tyrosine Hydroxylase (Connor, Wang et al. 2004).
c) In comparison to the brains of control subjects, iron concentration in the neuromelanin cells of the substantia nigra of RLS patients is decreased (Connor, Boyer et al. 2003).
d) In RLS patient brains, IRP1 activity is decreased (Connor, Wang et al. 2004).
e) In RLS patient brains, IRP2 activity is normal/increased. (This may be a compensatory mechanism in response to the decreased IRP1) (Connor, Wang et al. 2004).
f) In comparison to the brains of control subjects, the expression of the TfR on the neuromelanin cells of the substantia nigra of RLS patients is decreased, thereby consistent with the reduced activity of IRP1 (Connor, Wang et al. 2004).
g) Thy-1 is a T-cell protein which is expressed on some neuronal cells i.e. at the dopmainergic synapses (Morris 1985). The function of Thy-1 is believed to be in the association of pre-synaptic and post-synaptic membranes, and the fusion of vesicles to the pre-synaptic membrane (Jeng, McCarroll et al. 1998). Thy- 1 is responsive to iron status (Ye and Connor 2000). It is thought that a Thy-1 deficiency would lead to the decreased structural integrity of the cells on which it is expressed.

It was found that Thy-1 expression is decreased not only in RLS brains but in models which were iron deficient. If this deficiency results in loss of structural integrity, this might explain why no morphological differences have been observed between the brains of RLS patients and controls, and the reason that RLS symptoms appear to improve with the treatment of dopmainergic agonists (Wang, Wiesinger et al. 2004).

Cerebrospinal Fluid (CSF)

CSF was utilised for the indirect measurement of brain iron status. As previously mentioned, in iron deficiency, serum ferritin levels are usually low and serum transferrin levels high. In 2000, Earley and associates carried out a study which analysed the CSF concentrations of ferritin and transferrin in RLS patients (Earley, Connor et al. 2000). They discovered that CSF ferritin concentrations were low and transferrin levels were high, thus indicating brain iron insufficiency in RLS patients. In 2005 these results were further verified (Mizuno, Mihara et al. 2005).

In the report published by Earley and associates in 2000, it was found that the CSF ferritin levels of the controls improved as their peripheral ferritin status moved closer to the normal $100 \mu \mathrm{~g} / \mathrm{L}$. However, the normal CSF ferritin concentrations observed in the control subjects with normal serum ferritin concentrations would only occur for the RLS patients with a serum ferritin of over $400 \mu \mathrm{~g} / \mathrm{L}$. This would be considered highly elevated in a normal population. Therefore one hypothesised that RLS patients had an impaired ability to get brain iron from peripheral iron, which could in turn be due to impaired iron transport in the brain and/or an impaired ability of the brain to retain iron.

Neuroimaging Studies

To date Neuroimaging studies include Magnetic Resonance Imaging (MRI) which measures the brain iron status; Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT), both which examine the dopaminergic system;.MRI studies have been performed to measure regional brain iron concentrations. These studies found that decreases in iron concentration in the
nigrostriatal areas correlate with RLS severity (Allen, Barker et al. 2001). The decrease in iron was most evident in patients with a severe form of RLS. This finding was confirmed in a large sample of subjects with early-onset RLS (Earley, B. Barker et al. 2006), as well as in other studies using a transcranial ultrasound method (Schmidauer, Sojer et al. 2005). A more recent MRI study showed that the decrease in brain iron insufficiency extended beyond the nigrostriatal areas, indicating that the iron deficiency was brain-wide, thus emphasizing the importance of correct interpretation of MRI results when assessing brain iron status (Lee 2007).

### 1.6.5 Role of Iron in the Dopaminergic Pathway

Iron distribution in the brain is highly heterogenous and is higher in the dopaminergic regions such as the substantia nigra and striatum. The strong evidence of the role of iron deficiency and the role of the dopaminergic system raises the question of the relationship between iron and dopamine. There are two leading arguments indicating that decreased iron would lead to abnormal dopaminergic transmission (Krieger and Schroeder 2001). Firstly, iron is a known co-factor for tyrosine hydroxylase, a rate-limiting enzyme involved in the synthesis of dopamine. This enzyme catalyses the hydroxylation of troine into dihydroxyphenylalanine (DOPA) which is ultimately transformed into dopamine. The second is that iron is involved in the physiological regulation of the dopamine D2 receptor binding sites and the effect of iron deficiency is specific for these receptors, however, the exact mechanism remains to be resolved (Earley, Allen et al. 2000). Iron deprivation in rats results in a $40-60 \%$ reduction of D2 postsynaptic receptors, while dopamine type-1 receptors are not affected (Erikson, Jones et al. 2001).

### 1.7 Circadian Pattern of RLS

RLS symptoms display a true circadian pattern, with an increase in both sensorial and motor symptoms occurring most frequently in the evening and at night. Typically, the untreated patient reaches a maximum symptomology between 11 pm and 4 am , with the symptoms being reduced between 6 am and 12 pm (GarciaBorreguero, Larrosa et al. 2002). Since the dopamine system is proposed to play a central role in the pathophysiology of RLS, it is likely that alteration in the circadian variation of the dopaminergic system or related compounds might account for RLS symptom fluctuations. For example, changes in melatonin secretion, as a maker of the circadian rhythm, preceed the increase in sensory and motor symptoms in patients with the syndrome (Trenkwalder, Paulus et al. 2005). Nevertheless, the circadian rhythymicity is overridden and symptoms are present 24 hours a day in patients with increased disease severity.

In 2009, Sharifan et al published a study which investigated the relationship between shift work patterns and the occurrence of RLS. The study was conducted in an automobile manufacturing factory in Iran. During the study they noted that there was a greater percentage of antihitamanic and antidepressant drug consumption, a higher percentage of chronic disorders and a higher percentage of smokers among the RLS individuals than in controls. The study found that the prevalence of RLS was significantly higher in rotational shift workers (15\%) than in worker with permanent morning work schedules ( $8.5 \%$ ). Work during the night shift is much more likely to have adverse effects on health than work during morning or afternoon shifts because the circadian organisation of the body is disrupted. This indicates that the association
between rotational shift work and RLS is most likely mediated by disruption of circadian rhythmicity (Sharifian, Firoozeh et al. 2009).

### 1.8 Periodic Limb Movements during Sleep (PLMS)

Periodic limb movements during sleep (PLMS) have been classically described as rhythmic extension of the big toe and dorsiflexion of the ankle, with occasional flexion at the knee and hip (Allen, Picchietti et al. 2003). PLMS may be accompanied by brief awakenings that result in sleep fragmentation and subsequent excessive daytime sleepiness. Diagnosis of periodic limb movements during sleep is based on the definition of the American Sleep Disorders Association (Trenkwalder, Paulus et al. 2005). These movements are measured by surface electromyography from the tibialis muscle and are scored only if they occur in a series of four consecutive movements lasting $0.5-5 \mathrm{~s}$ and recurring at intervals of 5-90 s . The muscle contractions must have amplitude of at least $25 \%$ of the toe dorsiflexion during calibration. An index (number of PLMS per hour of sleep) greater than five for the entire night is considered pathologic.

The presence of PLMS in patients with RLS was first documented in 1965, and was originally called "nocturnal myoclonus". Since then, several studies have been carried out, and it was estimated that about $80 \%$ of patients with RLS have periodic limb movements during sleep (Montplaisir, Boucher et al. 1997). A recent study carried out by Boehm et al., (2008) characterised PLMS and their association with sleep disturbances in RLS patients and healthy controls without sleep complaints (Boehm, Wetter et al. 2008). Measures of PLMS showed that the RLS patients have a significantly longer mean duration of single PLM during wakefulness and non rapid eye movement (NREM) sleep. For future studies this mean duration of single PLM might be an appropriate parameter to discriminate between RLS patients and
healthy subjects with PLM. Decreased sleep efficiency was associated with a higher number and shorter duration of PLM sequences (Boehm, Wetter et al. 2008).

It should be emphasized that RLS remains a distinct clinical diagnosis. However, PLMS occur frequently in several other sleep disorders, including narcolepsy, rapid eye-movement sleep behaviour disorder, and obstructive sleep apnoea; as well as an isolated occurrence (Trenkwalder, Paulus et al. 2005). PLMS also appear more abundant among the elderly, possibly indicating a general aging process. Therefore, although the presence of PLMS is not specific to RLS, an elevated PLMS is supportive of the diagnosis of RLS. Effective treatments for RLS generally decrease episodes of PLMS.

### 1.9 Genetics of RLS

### 1.9.1 Familial Aggregation

Primary (genetic or familial) RLS may be difficult to distinguish from RLS associated with uraemia, iron deficiency, neuropathy, or other disorders (secondary RLS). Ekbom first described the familial component of RLS in 1945, estimating the frequency of hereditary RLS compared to sporadic cases as 'one-third' (Ekbom 1945). In 1960, Ekbom also reported that 49 out of 112 unselected probands with severe, typical RLS knew of one or several close relatives with creeping sensations in their legs (Ekbom 1960). Since then many studies have been published on the substantial genetic contribution in the aetiology of this sleep disorder. Clinical surveys have shown that up to $60 \%$ of idiopathic RLS patients report a positive family history (Walters, Hickey et al. 1996). Furthermore, RLS is 3-5 times greater amongst first degree relatives of subjects suffering from RLS than relatives of subjects without RLS. Clinical manifestations and the course of the disease appear similar in familial and sporadic cases (Winkelmann, Wetter et al. 2000). However, familial RLS undoubtedly correlates with an earlier age at onset, a much slower progression of symptoms, and a limited relation to serum iron status in contrast to late-onset RLS patients (Walters, Hickey et al. 1996; Allen and Earley 2000).

One of the best methods for evaluating the genetic and environmental contributions to a disease is twin study. Two twin studies have been published to date (Ondo, Vuong et al. 2000; Desai, Cherkas et al. 2004). One study examined twelve pairs of monozygotic (MZ) twins in which both members of ten pairs had definite RLS based on the IRLSSG criteria. The high concordance rate suggested, similar to other investigations, an autosomal dominant mode of inheritance with a high penetrance.

Despite this, however the symptom descriptions and age of symptom onset varied markedly (Allen and Earley 2001). Desai and associates evaluated RLS symptoms in 933 MZ pairs and 1004 dizygotic (DZ) pairs. Concordance rates were found to be $61 \%$ and $45 \%$ respectively (Desai, Cherkas et al. 2004).

### 1.9.2 Pattern of Inheritance

Pattern of inheritance or mode of inheritance, refers to the way in which genes are passed down from generation to generation. By analyzing the pattern of inheritance, the relative risk for and penetrance of an illness can be estimated. An autosomal dominant mode of inheritance with variable expressivity was suggested following several investigations of single families with RLS (Walters, Picchietti et al. 1990; Trenkwalder, Seidel et al. 1996). The possibility of anticipation - i.e. the disease starts earlier with each new generation - has been described in three large pedigrees of familial RLS. In 1996, Trenkwalder et al., (1996), demonstrated evidence of anticipation in one large German pedigree. In this German kindred which included 20 affected members, the mean age of onset fell from 51.5 years in the second generation to 19.8 years in the fourth generation (ANOVA, $\mathrm{p}=0.025$ ) (Trenkwalder, Seidel et al. 1996).

### 1.9.3 Linkage studies

To identify the gene(s) involved in a disorder the most powerful method is pedigree analysis. This involves using linkage statistics to compare the distribution of disease inheritance in high-risk families with the inheritance of a specific chromosomal region (locus) and disease-causing gene mutations. Linkage studies typically use polymorphic microsatellite repeat markers, spaced evenly at about $10 \mathrm{cM}(10 \mathrm{Mb})$ intervals. A likelihood of $\sim 3.3$ (described in section 1.12), at or adjacent to a marker in a genome wide study, is considered significant evidence that a disease and a chromosomal region co-segregate within a family (P-value <0.05). Linkage studies in families with RLS have identified ten loci that are associated with RLS, however no causal sequence variant has yet been recognised.

The first major susceptibility locus for RLS was identified in a French-Canadian family in 2001 (Desautels, Turecki et al. 2001). About 380 genetic makers spaced throughout the genome, were analysed in 25 individuals (14 affected, 4 at risk and 7 healthy). The locus was found on the long arm of chromosome 12 and connected with a series of adjacent microsatellite markers with an autosomal recessive mode of inheritance. The linkage was followed up by a study of 276 individuals from 19 families. Five of these lineages, which had a French-Canadian origin, were consistent with the chromosome 12 q linkage. The results also indicated the presence of other loci involved in this disorder due to six of the larger pedigrees not demonstrating any linkage to chromosome 12 (Desautels, Turecki et al. 2005). Furthermore a linkage signal was found in the Icelandic population (D.B. Reye 2005), and in a number of German families using the transmission disequilibrium test (Winkelmann, Lichtner et al. 2006).

Almost two years later, a second locus was identified on chromosome 14. In this study, 24 individuals (13 affected) in a large family from Northern Italy were genotyped for 382 polymorphic microsatellite markers. Five potential candidate loci were recognized. However, only a locus on chromosome 14 remained significant following the incorporation of more markers and increasing the number of individuals analysed. An autosomal dominant model was obtained, with variable penetrance depending on whether patients have periodic limb movements with or without RLS symptoms. The locus, which spans 9.1 cM on chromosome 14, contains at least 60 genes (Bonati, Ferini-Strambi et al. 2003).

In 2004, Chen and associates identified significant linkage to a third locus on the short arm of chromosome 9 (Chen, Ondo et al. 2004). In this investigation, 15 multiplex American families were genotyped for more than 400 markers and significant linkage to a third locus was found in two of the larger families. Chen and colleagues (2004) used an autosomal-dominant model-based analysis for this study. This study represented the first model-free linkage analysis used to genetically dissect RLS. The locus spans 34.43 cM and is located on chromosome 9p24-22.

In 2005, a large Irish family with autosomal dominant RLS was studied with the subsequent identification of a novel locus on chromosome 5q. Microsatellite markers spaced throughout the genome were analysed in 29 family members (12 affected). A genome scan for linkage was performed. Recombination events in two of the affected family members defined the flanking markers, thus placing the locus in an 11.5 cM region. This interval is 16.8 Mb long and contains approximately 120 known or predicted genes. The authors also highlighted four candidate genes for mutation analysis. These genes included the neurolysin ( $N L N$ ) gene, which is involved in
central dopaminergic regulation; the $\operatorname{CART}$ gene involved in the regulation of dopamine levels; the HTR1A gene that encodes the 5-hydroxytryptamine (serotonin) receptor; and the PMCHL2 gene, which exhibits diurnal variation and is active during certain phases of sleep (Parfrey et al., 2006).

In 2006, Levchenko and associates reported the first autosomal dominant locus in the French-Canadian population, mapping to chromosome 20p13. Desautels et al., had previously described an RLS locus in a French-Canadian population, but under an autosomal recessive model. Genomic DNA from fifty-six members of this FrenchCanadian family was used, and investigators performed a $10-\mathrm{cM}$ genome-wide scan using a panel of 377 microsatellite markers. The chromosome 20 candidate region is flanked by the telomeric end of chromosome 20 p and by a 0.56 cM interval between markers D20S849 and D20S835. This region spans approximately 16 cM or 5.2 Mb containing 84 annotated genes (Levchenko, Provost et al. 2006).

Later that same year Pichler et al., (2006) published a paper in The American Journal of Human Genetics providing strong evidence for the identification of a new susceptibility locus for RLS at 2q33. An isolated population in the South Tyrolean Alps was identified and 530 adults took part in the study, 37 of which were identified with having idiopathic RLS. A 4-cM genome-wide linkage scan was performed on the DNA from all the 530 participants and the data was analysed using both nonparametric and parametric analysis. A shared disease haplotype from two of these families defined a candidate region of 8.2 cM on chromosome 2 q . This study illustrates the use of isolated populations i.e. the western Alps of South Tyrol (Italy), where there are few founders, expansion is slow, traditional lifestyle has been maintained, and historical documents are highly available and accessible. The use of
such population can reduce genetic complexity and potentially the environmental heterogeneity (Pichler, Marroni et al. 2006).

Two loci were identified in a single RLS family on chromosome $4 q$ and $17 p$ under the assumption of an autosomal-dominant model (Winkelmann J 2006). A genomewide linkage analysis was conducted on a large RLS family of German origin. Twopoint linkage analysis of this pedigree revealed a LOD score of 2.3 and 2.1 and a multipoint LOD score of 3.08 and 2.78 on chromosome 4 and 17 respectively. The disease associated region on chromosome 4 was refined between D4S406 and D4S402, defining a $8.4 \mathrm{Mb}(7.39 \mathrm{cM})$ region. The disease associated region on chromosome 17 was located between markers D17S1857 and D17S1294, thus defining a $9.05 \mathrm{Mb}(7.73 \mathrm{cM})$ region. The latter finding was replicated in an independent family-based linkage study of 159 RLS trios from 8 European countries.

A novel locus for RLS on chromosome 16p12.1 was reported by Levchenko and colleagues in October 2008 (Levchenko, Montplaisir et al. 2008). The locus was identified in a French-Canadian family and supporting evidence of linkage was presented in a smaller pedigree of similar origin. An overall candidate region of 1.18 Mb was revealed. This region was divided in two linked regions of $\sim 850 \mathrm{~kb}$ and 350 kb each by an uncommon and rare double recombination in one individual. It was hypothesized that this recombination may have taken place in the situation of genomic rearrangement, e.g. duplication, therefore it was decided to follow with a karyotype analysis and copy number variation (CNV) analysis. Both analyses showed that the region has a normal chromosomal region. Furthermore, no diseasecausing variants were uncovered following sequencing of eight positional candidate genes (Levchenko, Montplaisir et al. 2008).

Kemlink and associates (2008) provided suggestive evidence for a new susceptibility locus for RLS on chromosome 19p. A genome-wide linkage study was conducted in a large RLS family of Italian origin with 12 affected members in 3 generations. 5,861 SNPs were used initially, and 13 additional microsatellite markers on chromosome 19p and 7 markers on chromosome 10p were subsequently genotyped to confirm the SNP genotyping and to reduce the candidate regions. The genomewide scan resulted in suggestive linkage on chromosome 19 p with a maximum multipoint logarithm of the odds score of 2.61. Although this value is below the threshold typically used for a Mendelian trait (i.e., a LOD > 3), Kemlink and associates did not observe a simulated result above this threshold. This may be due to the small pedigree used for the study. To confirm linkage to chromosome 19p, the locus was replicated in a family-based association study in a set of 159 trios of European origin. This study presents a further susceptibility locus for RLS mapped within a $1.90 \mathrm{cM}(0.3 \mathrm{Mbp})$ interval between D19S429 and D19S915. Two candidate genes, RAB3A and KCNN1 were subsequently chosen based on location and the believed pathophysiogy of RLS. However, no mutation was identified (Kemlink, Plazzi et al. 2008).

### 1.9.4 Molecular genetic studies

Few molecular genetic studies have been undertaken to determine genes that predispose to RLS, despite the many reports that have been published suggesting a genetic contribution.

In the studies that were performed, emphasis was put on the genes encoding for the GABA A receptor subunits and the gene for the alpha1 subunit of the glycine receptor (Winkelmann and Ferini-Strambi 2006). However, no significant findings were reported.

Desautels and colleagues (2002) investigated the role of Monoamine Oxidase (MAO) A and B genes in association with the RLS phenotype. Females were found to have high activity alleles, consequently resulting in an elevated $M A O-A$ activity. It was concluded that an elevated MAO-A activity would lead to an increased dopaminergic catabolism and cause a reduction in synaptic levels of this neurotransmitter. It is postulated that the high activity of the $M A O-A$ gene may represent a modifying factor in the severity of the RLS manifestation. Estrogens may in turn interact with specific MAO alleles (Desautels, Turecki et al. 2002). The involvement of the dopaminergic system in the etiology of idiopathic RLS has also been suggested. Eight relevant candidate genes involved in metabolism and dopaminergic transmission were examined in a study. The study was carried out on 92 patients and 182 controls; however, no significant difference was established in the genotypic or allelic distribution between the two groups. Nonetheless, the significance of the dopaminergic system in the pathogenesis of RLS has not been ruled out. Other loci implicated in the dopaminergic system remain to be studied (Desautels, Turecki et al. 2001).

### 1.9.5 Genome-wide Association Studies

Association studies compare the frequencies of alleles in case and control populations. A higher frequency of the allele tested in the cases is accepted as evidence that the allele or genotype is associated with an increased risk for the disease.

In 2007, Stefansson et al., reported the results of a genome-wide association study in an Icelandic discovery sample of patients with RLS and PLMS. The study revealed that one single nucleotide polymorphism (SNP) in the BTB (POZ) domaincontaining 9 (BTBD9) gene was associated with RLS. This association was replicated in a second smaller sample from Iceland and a United States sample. It showed that the discovery originated form a subgroup of patients with RLS who presented with PLMS. The absence of such an association for RLS without PLMS suggests that a genetic determinant of PLMS has been identified. Furthermore the serum ferritin levels were decreased by $13 \%$ per allele of the at-risk variant (95\% confidence interval, 5 to $20 ; \mathrm{p}=0.002$ ). This inverse correlation of the variant with iron stores is consistent with the suspected involvement of iron reduction in the pathogenesis of the disease (Stefansson, Rye et al. 2007).

Also in 2007, Winkelmann et al. performed a similar analysis for patients with RLS who were of European decent. A total of 236,758 SNPs were used for the first stage of the study. For stage 2, 13 SNPs (based on P value) and 15 neighbouring SNPs, were replicated in a German and French-Canadian case/control sample. The study highlighted SNP associations in four genes in three chromosomal loci; MEISI, BTBD 9 and MAP2K5/LBXCOR1. The second region mentioned here is interesting as it confirms the association with BTBD9 found also by Stefansson et al.,(2007).

BTB stands for broad complex, tramtrack and bric a brac, genes that in Drosophila melanogaster are required for embryonic development, cell fate determination in the eye and pattern formation in the limbs. In humans, BTBD9 is widely expressed in parts of the brain, such as the amygdala, cerebellum, hippocampus, and caudate and subthalmic nuclei, and in other organs, such as the heart, kidneys, pancreas and liver. The BTBD9 protein is not well characterised, and its function has not yet been determined (Winkelmann, Schormair et al. 2007).

Further analysis was carried out in the Mayo clinic in 2008, and genotyping was carried out on 11 SNPs spanning all three reported loci for disease susceptibility. The subjects used in this study consisted of 244 patients with RLS and 497 controls. Statistical analysis verified the association with MEIS1 and BTBD9 genes but showed only a trend for variants in MAP2K5/LBXCOR1 and RLS. The odds ratio for the associated risk alleles ranged from 1.8 to 2.9 for $B T B D 9$ and from 2.6 to 3.7 for MEIS1. It is interesting that MEIS1 was not detected in the original study of the Icelandic population, given the magnitude of the results. There could be many reasons for this such as, the use of different genotyping platforms or the diversity between populations (Vilarino-Guell, Farrer et al. 2008).

Furthermore, Kemlink and associates investigated if these three variants, which had been previously identified in German and Canadian RLS cases, were relevant among other European (Czech, Austrian, and Finnish) RLS cases (Kemlink, Polo et al. 2009). The study proved that these three loci present consistent disease risks in patients of European descent. Therefore these genetic determinants, observed in BTBD9, MEIS1, and MAP2K5/LBXCOR1, are risk factors for RLS in multiple populations. The study also found that $B T B D 9$ was the most consistent in its effect
on RLS across populations and was also more independent of familial clustering than the other two variants.

The genome-wide association studies that were carried by Winkelmann and associates and by Steffansson and colleagues (2007) identified four genes in total. However none of these genes are located in any of the previously described linkage regions for RLS. Schormair et al., (2008) analysed the five known loci (RLS1RLS5) in a genome-wide association study, which resulted in significant signals in RLS3 on 9 p23-24. From this 31 Mb region ( $9 \mathrm{p}, 0.5-31.5 \mathrm{Mb}$ ), an association study with 3,270 SNPs was carried out. For the exploaratory genome-wide scan, 628 RLS cases and 1,644 population-based controls were genotyped from the KORA-S3/F3 study, as previously used by Winkelmann et al., 2007. Of the 3,270 SNPs analysed in RLS3, 8 SNPs passed the criterion for replication. In the replication phase, these SNPs were genotyped in German, Czech, and Canadian samples. Two independent SNPs (rs4626664 and rs1975197) in the $5^{\prime}$ ' UTR of splice variants showed highly significant $P$ values. The association signals were located 0.41 Mb apart and mapped to introns 8 and 10 of protein tyrosine phosphatase receptor type delta (PTPRD) gene within two separate linkage disequilibrium (LD) blocks. Several PTPRD mRNA isoforms are expressed in developmental and tissue-specific manner. Both RLS-associated SNPs are located within the $5^{\prime}$ UTR, which consists of ten noncoding exons contained in two known long splice variants expressed predominantly in fetal and adult brain tissue. The involvement of $P T P R D$ in RLS remains unknown. These two RLS-associated SNPs are common. Rare alleles with strong effects within this gene that could explain the linkage signal were not detected (Schormair, Kemlink et al. 2008).

To further identify genes related to RLS Winkelmann and associates (2008) performed another association study. This time they carried out a three-stage association study in two Caucasian RLS case-control samples, which contained 918 independent cases and controls. In the first stage (explorative study), 1536 SNPs in 366 genes in a 21 Mb region encompassing the RLS1 critical region on chromosome 12 were screened. Three genomic regions were identified which were significant ( $P$ < 0.05). The second stage (the replication stage) consisted of genotyping the most significant SNPs of stage 1 . Following correction for multiple testing, an association was found with SNP rs7977109. This SNP is found in the neuronal nitric oxide synthase (NOS1) gene. In addition to the five SNPs typed in the first two stages, an extra 29 SNPs were used for fine mapping the NOS1 gene in the third and final stage, thus further confirming the significant association results to NOS1. Nitric oxide synthase action in the CNS has been associated with pain perception as well as the control of sleep regulation. Nitric oxide (NO) performs as an intracellular messenger in the CNS, and moreover the NO-arginine pathway is intimately connected to the modulation of the dopaminergic transmission. A positive response to dopaminergic agents supports the diagnosis of RLS. The pathophysiology of the disorder is thought to be related to an alteration of the dopaminergic neurotransmission. A relationship between the opioidergic system and NO could also be relevant in the pathophysiology of RLS. In conclusion this study provides indication for an association of variants in the NOS1 gene and RLS, and points to the involvement of the NO-arginine pathway in the pathogenesis of RLS (Winkelmann, Lichtner et al. 2008).

### 1.10 Management

The high prevalence of RLS does not necessarily mean that all patients with RLS should be treated with pharmacological therapy. Patients with sporadic or only mild symptoms who do not suffer from considerable impairment are unlikely to require pharmacological treatment. The proportion of such patients within the whole RLS population is currently the subject of ongoing studies. According to previous studies it is estimated that approximately $2-3 \%$ of the population have RLS that is severe enough to merit treatment (Trenkwalder, Hogl et al. 2009). The latest epidemiological study from Germany reported that among 1312 individuals the prevalence of RLS was $8.8 \%$; and only $1.6 \%$ of the study population wanted treatment. Unfortunately, there is no data available for mild to intermittent RLS and its response to treatment. Before beginning pharmacological treatment, it is important that sleep hygiene measures be suggested, and that all causes of secondary RLS, such as iron deficiency, be ruled out.

### 1.10.1 Nonpharmocological Treatments

Behaviour therapy- Many physicians recommend certain lifestyle changes and activities to reduce or eliminate symptoms. Researchers have found that alcohol, tobacco, and caffeine may aggravate or trigger symptoms in RLS patients. Decreased use or elimination of such substances may relieve symptoms. Physicians suggest a program of regular moderate exercise. A small randomized controlled trial of 23 patients illustrated improvement in symptoms of RLS with a program consisting of lower body resistance training and aerobic exercise (Aukerman, Aukerman et al. 2006). Some patients also benefit from hot or cold baths, whirlpool baths, acupuncture, chiropractic therapy, stress/relaxation therapy, and mental alerting
activities such as math/computer work. More recently, advances in additional psychotherapeutic interventions have been described and are presently under investigation (Hornyak, Grossmann et al. 2008).

Iron supplementation - Treatment of RLS should initially be directed toward any underlying illness, if possible. For example, clinicians should consider determining the serrum ferritin levels in RLS patients, especially those with a history of gastrointestinal blood loss, frequent blood donation or menorrhagia. A serum ferritin concentration lower than 45 to $50 \mu \mathrm{~g} / \mathrm{ml}$ has been observed in patients with increased severity of RLS (O'Keeffe, Gavin et al. 1994; Sun, Chen et al. 1998). If the serrum ferritin level is low, or percent iron saturation is low, replacement treatment is recommended.

Transcutaneous Electric Nerve Stimulation (TENS) - TENS is a non-invasive pain control treatment, which involves applying electrical stimulation to an area of the feet or legs. A session typically lasts from five to fifteen minutes and treatment may be applied as often as necessary depending on the severity of the pain.

Clinicians should also consider if certain medications (e.g. antidepressants, neuroleptic agents or dopamine-blocking antiemetics) are associated with initiation or worsening of RLS. Discontinuation of such medication should be considered, ensuring no harm is caused to patients.

### 1.10.2 Pharmocological Treatment

If pharmacological treatment is necessary, the medication that is initially selected is based upon individual's specific RLS symptoms. It is recommended that medications should be initiated at a low dose and be taken an hour or two before bedtime, thus to allow sufficient absorption and commencement of a reaction. If symptoms cause awakening during the night, additional doses can be taken. To prevent development of tolerance to one drug, monthly rotation of two to three drugs may be effective. In severe cases, a combination of drugs may be favorable. It is important to recognize that a variety of medications may be prescribed and eventually discontinued before the most effective $\operatorname{drug}(\mathrm{s})$ - and the suitable dosage level(s) - are determined. It is accepted that dopamine is the neurotransmitter most closely associated with the pathophysiology of RLS based on the class effect of levodopa (L-dopa) and Dopamine Agonists against RLS symptoms Generally, physicians chose from dopaminergics, dopaminergic agonists, opioids and benzodiazepines.

Dopaminergics - The first-line drug for most RLS patients are dopaminergic agents, which are largely used to treat Parkinson's disease. Treatment with levodopa plus carbidopa, have received positive results when used for RLS that occurs intermittently in the evening, at bedtime, or during specific activities such as airplane or lengthy car journeys. Studies have consistently shown that Levodopa reduces PLMS and nocturnal RLS symptoms (Stiasny, Wetter et al. 2001). For maximal absorption, it is recommended that levodopa be not taken with high-protein foods.

The manifestation of augmentation and rebound are the two most frequently encountered problems associated with Levodopa treatments.

Rebound refers to the appearance of symptoms at a time coinciding with the end of the half-life period of the drug. The recurrence of RLS in the early morning occurs in $20 \%$ to $35 \%$ of patients taking levodopa (Hening, Walters et al. 1999; Hening, Walters et al. 2004).

Augmentation is the most important complication of dopaminergic therapy. It involves earlier onset of RLS symptoms during the day, commencement of symptoms during the day, an involvement of other body parts such as the arms, an increased severity, or a shorter latency to the beginning of RLS symptoms while at rest (Allen and Earley 1996). Up to $70 \%$ of patients, taking levodopa daily will develop augmentation, and the risk increases with daily doses of 200 mg or more. Augmentation, however, can start soon after therapy is begun or not until months or years later. The mechanisms causing the development of augmentation are still unknown. The main hypothesis is that a dopaminergic overstimulation of the striatal dopamine D1-receptors may play a role (Paulus and Trenkwalder 2006). Low serum ferritin levels may also enhance the symptoms of RLS (Trenkwalder, Hogl et al. 2008). Furthermore, regardless of the difficulty of comparing augmentation rates from different studies it has become evident that higher dopaminergic doses must be considered as a risk factor for augmentation (Allen and Earley 1996).

Tolerance to treatment has been described in some cases of dopaminergic therapy. It is the loss of therapeutic efficacy of an RLS treatment that had previously been efficacious.

Punding refers to an intense fascination with complex, stereotypical, purposeless, and repetitive behaviours e.g. sorting and hording of objects, cleaning and grooming. It has been previously described in high-dose psychostimulant users and patients with PD on dopamine agonists or levodopa. In October 2008, Evans and associates described two individuals with RLS who were treated with dopamine agonists and developed punding along with a variety of impulse control disorders (ICDs). Nevertheless, patients treated with agonists for RLS generally show a predisposition to punding (Evans and Stegeman 2009). Further studies are needed to fully evaluate punding and other ICDs with regards to RLS.

Dopaminergic Agonists stimulate dopaminergic receptors. Generally, agonists which do not cross the blood-brain barrier have peripheral effects and agonists which penetrate well into the brain have mainly central effects. Agents such as mesylate, pramipexole, and roprinirole hydrochloride have proved to be effective in some patients. Although augmentation may be observed in some patients, it does not appear to be a major problem (Odin, Mrowka et al. 2002).

Pergolide mesylate (Permax) is a potent, long acting dopamine agonist, which has been proven effective in RLS (Walters 1995). Pergolide was found to deliver better subjective relief and greater decreases in PLMS when compared with Levodopa (Staedt, Wassmuth et al. 1997). It was also found to be effective in patients who are unresponsive to levodopa (Earley and Allen 1996). However, some patients taking pergolide to treat the symptoms of Parkinson's disease have been shown to develop valvular heart disease, even though this is usually associated with higher doses than those used in RLS (Van Camp, Flamez et al. 2004).

Pramipexole (Mirapex) is a D3 and to a lesser extent a D2 dopaminergic receptor agonist. According to experimental studies, Pramipexole could have a neuroprotective effect. It can induce various adverse effects which include hallucinations and sudden onset of sleep. A recent study by Inoue and associates, was performed to evaluate the safety and efficacy of the long term use of Pramipexole in patients with primary RLS. The study was carried out in a cohort of 141 Japanese patients. The IRLS score improved from $22.3+/-4.7$ to $11.1+/-7.7$ at week 8 and $4.9+/-5.9$ at week 52 . Adverse events were typical of nonergot dopmamine agonists, mild in intensity, and decreased in frequency as the study progressed. RLS augmentation was not observed. Overall the study found that Pramipexole showed good efficacy, particularly in patients with an IRLS total score <20 (Inoue, Kuroda et al. 2010).

Bromocriptine mesylate (ParLODel), which was first reported in 1988, was reported to decrease PLMS and provide subjective relief in RLS patients.

Cabergoline (Dostinex) is an ergot derivative, and a rather selective D2 receptor agonist. It is the strongest acting dopamine agonist available, with a half-life of at least 65 hours. It has the advantage of giving a 24 -hour effect, which is useful for reducing/preventing the effects of augmentation.

The action of dopamine agonists does not commence for 90 to 120 minutes after ingestion; thus, these agents cannot be used effectively once symptoms have started. In addition, agonists can cause nausea; therefore, slow dosage increase is important, especially for pergolide.

Opioids - These agents are usually recommended for the treatment of more severely affected patients, patients who are unresponsive to other medications or the uncommon patient with pain as a prominent symptom (Clark 2001). Although never formally investigated in large scale trials, opioids are often used following unsuccessful treatment with dopaminergic agents. Low-potency opioids include codeine and propoxyphen, and have proven to benefit patients with mild or intermittent symptoms. Higher-potency agents, such as methadone hydrochloride and oxycodone hydrochloride are thought to have a role in refractory cases. Oxycodone at an average dose of 15.9 mg reportedly decreases PLMS, and provides decreased arousals and improved sleep efficiency. Nevertheless, because of the recognized risk of addiction, most physicians are reluctant to use opioids.

Benzodiazepines - Studies of benzodiazepines have focused on clonazepam therapy. Clonazepam has shown to ease the sensory symptoms and decrease PLMS. Other benzodiazepines include temazepam and alprazolam. Although this drug has been proven to be well tolerated in older patients, its long duration of action may cause effects that are more adverse. Such side effects include daytime drowsiness and confusion, unsteadiness and falls, and cognitive impairment in the morning.

Gabapentin and other anticonvulsants - Anti-epileptic medications such as carbamazepine and gabapentin can also provide a therapeutic effect, especially in patients with painful RLS. Gabapentin has been shown to be an effective treatment, however, similar to opioids, large scale trials and long-term observations are minimal. Anticonvulsants such as carbamazepine and valproic acid, which have been previously investigated for the treatment of RLS, are no longer regarded as first-line
options due to their side effects. Dizziness, fatigue, and sleepiness are among the possible side effects (Happe, Klosch et al. 2001).

Yokukansan (Herbal) - Yokukansan (YKS) was developed as a remedy for restlessness and agitiation. It has been approved for patients with insomnia in Japan, and in 2008, Shinno and associates reported that YKS improved sleep quality in patients with dementia (Shinno, Inami et al. 2008). In 2010, Shinno reported that three patients with RLS were successfully treated with YKS. It was speculated in the paper that actions on GABAergic, serotonergic and dopaminergic systems might account for some of the therapeutic effects of YKS in RLS patients (Shinno, Yamanaka et al. 2010).

### 1.10.3 Medications that may exacerbate RLS

RLS as a side effect is ascribed to a number of drugs. Prior reports exist on RLS associated with drugs such as olanzapine (Kraus, Schuld et al. 1999), risperidone (Wetter, Brunner et al. 2002), and clozapine (Duggal and Mendhekar 2007). A number of case reports have been published on the provocation of RLS by "modern antidepressants (AD)" with specific receptor binding profile. One case was reported for each one of the selective serotonin reuptake inhibitors (SSRIs) sertraline, fluoxetine, paroxetine and citaloprame. For the nonadrenergic-specific serotenergic AD (NASSA) mirtazapine, a total of seven cases were reported (Rottach, Schaner et al. 2008). Some papers focused on the relationship between AD and RLS. Brown et al., investigated the possible association between RLS and AD, in 200 patients with sleep onset disturbance. Of these 200 patients, $45 \%$ met the diagnostic criteria of RLS, $56 \%$ suffered from depression, and $38 \%$ were on AD. A statistically significant association between RLS and AD was not demonstrable (Brown, Dedrick et al. 2005).

In 2007, Kang et al., reported that the prevalence of RLS was more than twofold higher (21.4\%) in schizophrenics taking antipsychotics than in healthy individuals (Kang, Lee et al. 2007). However these reports are often disregarded or believed to be psychotic agitation or a somatic copmplaint because its symptoms are nonspecific, unclear and diverse, and are aggrevated or even happen exclusively at night time.

In 2008, Kang et al., published another report in the Journal of Psychopharmacology in which five case studies were reported of RLS and PLMS that were probably associated with olanzapine. For example, the first case, a 36 -year-old Korean female
with schizophrenia, showed a good response to olanzapine. However the RLS symptoms associated with olazapine resulted in poor long-term compliance, which ultimately led to the recurrence of psychotic symptoms. These report high-lights the fact that antipsychotic-induced RLS and PLMS are under-recognized side effects of antipsychotics, with the symptoms often misdiagnosed as psychotic agitation. It also aims to make clinicians aware that although the antipsychotic olanzapine has few extrapyramidal symptoms, it can be the cause of RLS and PLMS and therefore affect a patients compliance with the medication and lead worsening of psychotic symptoms. Even though the cause of RLS and PLMS caused by antipsychotics has not been established, it is thought to be due to the dopamine receptor blocking of antipsychotics.

### 1.11 Prognosis and Impact of RLS

### 1.11.1 Prognosis

RLS is generally a lifelong condition. Symptoms tend to gradually progress with advancing age. At times individuals may experience spontaneous improvement, in which symptoms decrease or disappear for days, weeks, or months, although symptoms tend to recur. Improvement or resolution of symptoms is often observed in patients with RLS secondary to an underlying condition, if the underlying condition is treated.

### 1.11.2 Impact on quality of life

Depression is common in RLS patients and may be related to the chronic unrelenting nature of symptoms, to the persistent sleep loss or to the underlying brain abnormalities that cause RLS. In a study carried out on a headache and control groups, higher scores for depression and anxiety were more frequent in subjects with RLS compared to those without (d'Onofrio, Bussone et al. 2008). Treating depression patients with RLS may be difficult since, as mentioned above, some of the antidepressants may individually exacerbate RLS symptoms.

The most disturbing feature of RLS may be the social disruption which RLS sufferers experience. This is based on the need to decrease evening social or work engagements that involve inactivity. Social dinners, board meetings or theatre can be very challenging. Furthermore, individuals often have difficulty concentrating on daily tasks such as those demanded by their work. Problems with short term memory loss are often encountered, which can lead to cognitive deficits i.e. learning difficulties (Pearson, Allen et al. 2006).

RLS patients are more prone to headaches than healthy subjects. This is most likely secondary to the sleep disturbances and sleep loss. Headaches are usually comparable to migraine in character, though with an element of tension-type headache (d'Onofrio, Bussone et al. 2008). In a study conducted between headache and control groups, headache patients with RLS reported sleep disturbances more frequently compared to those without RLS (50.0\% vs. $32.7 \%$; p<0.0001) (d'Onofrio, Bussone et al. 2008).

### 1.12 Linkage analysis

### 1.12.1 Basic concepts in Genetics

For centuries the hereditary basis of human disease has fascinated both scientists and the general public. An Austrian monk, Gregor Mendel provided the foundation for the study of human genetics. By carefully creating crossing experiments with peas and recording observations of the frequency of variable characteristics in the pea plant (e.g. seed texture, colour, plant height), he hypothesised what are now called Mendel's laws (Mendel 1866). Mendel's fundamental unit of inheritance is named the gene, which contains the information necessary for manufacturing proteins. DNA or deoxyribonucleic acid is the molecule that contains the gene and encodes information for producing both proteins and RNA. The locus is the physical location of a gene. Each individual carries two copies of each gene of which one was received from the mother and the other received from the father. At any particular locus there can exist a number of different forms of the gene, known as alleles.

Normal chromosomes have a single centromere that is seen under the microscope as the central constriction. It separates the p (short) arm from the q (long) arm. It is essential for segregation during cell division. The normal human chromosome consists of 23 pairs of chromosomes, with one member of each pair inherited from each parent. Non-gamete cells are diploid as there are two copies of each chromosome in the cell. The first 22 pairs of chromosomes are called autosomes. The two remaining chromosomes, X and Y , are called sex chromosomes of which females carry two X and males carry one X and one Y .

Genes located on the same chromosome are said to be syntenic. Although two genes may be syntenic they may be far enough away from each other on the chromosome to segregate independently from one another i.e. unlinked. If two syntenic genes fail to be passed on to gametes independently from one another they are said to be linked.

In sexual organisms such as humans the reproductive method involves the union of haploid gametes (sperm and egg cells). The process by which these haploid gametes are formed is termed as meiosis. Meiosis consists of meiosis I and meiosis II. In meiosis I each chromosome in the cell is replicated to generate two sets of duplicated homologous chromosomes. Each homologous chromosome therefore consists of two strands known as chromatids. Physical contact may occur between the chromatids, resulting in the formation of chiasmata. Chiasmata are a sign of the occurrence of crossing over (recombination) between two chromatids, thus leading to the exchange of DNA between two of the four chiasmata. A chiasma occurs at least once per chromosome pair, resulting in recombinant haplotypes. Meiosis II is identical to a mitotic division in which genetic material is transmitted equally and without recombination to daughter cells.

As a consequence of the process of meiosis, gametes contain only a haploid set (one copy) of chromosomes. This explains why parents pass only a single gene onto their offspring. It also explains why the gender of an individual is determined by the father of the child.

### 1.12.2 The principles underlying linkage analysis

In the accompanying figure (Figure 1.3), each bar represents a chromosome, paternal ( P ) and maternal (M). "D" represents the disease gene in a disorder with an autosomal dominant mode of inheritance and "d" is its normal correspondent. The numbers denote particular alleles at a series of genetic markers that span the chromosome (genetic markers will be explained later in section 1.11.3).

When a gamete is produced, crossover events (recombinations) can occur at the points indicated with arrows during meiosis. (Four chromatids are formed during meiosis, but it is not shown here for the purpose of simplicity). The resulting offspring will inherit a "new" sequence of alleles.

Subsequently a different pattern of marker alleles is seen in the next generation. Only the markers adjacent to the disease gene "D" (marked with a red bar) remain unchanged. During gamete formation in this individual, a


Figure 1.3: The principles underlying linkage analysis
Each bar represents a chromosome, paternal (p) and maternal (m). The numbers denote particular alleles at a series of genetic markers that span the chromosome. Crossover events (recombinations) have occured at the points indicated with arrows during meiosis. Thus, it is only the marker which are adjacent to the disease gene 'D' which remain unchanged i.e. are inherited with the disease gene.
further recombination event may occur, thus separating some alleles from the disease gene.

It is only the marker alleles closest to the disease gene " $D$ " that remain unchanged. It is clear that allele 5 of the marker above the disease gene and allele 6 of the marker below the disease gene have remained "associated" with the disease gene "D".

These two markers are said to be linked to the disease gene because recombination events are not likely to isolate them from it. This is because of their close physical proximity.

From figure 1.3 it can be perceived that if all the numbers in the bars were " 2 " it would be impossible to demonstrate the recombination events. This is an essential detail and indicates why genetic markers and in particular microsatellite markers are so valuable in linkage studies.

### 1.12.3 Genetic Markers

If, in the above example the alleles were all numbered " 2 " i.e. if all individuals were homozygous for the allele 2 , then no information on recombination events could be acquired. The rationale behind why the example shown above succeeds is that two different alleles are present at each locus, in other words the individuals are heterozygous. When individuals are heterozygous at these locations, recombination events can be identified and information can be obtained from the family.

Genetic markers can be defined as sequences of DNA inherited in a mendelian manner. They demonstrate polymorphism, which means a difference in DNA sequence is observed among individuals, groups or populations that gives rise to different forms of the marker. Traditionally autosomal marker traits included the blood groups and the ability to taste phenylthiocarbamide (PTC). The X-linked marker groups include the Xg blood group and colour blindness. The demonstration of genetic linkage by pedigree studies was first described by Bell and Haldane in the 1930's, when they showed that the locus for haemophilia was close to that for colour vision (Bell and Haldane 1986).

## Microsatellite markers

The genetic markers used in this study were polymorphic repeats which belong to a group of genetic markers called microsatellite repeats. Microsatellites were first described in 1989 as small blocks of tandemly repeated DNA in which the repeated element is usually a di-, tri-, or tetra-nucleotide sequence (e.g. [CA]n, [CAG]n, [CAGT]n, where n represents the number of repeats). The number of repeat elements in these blocks is often highly polymorphic, and show simple Mendelian inheritance. Information on several of thousands of microsatellite repeats is now electronically accessible to investigators through databases such as the Marshfield Institute, Entrez (at NCBI), the Whitehead Institute, the Genome Database and Généthon. Although these highly polymorphic microsatellites supply the investigator with important maps for linkage analysis, it is vital to remember that all genetic and physical maps are not constructed alike or with the same reliance.

## Dinucleotide Repeats

The most common type of repeat is the dinucleotide CA (GT) repeat, occurring on average every 0.4 cM (Weber, 1990). The function of these repeats is unknown. A suggestion that they may be "hot-spots" for recombination is unlikely because of the linkage disequilibrium seen between them. Dinucleotide repeats have been used extensively as genetic markers. Two factors determine whether these repeats are polymorphic. Firstly the size of the repeat, and secondly whether it is an imperfect repeat (with interruptions) or a perfect repeat. A large percentage of the markers with 15 or more perfect repeats are polymorphic. Dinucleotides while powerful have several technical disadvantages. Often dinucleotides have a "stutter" or "shadow band" that produces a background ladder of bands two base pairs apart. Also since
the alleles are only two base pairs apart, it can be difficult to make a distinction between one allele and the next.

## Tri- and Tetranucleotide repeats

Trinucleotide repeats are approximately ten times less common than dinucleotides. They occur once every $300-500 \mathrm{~kb}$. The most common form is [AAB]n (where B is $\mathrm{C}, \mathrm{G}$ or T$)$. Although certain trinucleotide repeats which are prone to expansion have warranted a lot of attention because of their disease causing characteristics (e.g. fragile X syndrome, Huntington's disease, myotonic dystrophy), the majority of trinucleotide repeats are not associated with disease and are very stable. Tetranucleotide are even more rare, and the most common form is [AAAB]n (where $\mathrm{B}=\mathrm{C}, \mathrm{G}, \mathrm{T}] \mathrm{n}$. Tri- and Tetranucleotides have fewer stutter bands than dinucleotide repeats, typically generating unique PCR alleles without the laddering artefact. They are also easier to read when using fluorescently labelled primers and automated detectors such as the ABI 3130. Accurate allele identification is imperative, making these markers more favourable than their di-nucleotide match. However their lower occurrence in the genome restricts their extensive use.

### 1.12.4 Centre d'Etude du polymorphisme Humain (CEPH)

Centre d'Etude du polymorphisme Humain (CEPH) is a centre for genetic studies in Paris, France. The primary URL for CEPH is http;//www.cephb.fr. This website provides information about CEPH and provides access to the CEPH genotype database which contains data on polymorphic markers. Researchers from over 100 laboratories around the world have submitted genotyping data and from this CEPH have assembled a large panel of families to construct maps of genetic markers. These large families generally consist of pedigrees with three generations, living grandparents and an average sibship size of 8.5 . The standard family set comprises of 40 families, while an extended family set of 64 families is available. These families are generally of European descent (Dausset, Cann et al. 1990).

The CEPH database provides information on the marker including allele sizes and frequencies, and heterozygosity. It can also provide the actual genotypes for individuals in the CEPH pedigrees. Blood from each member is stored at CEPH and can be made available to researchers around the world, therefore being able to access the actual genotypes of these individuals can greatly improve the consistency of allele calling between gels, projects and laboratories. CEPH however does not provide primer sequence data or the position of the marker on the chromosome in question.

### 1.12.5 Using Microsatellite Markers to find Disease-causing Genes

When the disease causing gene in a family is unknown, a common technique used to find the gene is to genotype family members for a collection of microsatellite markers dispersed throughout the genome. If a distinct allele of one of these makers is found to be "linked" with the disease phenotype, then the disease gene is likely to be physically close to that marker. Complex statistical analyses are required to determine the level of linkage between the disease phenotype and the marker. A number of factors such as family structure, mode of inheritance and marker allele frequencies are all taken into account.

Following sufficient evidence that the disease is linked to a given marker, other markers in that area need to be genotyped. When a set of alleles from adjacent microsatellite markers are found on a short segment of the same chromosome and tend to be transmitted together through a family, it is referred to as a haplotype. Within this haplotype there will be a number of candidate genes present, one of which will be the disease gene.

### 1.12.6 LOD scores

By analysing the pattern of segregation of a gene and potential marker genes in families, it is possible to generate a mathematical measure of the likelihood of linkage, known as the LOD score or Z score. The LOD score was devised by Morton in 1955 and is defined as the logarithm of the odds (LOD) ratio (R) that the loci are linked rather than unlinked. The LOD score is therefore a statistical estimate that measures the probability of two loci being physically close together and thus being inherited together.

## The recombination fraction and genetic distance

Recombination will rarely separate loci that lie very close together on a chromosome, as only a crossover located precisely in the small space between the two loci will produce recombinants. Thus sets of alleles on the same small chromosomal segment tend to be transmitted as a block through a pedigree. This block of alleles is known as a haplotype. The further apart two loci are on a chromosome the more likely it is that a crossover will separate them. The recombination is a measure of the distance between two loci. The further apart two loci are on a chromosome, the greater will be the frequency of recombination between them up to a maximum of $50 \%$. Recombination fractions ( $\theta$ ) never exceed 0.5 , however far apart the loci. If there are no recombinants, the LOD score will be maximum at $\theta=0.00$. Recombination events can therefore be used as a measure of how close two loci are (genetic distance). Two loci that show $1 \%$ recombination are defined as being 1 centimorgan (cM) apart on a genetic map (Strachan and Read 2003).

Genetic distance and physical distance are not equivalent. Genetic maps show the order of features along the chromosome and the chances that they will be separated by recombination; however, physical maps show the distance of features along the chromosome in kilobases (kb) or megabases (Mb). Areas such as the centromere show very few crossovers; therefore, genetic distances in these areas would be small in comparison to physical distance. In contrast, areas of equivalent physical size in crossover "hotspots" such as the telomeres would show large genetic distance. For large distances i.e. two loci that have a recombination value greater than $\Theta=0.50$, it is not possible to use the recombination fraction as an additive distance measure. It must therefore be transformed by a map function into a certain additive map distance, under the assumption of a certain level of genetic interference.

Interference is thought of as the process whereby the presence of one cross-over in an interval inhibits the formation of another cross-over in the same region. Subsequently negative interference suggests that the presence of one cross-over will in turn encourage the formation of another cross-over event within an interval. Numerous levels of interference in humans have been hypothesized; however, the level of interference used in this study was the Kosambi (Kos) level. This method adopts an intermediate level of interference whereby double cross-over are seldom observed at small genetic distance, although the probability increases at greater distances (Keats, Ott et al. 1989). Another common map function employed to covert the estimated recombination fractions to map distances is the Haldane level, however it appears to produce less realistic values than does Kosambi's formula. For example the Haldene map function converts $\Theta=0.27$ into 39 cM and the Kosambi map function translates $\Theta=27$ into 0.30 Morgans ( 30 cM ). Higher recombination rates are seen in the female than in the male. (Kosambi 1944)

Some allowance must therefore be made for the likely distance (in addition to recombination fraction) that may be present between a particular marker and the disease locus. Hence, it is normal practice to calculate all LOD scored at a range of recombination fractions.

### 1.12.7 Calculating LOD scores

To find the odds of linkage it is necessary to calculate the overall likelihood that the two loci are linked $(\Theta=0.00)$ compared to the likelihood that they are not linked $(\Theta$ $=0.50$ ). The ratio of the latter probability to the former, expressed as a logarithm (base 10) is then the 'LOD score' between those two markers. Therefore, the LOD score is a statistical estimate that measures the probability of two loci being physically close together and thus being inherited together.

Each meiosis in the family is looked at in turn and the likelihood of the observed genotype and disease gene being transmitted together is calculated. This is moderately straightforward when all individuals are genotyped and recombinations are easily determined. Nonetheless problems do arise when recombinations cannot be easily identified and when gaps arise in a pedigree e.g. when individuals are unavailable for DNA sampling or perhaps deceased. Therefore when uncertainties like this arise the meiosis must be examined under the various alternatives that exist, and each alternative given an appropriate weighting. In order to calculate the full LOD score for a family various factors exist that need to be taken into account e.g. the genotypes of other individuals and population allele frequencies. Consequently this results in a large "branching tree" of genetic probabilities (Strachan and Read 2003). Therefore, apart from very simplistic cases, human linkage analysis is completely dependent on computer programs.

### 1.12.8 LOD score values for linkage and exclusion

The degree of linkage is a function of the distance between the disease gene locus and the maker locus. This can be measured by the number of crossovers between the two loci among the observed meioses (i.e., recombination fraction, $\theta$ ). If there is no recombination, $\theta=0.00$, there is "complete linkage" and observed meiosis $(\theta<$ $0.50)$. However if the observed recombination fraction is $50 \%(\theta=0.50)$, there is no linkage. LOD scores $(\theta)$ are usually recorded at $\theta=0.00,0.05,0.10,0.20,0.30,0.40$ and 0.50 .

If all chromosomes were of equal size and if two loci were picked randomly, the chance that they were found on the same chromosome would be 1 in 23 . However the probability that two randomly picked loci would be close together and likely to be linked is estimated to be a one in fifty chance, thus the prior probability of linkage. Therefore, a large amount of evidence would be required to confirm linkage if there were only a one in fifty chance the two loci were actually linked.

The LOD score is the logarithm of the odds that the loci are linked rather than unlinked, therefore, $\log _{10} 1000=3$ represents the minimum LOD score required for statistical proof of linkage.

As stated the conventional threshold for claiming linkage is a LOD score of 3.0, corresponding to 1000:1 odds that the loci are linked. LOD scores of -2.0 or less are indicative of nonlinkage (Morton, 1955). Values between -2.0 and 3.0 are inconclusive and require additional data before an assumption can be made. These criteria are based on the probability of type I and type II errors. Type I errors refers to the probability of concluding that there is linkage between the loci in question
when in fact there is no linkage (false-positive); and type II refers to assuming that there is no linkage when in fact there is (false-negative). A cautious value of $z>3.00$ for inferring linkage was chosen by Morton (1955) to prevent the risk of false positives. Therefore, if the total LOD score for one family or over all families is 3.0 or more, one can conclude that there is extensive evidence for linkage.

### 1.12.9 Drawing family trees

There are a number of computer programs available for pedigree drawing, and they have huge advantages over some of the older methods such as by hand. The pedigree drawing package used for this study was Cyrillic v2.01. Aside from being a drawing program Cyrillic v2.01 has a number of useful features. Such features include the ability to store vital information about individual patients and their families; to scale pedigree drawings to fit on one page; and to store up to 10000 individuals per family, up to 250 markers per chromosome and up to 150 alleles per marker. It can also colour haplotype bars to show inheritance and multiple crossovers.

### 1.13 Haplotype Analysis

A haplotype is a set of alleles on the same small chromosomal segment that tends to be transmitted as a block through a pedigree. A haplotype may refer to as few as two loci or to an entire chromosome depending on the number of times recombination takes place between a given set of loci. Although a LOD score can point us in the direction of whether linkage has taken place or not, it is simply a statistical estimate and does not give us a mental picture of what is occurring.

If a particular candidate locus is being examined with a series of markers, it is often useful to draw the haplotype at this locus for the family in the study. It can provide a visual explanation of the LOD score. (It may also be useful in identifying cases with possible reduced penetrance or other useful information.) Figure 1.4 explains this concept. Each individual in the pedigree has two columns of numbers, one column representing a paternal chromosome and the other representing a maternal chromosome. The numbers signify the set of alleles found using a series of adjacent markers. Therefore, each column is a haplotype and using colours for different haplotypes makes it easier to follow through the family. In the figure, one can see that the affected daughter has received the haplotype 2-1-5-7 from the father and haplotype $4-5-7-6$ from the mother. Nonetheless there are times when it is not possible to determine which parent the haplotype has been passed on from. For example, for marker D in figure 1.4 the father is homozygous for allele 7, therefore, we are unable to determine if the allele 7 inherited by the affected son comes from the "red" chromosome or the "green" chromosome.


Figure 1.4: The concept of haplotypes and haplotye analysis
This figure shows an affected father (filled square), an unaffected mother (circle) and an affected daughter. Four adjacent markers are examined here, marker A, B, C and D. The resultant allele for each marker is given by the numbers under each individual. The series of alleles (haplotypes) is given a coloured vertical bar. In this example, the daughter has inherited the red haplotype from the father and the blue haplotype from the mother. However for marker D, it is not possible to determine if the allele 7 inherited by the affected daughter comes from the fathers 'red' chromosome or the 'green' chromosome as the father is homozygous for this marker.

### 1.14 Summary

Restless legs syndrome (RLS) is one of the most common neurological sensorimotor disorders in Western countries, affecting approximately $10 \%$ of the population. It is often associated with periodic limb movement during sleep (PLMS) leading to severe insomnia. However, it remains largely underdiagnosed and its underlying pathogenesis remains to be elucidated.

RLS is generally idiopathic, with familial association in 40-60\% of the cases, but may also be symptomatic of such associated conditions (secondary forms) as peripheral neuropathies, uremia, iron deficiency, diabetes, Parkinson's disease and pregnancy. In comparison to non-familial cases, familial RLS usually has a younger age at onset and a more slowly progressive course. The symptoms within a single RLS family can vary significantly, with some members displaying mild symptoms on only a few occasions during their life, while those more severely affected suffer from restlessness and sleep disturbances.

Linkage studies in RLS families have revealed ten loci to date, however, no causally related sequence variant has yet been identified using this approach. Based on the hypothesis that RLS follows a recessive mode of inheritance, linkage to chromosome 12q (RLS1) was identified in 2001 in a French-Canadian family. All of the nine other loci identified followed a dominant mode of inheritance.

Genome wide association studies have identified variants within intronic or intergenic regions of MEIS1, BTBD9, PTPRD, MAP2K5/LBXCOR1, which has raised new pathological hypothesis for RLS.

Treatment options for RLS are advanced and have been investigated in large trials. In order to optimize treatment strategies, future studies should concentrate on the remaining problems of augmentation, rebound and its pathomechanism. Furthermore, genetic insights could reveal further specific treatment regiments other than the present therapeutic strategies.

Notable progress has been made in distinguishing the diagnostic criteria of RLS from mimic conditions. However, much more must be done to fully elucidate the pathophysiology and etiologies of both primary and secondary forms of RLS.

## Chapter 2

## Materials and Method

### 2.1 Family recruitment and clinical assessment

In the University College Cork (UCC) Department of Pathology, research into the genetic features of RLS is continuous. Families for this ongoing study are recruited by various methods e.g. word of mouth, contact with GPs, and articles in the press. Contact was made with the department by one member of this family interested in participating in the study. Subsequently other family members were primarily contacted by phone, and those with interest in participating were visited individually in their homes located in various parts of Ireland. The requirements and aims of the study were explained to the members of the kindred and those willing to take part were asked to sign a consent form. Participants were then questioned as to the presence, frequency and severity of symptoms suggestive of RLS with particular reference to:

- The presence of the revised essential diagnostic criteria proposed by the RLS epidemiology and diagnosis workshop with IRLSSG, 2002.
- Obtaining RLS severity rating scale (proposed by IRLSSG).
- Periodic limb movements during sleep and sleep disturbance.
- The duration, frequency and severity of these symptoms.
- The effect of pregnancy on symptoms (female patients).
- Their past medical history.
- Current medications and drug history.
- Family history including anecdotal information on the affection status of deceased family members.

Physical examination included a full neurological examination. Venous blood samples (approximately 20 mls ) were obtained from affected and unaffected family members. Blood samples were collected in EDTA coated Vacutainers (to prevent clotting) and subsequently stored at $-20^{\circ} \mathrm{C}$.

### 2.1.1 RLS3002 Pedigree

The pedigree, name RLS3002, is outlined in figure 2.1 below. In this family RLS appears to follow an autosomal dominant mode of inheritance. Blood samples were collected from 18 members of the family, and following genomic extraction each family member was assigned their own unique "PATH" number. Of the 18 family members 11 are affected, all of whom met the essential diagnostic criteria. The age of onset was seen to vary from childhood, to teenage years and some early twenties. Although the majority of affected individuals did not endure sleep disturbances, one individual did experience severe disturbance and another had moderate disturbance. 4 of the 14 family members remained unaffected. Table 2.1 lists the clinical data from all 11 affected members of RLS3002


Figure 2.1: RLS3002 Pedigree
This is a three generation family. Circles represent females, squares represent males. Coloured symbols represent affected members and symbols with the letter N represent unaffected members. The lines through the grandparents symbols indicate that they are deceased. Members of the family were each assigned their own unique PATH number.

Table 2.1: Clinical Features of RLS in the affected members of Family RLS3002.

| Individual | Sex | Age at study (yr) | Age at onset (yr) | Severity (IRLS) | Parasthesia in legs | Parasthesia in arms | Symptom Prevalence |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| PATH046 | F | 58 | 3 | 4 | Yes | No | Night |
| PATH048 | F | 31 | 10 | 14 | Yes | No | Evening/ Night |
| PATH050 | F | 21 | 11 | 13 | Yes | No | Evening/ <br> Night |
| PATH051 | M | 57 | 20 | 15 | Yes | No | Sitting for long periods/ Evening/ Night |
| PATH053 | F | 25 | 13 | 17 | Yes | No | Sitting for long periods/ Evening/ Night |
| PATH054 | M | 23 | 15 | 11 | Yes | No | Evening/ Night |
| PATH055 | M | 53 | 10 | 10 | Yes (Lateralization of Symptoms; left leg) | No | Evening/ Night |
| PATH083 | M | 27 | 13 | 10 | Yes | No | Night |
| PATH185 | M | 57 | 13 | 16 | Yes | No | Evening/ Night |
| PATH187 | F | 29 | 13 | 8 | Yes | No | Evening/ Night |
| PATH207 | F | 19 | 15 | 10 | Yes | No | Sitting for long periods |

### 2.2 DNA Extraction

DNA was extracted from venous blood using a QIAamp Blood Kit from Qiagen. It involves cell lysis, nuclear lysis and protein salt presipitation stages. This method retains the DNA in a QIAamp spin column while contaminants are washed out and typically yields approximately $3-12 \mu \mathrm{~g}$ in $200 \mu \mathrm{l}$ of final volume per $200 \mu \mathrm{l}$ of whole blood. Before the extraction process the blood samples were allowed to thaw at room temperature, and a check was performed to ensure that the Buffer AL, Buffer AE and QIAGEN Protease were prepared correctly, according to the accompanying manual. The extraction was carried out according to the manual. The DNA was eluted with Buffer AE or distilled water and a NanoDrop was used to measure the DNA concentration. The DNA extraction was also confirmed by amplification using the polymerase chain reaction and efficient primers, then checked by agarose gel electrophoresis. The manufacturers state that the typical DNA yield from a $200 \mu \mathrm{l}$ sample volume of Human Whole Blood is $3-12 \mu \mathrm{~g}$ of DNA in $200 \mu \mathrm{l}$ water. The yield however depends on the quantity of white blood cells present and the performance of the procedure.

### 2.2.1 Determination of DNA Concentration

A NanoDrop ND-1000 spectrophotometer was used to measure the DNA concentration following extraction. A NanoDrop is a cuvette-free spectrophotometer. It uses just $1 \mu \mathrm{l}$ of sample and is accurate from $5 \mathrm{ng} / \mu \mathrm{l}$ up to $3,000 \mathrm{ng} / \mu \mathrm{l}$. By eliminating the need to carry out dilutions, and thus reducing human error and variability factors, analyses can de done quickly and easily. The NanoDrop Spectrophotometer is linked to the computer that also runs the digital imaging system.

The NanoDrop was blanked using buffer as the DNA was eluted with Buffer TE. When adding the sample it is essential that the liquid column is formed between the upper and lower measurement pedestals.

Operation: A $1 \mu \mathrm{l}$ sample is pipetted onto the lower pedestal (the receiving fiber). The second fiber or the upper pedestal is brought into contact with the liquid. A pulsed xenon flash lamp within the machine provides the light source and a spectrometer is used to analyse the light passing through the sample. As previously mentioned, the instrument is controlled by special software run from a PC. The data is then saved in an archive file on the PC. The sample concentration is recorded in $\mathrm{ng} / \mu \mathrm{l}$ and is based on absorbance at 260 nm and 280 nm .

### 2.3 Polymerase Chain Reaction (PCR)

The polymerase chain reaction is a rapid and versatile cell free procedure for in vitro enzymatic amplification of specific segment's of DNA. The template DNA is usually genomic DNA from a particular tissue or cultured cells, in which case the target DNA is typically a tiny fraction of the total DNA. In order to permit selective amplification of the target DNA, some prior DNA sequence information from the target sequence is required. This information is used to design two oligonucleotide primers, usually about $18-25$ nucleotides long. These primers are specific for sequences flanking the target sequence. For the majority of PCR reactions, the goal is to amplify a single DNA sequence and so it is important to reduce the possibility of the primers binding to other locations in the DNA than the desired ones. Therefore, in order to avoid this a variety of considerations need to be factored into primer design e.g. the melting temperature $\left(\mathrm{T}_{\mathrm{m}}\right)$ of the two primers should not differ by $>5^{\circ} \mathrm{c}$, self-complimentary sequences should be avoided, and the GC content should be between 40 and $60 \%$.

In addition to template DNA and primers, a PCR needs other reagents in order to be successful. A heat stable enzyme known as DNA polymerase, e.g. Taq polymerase, is employed. Small segments of DNA are generated when the polymerase adds complimentary deoxynucleotides (dNTPs) to the template DNA. The primers in this case are used as the starting point for the polymerase by providing double stranded DNA. During PCR, the DNA is heated and the double strands separate. Upon cooling, the primers bind to the template DNA thus providing a region of double stranded DNA from which the polymerase can begin to synthesise the new strand.

The PCR process usually consists of a series of twenty to thirty-five cycles. Each sequential cycle is composed of three steps:

Denaturation: The double-stranded (template) DNA has to be heated to $94-96^{\circ} \mathrm{C}$ in order to separate the strands. This is referred to as denaturing; it breaks apart the hydrogen bonds that connect the two DNA strands. Also certain polymerases are activated at this time.

Annealing: After separating the DNA strands, the temperature is lowered. This allows the oligonucleotide primers to bind to the single stranded DNA template. The two oligonucleotide primer sequences are specific for the taget DNA and are constructed using prior knowledge of the DNA sequence of the target sequence. The annealing temperature depends on the primers and is usually $5^{\circ} \mathrm{C}$ below their melting temperature $\left(45-60^{\circ} \mathrm{C}\right)$.

Extension: This is the final stage which involves the DNA polymerase copying the template DNA strand. The heat stable DNA polymerase initiates the synthesis of new DNA by adding the four bases, the deoxynucleoside triphosphates - dATP, dCTP, dGTP and dTTP, to the annealed primers in the order complimentary to the single stranded DNA template. The elongation (extension) temperature depends on the DNA polymerase. Taq polymerase elongates optimally at a temperature of $72^{\circ} \mathrm{C}$. The thermostable DNA polymerase is possibly the most important component of the PCR technique as it remains active despite being repeatedly exposed to high denaturing temperatures. Taq polymerase isolated from the bacteria Thermus aquaticus, is thermostable up to $94^{\circ} \mathrm{C}$. The Taq polymerase and buffers used in this study were obtained from Promega.

The newly synthesized DNA strands act as templates for further DNA synthesis in subsequent cycles. After about 30 successive cycles of DNA synthesis, the products of the PCR will lead to a $10^{5}-10^{6}$ fold increase in the amount of target DNA. This amplification results in sufficient quantities of the target DNA for direct visualization by ultraviolet fluorescence after ethidium bromide staining.

## The PCR Process



Figure 2.2: The PCR process
Double-stranded DNA in the sample is heated to generate single strands. Sequence specific primers are added, which anneal to desired sites on the DNA. Nucleotides and heat-tolerant DNA polymerase allow for primer extension at elevated temperature. The result is two new copies of double-stranded DNA. The process is repeated to generate multiple specific dsDNA molecules (www.universe-review.ca).

### 2.3.1 Determination of optimal PCR conditions

When carrying out a PCR reaction some variables may need to be optimized as conditions are specific to each primer pair. The most important variables are the $\mathrm{MgCl}_{2}$ buffer concentration and the cycling temperatures. The number of cycles, amount of template DNA and the primer concentration may also need to be optimized.

The selection of the annealing temperature is one of the most critical factors for optimizing the specificity of a PCR reaction. The higher the temperature the more stringent the base-pairing; hence, the more specific the PCR amplification. However, if the temperature is too low non-specific priming may occur. The choice of optimum annealing temperature for each primer depends on the Tm (melting temperature) of the expected duplex between primer and the DNA. This is the temperature in which half of the primer binding sites are occupied. The melting temperature increases with the length of the primer. The $\mathrm{T}_{\mathrm{m}}$ is based on the nucleotide composition of the primer sequence and can be calculated using the following formula:
$\mathrm{T}_{\mathrm{m}}\left({ }^{\circ} \mathrm{C}\right)=2(\mathrm{~A}+\mathrm{T})+4(\mathrm{G}+\mathrm{C})$.

A preferred method of determining the optimum annealing temperature is when a temperature gradient is generated across a "gradient" block. This can be carried out on a thermocycler. A temperature gradient is built up across the thermoblock, where multiple reaction mixtures can be simultaneously run at temperatures that differ only slightly. With the aid of only one single PCR reaction an optimum temperature for the reaction can be quickly identified.

Lower salt concentration gives a higher degree of specificity by 1) affecting the primer annealing and 2) lowering the activity of the Taq polymerase, which in turn correlates with higher accuracy of the polymerase.

PCR is a highly sensitive technique; therefore, it is important that sufficient measures are used to avoid contamination with other DNA (bacteria, viruses, own DNA etc.). Such measures include changing gloves for each PCR step; and storing aliquots separately from other DNA samples. A control reaction, omitting template DNA, should also be carried out, to ensure the absence of contamination.

### 2.3.2 PCR Method

The Polymerase chain reactions were carried out in $15 \mu 1$ reaction volumes. Genomic DNA at a concentration of $100 \mathrm{ng} / \mu \mathrm{l}$ was used as a template. The reagents required were gathered and thawed. The Taq polymerase was always kept on ice. Reagent volumes were calculated using the following table (Table 2.2), in order to prepare a premix solution covering the required number of samples (n). The table contains the name of the reagent, the stock concentration, the concentration used, and the amount of each reagent in a $15 \mu \mathrm{l}$ reaction volume.

Table 2.2: Reagents used for PCR in $15 \mu 1$ reaction volumes

| Reagent | Stock | Final | $\mathbf{n \times ( \mu l )}$ |
| :--- | :--- | :--- | :--- |
| DNA | $20 \mathrm{ng} \mu \mathrm{l}^{-1}$ | 100 ng | $\mathrm{n} \times 1.5$ |
| $10 \times$ Buffer | 10 x | 1 x | $\mathrm{n} \times 1.5$ |
| $\mathrm{MgCl}_{2}$ | 100 mM | 1.5 mM | $\mathrm{n} \times 0.225$ |
| dNTP | 10 mM | 0.2 mM | $\mathrm{n} \times 0.3$ |
| F/R Primer | $100{\mathrm{ng} \mu^{-1}}^{-1}$ | 100 ng | $\mathrm{n} \times 0.15$ |
| Taq Polymerase | $5{\mathrm{U} \mu \mathrm{l}^{-1}}^{0.75 \mathrm{U}}$ | $\mathrm{n} \times 0.05$ |  |
| $\mathrm{H}_{2} \mathrm{O}$ | Variable | Variable | $\mathrm{n} \times 11.35$ |
| Final Vol $(\mu \mathrm{l})$ |  |  | $1 \times 15$ |

The reagents (with the exception of the DNA) were added to a mastermix tube. The Taq polymerase was added last and kept on ice thereafter. Once a premix solution of reagents was prepared $13.5 \mu \mathrm{l}$ of this solution was transferred into each 0.2 ml labelled PCR tube. The $1.5 \mu \mathrm{l}$ DNA sample was then added to give the total $15 \mu \mathrm{l}$ reaction volume.

PCR was carried out on a G-Storm Thermal cycler, under tube temperature control using the following program:

- $95^{\circ} \mathrm{C}$ X 5minutes
- $95^{\circ} \mathrm{C}$ X 30seconds
- $40-60^{\circ} \mathrm{C}$ X 30 seconds
- $72^{\circ} \mathrm{C}$ X 45 seconds

- Incubate at $72^{\circ} \mathrm{CX} 5$ minutes
- Hold at $4^{\circ} \mathrm{C}$


### 2.4 Agarose Gel Electrophoresis

Agarose gel electrophoresis is the easiest and most common way of separating and analysing DNA. An electric current is applied and due to the fact that DNA has a negative electric charge, the DNA molecules move across the agarose gel. The DNA fragments are therefore, separated according to their size. By adding ethidium bromide to the gel the DNA is visualised. The ethidium bromide is an intercalating dye which means it binds strongly to DNA. It is also fluorescent, therefore, capable of absorbing invisible UV light and transmitting the energy as visible orange light.

Agarose gel electrophoresis was used after PCR had been performed on any given marker; the products from a number of samples together with the CEPH product and negative control were run on an agarose gel. This was to assess the success of that particular PCR and to confirm that there was no contamination of the reaction.

### 2.4.1 Procedure

A number of different gel concentrations were used (1.0 to $1.5 \%$ ), however a $1.5 \%$ gel will be described as it was used most often.

1. Molecular grade agarose $(1.5 \mathrm{~g})$ was dissolved in 100 ml of 1 X TBE by heating in a microwave until the agarose was seen to have dissolved.
2. The molten solution was poured into a gel plate and when set then tape and combs were removed.
3. Each sample was assigned an electrophoresis lane/well. For each sample $5 \mu \mathrm{l}$ of PCR product was mixed with $2 \mu \mathrm{l}$ of 2 x gel loading buffer (Promega) and loaded into its well.
4. A 100 bp ladder (Promega) was added allow determination of the PCR product size. The ladder contains multiple bands of known sizes, yielding 12 bands suitable for use as molecular weight standards for agarose gel
electrophoresis. The digested DNA includes fragments ranging from 100$1,517 \mathrm{bp}$. The 500 and $1,000 \mathrm{bp}$ bands have increased intensity to serve as reference points (Figure 2.3).

The DNA ladder also allowed for qualitative estimation of the level of staining and exposure of the gel that was independent of the PCR itself (i.e. if there were no bands seen in the sample lanes and the 100 bp ladder was seen to stain well, then this was due to the absence of the PCR product and not the under-exposure or under-staining of the gel).
5. A voltage of between 100 and 150 volts was applied to the gel for between 10 and 30 minutes.
6. The gel was then immersed in a solution of ethidium bromide $(0.5 \mathrm{ug} / \mathrm{ml})$ for between 30 and 50 minutes, depending on the quality and age of the ethidium bromide solution.
7. After staining the gel was examined with a UV transilluminator. (A UV protective visor was used when viewing to avoid eye injury caused by exposure to light from the UV light) Photographic record of the appearance of the gel was obtained and the gel was carefully disposed of.


Figure 2.3: 100 bp DNA Ladder visualised by ethidium bromide staining on an agarose gel
The 100 bp DNA Ladder consists of 10 blunt-ended fragments between 100 and $1,000 \mathrm{bp}$ in multiples of 100 , plus additional fragments at 1,200 and $1,500 \mathrm{bp}$. The 500 and $1,000 \mathrm{bp}$ bands are approximately 2 to 3 times brighter than the other ladder bands, and these serve as reference bands.

### 2.5 Genotyping

One of the essential components of any gene mapping study is obtaining the genotypes for the genetic markers used to test for linkage. Genotyping encompasses a range of applications used to analyse genetic differences between individuals. Amplification by PCR of microsatellite markers using oligonucleotide primers results in alleles of the microsatellite markers that differ by their number of integral repeat units. In this study, genotyping is used for analyzing the differences in microsatellite marker repeats between individuals.

### 2.5.1 ABI 3130 Genetic Analyser

Genotyping was carried out by direct electrophoreses on an ABI 3130 sequencer analyser (Applied Biosystems). Instead of an agarose gel there is a capillary containing a gel matrix through which the samples are electrophoreised. The fragments of DNA (and all other negatively charged molecules) are attracted to the platinum cathode electrode attached to the end of the capillary (4 capillaries in total), which are filled with Performance optimized Polymer (POP). POP Polymers dynamically coat the capillary wall to control electro-osmotic flow and are specifically formulated to separate DNA fragments of a known size range at a desired resolution and run time. A current is applied to the DNA fragments which travel through the polymer. The capillary has a glass window through which laser light is passed. The fluorescent labels on the PCR product are excited by laser resulting in the emission of light of a particular wavelength (wavelength depends on the fluorescent label). The light is collected and separated according to wavelength by a spectrograph. It is collected onto a charge-coupled device camera. The ABI 3130 has the ability to collect up to five types of fluorescent emissions simultaneously. Using software selectable filters, the data collection software gathers the emissions of light and stores them on the hard disk for eventual processing.

Within each sample a size standard is also run. The internal-lane size standard permits accurate estimation of the peak sizes. The internal-lane size standard used is GeneScan ${ }^{\text {TM }}-500$ ROX ${ }^{\text {TM }}$ developed by Applied Biosystems, which allows accurate sizing of DNA fragment in the $35-500 \mathrm{bp}$ range, and provides 16 single-stranded fragments of $35,50,75,100,139,150,160,200,250,300,340,350,400,450,490$, and 500 bases. These fragments are uniformly spaced to provide extremely accurate
base calling. Using this size standard for comparison, the analysis software assembles a graph with fragment size on the X -axis and coloured peaks complementary to the various fluorescent signals. The position of the peaks on the X -axis will depend on the time point these signals were detected in comparison to the time the size standards were detected. The smaller the DNA fragments the faster they will move through the gel and therefore, the earlier they will be detected. The height of the coloured peaks depend on the amplitude of the fluorescent signals, and therefore, on the quantity of the PCR product in the sample. Table 2.3 outlines the advantages of the ABI 3130.

Table 2.3: A list of the advantages of the ABI3130
Advantages of the ABI3130
Continuous, unattended 24-hour operation, with fully automated delivery system, sample injection, separation and data analysis.
Accurate determination of allele size. This is vital when dealing with dinucleotide or trinucleotide repeats, where some alleles may differ by only two or three base pairs.
Capillary electrophoresis is used, which is a significant advantage over slab-gel based technologies. Samples can be re-run and the arrays are guaranteed for 100 runs (rather than one slab per gel).
It is also less time consuming when compared with alternative techniques e.g. polyacrylamide gel electrophoresis succeeded by silver staining.
Markers of similar size labelled with different fluorescent labels can be analysed together, thereby increasing the efficiency of sample analysis.
The results may be saved, which allows easy review of previous ABI runs.

As previously mentioned it is possible to distinguish between the different markers because they have colours on the graph (due to fluorescent markers) or have different size ranges. The operator can accurately view allele sizes for each marker. However interpretations of the graph and deciding which are the true alleles resides with the operator.

For each microsatellite marker, the DNA samples from the family members was analysed with two positive controls in order to accurately determine the correct allele sizes. These positive controls were individual 1 and individual 2 of the CEPH 1331 family. These DNA samples were obtained from CEPH and amplified by PCR in the same manner as the family DNA. The genotypes of these CEPH samples is known for a large number of microsatellite markers and that data is available from the CEPH database. By comparing the known allele sizes with those obtained experimentally, corrections can be made to the allele sizes which ensures that the correct fequency data is applied in the statistical calculation of LOD scores. These controls were used for comparison when sizing the unknown alleles, as the genotype of the CEPH individual is known. The Foundation Jean Dausset-Centre d'Etude du Polymorphisme Humain (CEPH) maintains a database of genotypes for genetic markers that have been typed, and the allele sizes of the positive controls can be viewed on this database (section 1.11.4).

### 2.5.2 Preparing an ABI3130 run

To analyse samples on the ABI3130, the samples need to be prepared and then a Genemapper software plate record is created in order to inform the of the location of each sample within the sample plate.

## 1. Sample Preparation

- For each analysis, a premix was prepared for all samples in the run allowing $12 \mu \mathrm{l}$ of de-ionised formamide and $0.5 \mu \mathrm{l}$ of GeneScan ${ }^{\text {TM }}-500 \mathrm{ROX}^{\mathrm{TM}}$ size standard for each sample. [To maximise use on the ABI3130, multiple markers were analysed simultaneously. For each family member, $1 \mu \mathrm{l}$ of products from different PCR reactions were pooled together in one sample tube, making sure they were compatible i.e. if they could be distinguished from one another on the basis of different fluorescent primer labels, or different expected sizes.]
- $12.5 \mu \mathrm{l}$ of the premix (step 1) was then added and mixed with the pooled PCR products.
- All sample tubes were checked for air bubbles. Air bubbles can interfere with the injection step in the ABI analysis.
- The samples were then denatured by heating to $95^{\circ} \mathrm{C}$, for five minutes. After denaturing the samples were snap cooled by placing on ice before the samples were placed onto the autosampler tray of the machine.


## 2. Plate Preparation

- Before running the samples on the ABI3130 it was necessary to prepare a plate using GeneMapper softer, which told the machine the positions of the samples on the plate. To open a GeneMapper software plate information in the New Plate dialog must first be entered. This includes information on the name of the plate, description of the plate, the plate type (96-well or 384well), the name of the owner, and the name of the operator. This enables the operator to find and review previous ABI runs easily. Once this is completed the Genemapper software plate editor opens.
- Preparing a Genemapper software plate is simply a way of labelling each sample in the analysis software by indicating its position on the autosampler tray. The following information and conditions were also detailed: Sample Type (Positive control/Negative control/Sample), Size Standard and Analysis Method.

3. Analysis of samples

- When the ABI3130 run was finished, it was necessary to analyse the results and assign alleles to each individual. GeneMapper® Software was used to size and genotype the data. Fragment sizes were determined automatically relative to an the size standard. The software then allocated fragment sizes to the previously calibrated genotypes. The genotypes for each individual were then adjusted based on the two CEPH controls. The alleles for the family members were then recorded on an page which had the family pedigree. This was to ensure that each individual had been assigned the correct alleles, i.e. that a child had one allele from his/her father and one from the mother. Once alleles were assigned it was possible to generate LOD scores (Linkage analysis).


### 2.6 Programs for Linkage analysis

LOD scores can easily be calculated on any desktop PC. The original LINKAGE program was compiled by J. Ott. (Ott 1985), and this is the program most commonly used for linkage analysis. This program accepts genotype data as a direct input or from the Cyrillic pedigree drawing software. LINKAGE calculates LOD scores between a chosen marker (a disease locus) and one or more other markers, and gives an output which indicates where the chosen marker lies relative to the others. The so called direct method of linkage analysis proceeds by directly observing and counting recombinants and non-recombinants.

For this study two-point linkage analysis was performed using the subprogram MLINK of the Linkage program (version 5.0) as implemented in FASTLINK (ftp://linkage.rockefeller.edu/software/fastlink) (Lathrop et al., 1984: Lathrop et al., 1985). MLINK calculates the likelihood of the pedigree under user-specified values of $\theta$ for two different loci (http://linkage.rockefeller.edu/soft/linkage/sec3.3.html). Subsequently, these likelihoods are used to calculate the LOD scores. In this case, analyses performed with MLINK involved a disease locus and a marker locus.

Prior to running the MLINK program two input files are required;

1. A pedigree (marker.ped) file. This file contains numeric representation of the family structure, disease affection status and details the alleles present in each individual.
2. A parameter (marker.dat) file. This file includes information specific to the genetic model to be analyse such as disease and marker allele frequencies, and disease penetrance.

In this study, RLS was analysed as an autosomal dominant disease with the assumption of a disease-gene frequency of 0.001 , and a penetrance rate of $95 \%$ (Bonati, Ferini-Strambi et al. 2003). Microsatellite maker allele frequencies were obtained from the CEPH database (www.cephb.fr).

Using the above files, MLINK was used to generate LOD scores for specified $\theta$ values. The output from the two-point LOD score is put into the output (marker.out) file.

While genemappers hope for a positive LOD score i.e. linkage, negative LOD scores are not without value. Such values are informative and show where the disease gene is not located, otherwise known as exclusion mapping. Consequently if enough of the genome is excluded only a few possible locations may remain. To find the genetic distance actually excluded when a negative LOD score $<-2$ is observed, a program called the map function (mapfun) is used. This program is available at URL ftp://linkage.rockefeller.edu/ott/linkutil.htm. A map function allows conversion between recombination frequencies $(\theta)$ and genetic distance in centimorgans $(\mathrm{cM})$, under the assumption of a certain level of genetic interference (section 1.11.6).

### 2.6.1 Calculating sample size and power

Before commencing linkage analysis, it is important to know whether the available pedigree information is sufficient to allow detection of gene(s) causing the disease of interest. Computer simulation methods are often employed to investigate this. The simulation method can also be helpful in determining if an observed maximum LOD score is significant for a pedigree. For example, if a LOD score of 2.0 was found in the pedigree in question with a given marker and disease, according to the established LOD-score-of-3 criterion this result is not significant. However, in a small pedigree with a sample size that is not sufficient to generate a LOD score of 3 such a LOD score could be of interest. Such information can help the researcher decide whether or not to devote further time into typing more markers in this region, to try finding the disease causing gene in question.

For this study we used the SLINK package to investigate if the RLS3002 pedigree was powerful enough to detect linkage. The program SLINK is a general computer program that uses a variation of the algorithm described by OTT (1989). It is based on the LINKAGE programs version 4.91 (Lathrop, Lalouel et al. 1984). The SLINK package consists not only of the simulation program (SLINK) but also of several analysis programs (MSIM, LSIM, ISIM, ELODHET). It is essential to remember that the data are simulated conditional on the phenotypes given in the pedigree file, at the recombination fraction(s) given in the data file.

### 2.7 Primer Design

Primer design is aided greatly by software and includes checks for balanced annealing temperatures and for possible dimers and hairpins. There are many software packages available such as Primer Express (Applied Biosystems) and some can even be downloaded from the internet. All of the primers which were designed for this project were done so with the assistance of PrimerSelect, EditSeq and SeqBuilder, which are components of the DNAStar software package (Lasergene). This software supports a large number of criteria for primer design including checks for internal primer stability, energy profile, primer dimer and hairpin formation and a mismatch primer deign.

The following points should be adhered to when designing primers regardless of the software used:

- Primers should be between 16 and 24 bases long
- Avoid long runs of any single base, particularly the tetranucleotide GGGG as it frequently occurs throughout the human genome.
- If possible, the G and C content should be approximately $50 \%$ to prevent excessive primer dimer formation
- Ensure that the ends of the primers are not complimentary, thus avoiding excessive primer dimer formation
- Preferably the $\mathrm{T}_{\mathrm{m}}$ of the two primers should be within $2^{\circ} \mathrm{C}$ of each other.

Primers had to be designed for this project for two different purposes;

1. The first was when trying to minimize the RLS locus, by selecting repeats within specific areas of the locus in the hope that the number of repeats would differ between family individuals thus giving information.
2. The second was when selecting a gene for screening within the RLS locus.

When selecting an area for genotyping or a gene for screening:

- UCSC (www.genome.ucsc.org), ensemble (www.ensembl.org) and NCBI (www.ncbi.nlm.nih.gov/) websites were consulted.
- The location of interest was chosen and the DNA sequence was found using UCSC website.
- Di-, tri-, or tetranucleotide repeats in the area of interest were identified. This area containing the repeat region was selected, with approximately 200 bp flanking either side
- The sequence of interest was entered into EditSeq by a simple copy and paste procedure. The sequence became a (seq)-format when saved there.
- The sequence was imported to PrimerSelect.
- For each microsatellite marker primers were designed. Each of these primer files were saved as D (chromosome no.)S(physical position).
- The primers were located on the original SeqBuilder File and it was ensured that the amplified region contains the repeated region.
- The $5^{\prime}$ end of one of the primers was tagged with a fluorescent dye such as 6FAM or HEX as genotyping was carried out on an ABI automated DNA analyser.
- By using the PCR tool in UCSC or using BLAST from NCBI it was verified that the primer pairs were specific. It was vital to ensure that each primer would bind to the correct genomic location. If both primers also bound a location on another chromosome, it was essential to investigate if a PCR product would be generated.
- When all primers had been validated primers were ordered from www.biomers.net .
- Once a candidate gene was chosen for screening the UCSC (www.genome.ucsc.org), Ensembl (www.ensembl.org) and NCBI (www.ncbi.nlm.nih.gov/) websites were searched for gene information. If more than one transcript existed it had to be ensured that all exons were screened.
- The genomic DNA, mRNA and protein sequences for the gene was obtained, and saved using EditSeq. Looking at the genomic sequence the number of exons in the gene were confirmed i.e. the sequence in uppercase.
- It was important to consult another commonly used genetic website such as Ensembl, and check that no additional transcripts or alternate exons existed. Also it was confirmed that the protein sequence corresponded with the UCSC protein.
- Also it was examined if the exons found in the ensemble site were identical or different to the UCSC exons.
- Each exon sequence was saved as a file named GENEx1.
- All the exons were spliced together to make an in silico mRNA. This GENEspliced file was opened in MapDraw, the ORFs were found and the protein translated. It was confirmed that it was the expected protein.
- Once the exons were confirmed, the UCSC genomic seq file was revisited and each exon with approximately 300 bp flanking sequence was selected. Each of these files was saved as GENEx1 exonic region.
- Using primerSelect and the exonic region files, primers were designed for each exon. The primers had to be positioned so that at least 50bp upstream of the start of the exon would be sequenced
- If a large exon existed, a group of overlapping primer pairs was designed to ensure that the entire region was covered. These primers were labeled x3a, $\mathrm{x} 3 \mathrm{~b}, \mathrm{x} 3 \mathrm{c}$ etc
- Each of these primer pairs were saved as GENEx1 primer files.
- The primers were detected on the exonic region file.It was also confirmed that the exon was adequately covered. This file was saved with the sequence of the PCR product as a GENEx 1 amplicon file.
- A word file of primers to be ordered was generated and the primers were copied from PrimerSelect. It was confirmed that the orientation of the primers was correct by comparing them with the amplification summary in PrimerSelect.
- It was also confirmed that the primer pairs were specific for the selected gene by using the PCR tool in UCSC or using BLAST from NCBI selecting human only. It was important that each primer hit the correct location. If both primers also hit a location on another chromosome, it was necessary to investigate if a PCR product would be produced.
- When all primers had been validated primers were ordered from www.biomers.net.


### 2.8 PCR Purification

PCR Purification prior to sequencing was carried out using the JETQUICK PCR purification kit (Genomed). PCR purification kits provide rapid and efficient removal of short primers, dNTPs, enzymes, short-failed PCR products, and salts from PCR products. The kit contains two solutions: Solution H1 (binding) contains guanidine hydrochloride, isopropanol and Solution H2 (wash, reconstituted) contains ethanol, NaCl, EDTA and Tris/ HCl . Before starting the procedure, the solution H 2 is reconstituted in alcohol ( $96-100 \%$ ) as indicated in the instructions. The kit also contains spin columns or 'micro-spin units' which have a silica membrane in microcentrifugation units. These silica membranes initially trap the PCR products and during the final steps the PCR products are released from the membranes. All centrifugation steps are carried out at $>12,000$ RPM in a table-top microfuge.

## 1. Sample Preparation

$400 \mu$ l of solution H1 was added to $100 \mu$ l of PCR assay and mixed thoroughly.

## 2. Column Loading

A JETQUICK spin column was placed into a 2 ml receiver tube. The mixture from step 1 was loaded into the prepared spin column and centrifuged at $>12,000$ RPM for 1 min . The flowthrough was discarded.

## 3. Column Washing

The spin-column was inserted into an empty receiver tube. $500 \mu \mathrm{l}$ of reconstituted solution H 2 was added and centrifuged at $>12,000$ RPM for 1 min . The flowthrough was discarded. The JETQUICK column was placed back into the same receiver tube and centrifuged at maximum speed again for 1 min .

## 4. DNA Elution

The spin-column was inserted into a new 1.5 ml microfuge tube. $50 \mu \mathrm{l}$ of sterile water (or TE buffer or 10 mM Tris/HCL, pH 8.0 ) was added directly onto the center of the silica matrix of the spin column. It was then centrifuged at $>12,000$ RPM for 2 min. The eluted sample was stored at $1-5^{\circ}$ C.

### 2.9 Sequencing Reaction

Prior to sequencing the following steps were essential:

- The fragment was amplified by PCR ( $50 \mu \mathrm{l})$.
- Amplification was confirmed on an Agarose gel.
- PCR product was purified (JETQUICK kit).
- The purified product was then used as the template for sequencing. This step is based on Fred Sanger's dideoxy sequencing method which will be discussed in more detail in section $6.1 / 6.2$. This will result in differently sized DNA fragments of the desired sequence with each base in the sequence tagged with a different fluorescent label.

Table 2.4: Reagents used for PCR in $15 \mu 1$ reaction volumes

| Big Dye Ready Reaction mix | $0.5 \mu \mathrm{l}$ |
| :--- | :--- |
| Buffer | $1.0 \mu \mathrm{l}$ |
| Primer $\left(100 \mathrm{ng} \mu^{-1}\right)$ | $0.5 \mu \mathrm{l}$ |
| $\mathrm{H}_{2} \mathrm{O}$ | $0.5 \mu \mathrm{l}$ |
| Template DNA (purified) | $2.5 \mu \mathrm{l}$ |
| Total | $5.0 \mu \mathrm{l}$ |

Sequencing was carried out on a thermocycler, using the following sequence cycle programme:

- $93^{\circ} \mathrm{C}$ X 30 seconds
- $50^{\circ} \mathrm{C}$ X 15 seconds
- $60^{\circ} \mathrm{C} \times 4$ minutes
$\} x 25$ cycles
- Incubate at $10^{\circ} \mathrm{C}$ X 15 minutes


### 2.10 Post-Sequencing Purification

Purification of sequencing products was carried out using the AmpliPurify ${ }^{\mathrm{TM}}$ ExTerminator Kit (AME Biosciences). This optimised purification procedure will remove unincorporated dyes, dNTPs and excess salt. It will also remove the sequencing enzyme. All of these components, if not removed, will greatly reduce the quality of the capillary electrophoresis data.

The post-sequencing purification was carried out as follows:

1. $5 \mu \mathrm{l}$ of Mix Blue was added to the sequencing reaction mixture $(10-20 \mu \mathrm{l})$.
2. $100 \mu \mathrm{l}$ of bind/wash solution was added.
3. The sample was loaded onto a minicolumn and spun at $3,000-4000$ RPM for 30 seconds.
4. Another $400 \mu \mathrm{l}$ of bind/wash solution was added to the minicolumn and spun at $12,000-14,000$ RPM for 2 minutes.
5. The minicolumn was transferred to a new 1.5 ml tube and $25 \mu \mathrm{l}$ of was applied directly to the membrane.
6. The column and solution were incubated at room temperature for 2 minutes, and then spun again at $12,000-14,000$ RPM for 1 minute.
7. The samples were loaded directly onto the ABI3130 Sample Plate.

### 2.11 Sequencing analysis

After loading the samples onto the ABI3130 plate, a Plate Record was created as for genotyping (section 2.5.2), however the application "Sequencing" was chosen instead.

Following completion of the run, the generated sequences were analysed from raw data. This was carried out by opening the Sequence analysis software on the ABI3130 PC, adding the samples, and analysing. The success/failure of the ABI3130 system run was recorded. It was noted if the run was completed correctly or if there had been an interruption to the run. The success of the sequencing for each individual sample was also noted, e.g. failed to sequence or sequence present but short/unreadable etc.

### 2.11.1 Seqman Software

After a sequence was run successfully on the ABI3130, it was necessary to compare generated sequences to each other, and also to generate a contig of sequences. DNAstar has a software tool available to do this, known as SeqMan. It allows users to perform alignments and discover variants, along with many other analyses on de novo or template assemblies.

- The Seqman program was opened from the Lasergene menu.
- The sequences to be assembled were located and opened.

The following options for assembly were chosen:

1. Minimum match percentage $=80 \%$
2. Trace evidence (compare) $95 \%$
3. Majority (primary) $100 \%$

- To detect any variants between samples the traces were compared.
- The sequences were examined carefully and any changes were recorded. It was important to see if any of the variants observed were in fact SNPs, and this was discovered by consulting with either the dbSNP or UCSC databases.
- All files were saved so that additional sequences could be added as they were generated.


# Chapter 3 Investigation of 

## known RLS Loci and

Associated Regions

### 3.1 Introduction

Familial aggregation of RLS has been recognized since Ekbom formally described the condition in 1945 (Ekbom 1945). Since then genetic contribution to idiopathic RLS has been well-documented, is substantial, and has been consistently recognised from population and family studies. In 1960 Ekbom described familial aggregation in one-third of RLS patients (Ekbom 1960). A high concordance rate of $83 \%$ in monozygotic twins adds to the evidence(Ondo, Vuong et al. 2000).

To date several major susceptibility loci for RLS (termed RLS1-5 and RLS7) have been proposed by linkage analysis. In addition 3 other loci on chromosome $16 \mathrm{p}, 4 \mathrm{q}$ and 17 p were recently identified (table 3.1). A study of 25 individuals from a French-Canadian family was the first to identify a major genetic locus for the RLS phenotype. While findings were consistent with an autosomal recessive mode of inheritance, the researchers suggested a pseudodominant mode of inheritance may be more appropriate (Desautels, Turecki et al. 2001). Linkage to chromosome 12q was the only locus found under this model of inheritance. This locus has since been reported in several families as well as in the Icelandic population. According to Winkelmann et al the genetic traits found in these families could be explained by the described pseudominant model (described by Desautels et al) as well as by an autosomal dominant model (Desautels, Turecki et al. 2001; Winkelmann, Lichtner et al. 2006). Nonetheless, the susceptibility locus for RLS on chromosome 12 q could not be confirmed in a study of 51 South Tyrolean family members or in two Northern Italian families affected by RLS (Ferini-Strambi, Bonati et al. 2004). Linkage in all other instances assumed an autosomal dominant inheritance model and is limited to single or a small number of kindreds. Further studies in Italian,

American, Canadian and German families showed linkage to chromosome 14 q (RLS2), 9p (RLS3), 20p (RLS4), and 2q (RLS5). A study carried out in 2006 by Winkelmann and associates identified two loci in a single RLS family of German origin on chromosome 4q and 17p (Winkelmann J 2006). In 2008, suggestive evidence for linkage was found on chromosome 19p13 (Kemlink, Plazzi et al. 2008).

Genome-wide association studies are also providing a better picture of how variants in the sequence of the human genome impact RLS. Association studies compare the frequencies of alleles in case and control populations. A higher frequency of the allele tested in cases is taken as confirmation that the allele or genotype is associated with an increased risk for the disease. Two such studies used a large-scale high density genome-wide approach. The first study was performed using the German and Canadian population in 1600 RLS cases and 2600 controls. Three genomic regions were identified. The first was RLS6 which was found to be associated with SNPs in the $B T B D 9$ gene on chromosome 6 p21 (Stefansson, Rye et al. 2007; Winkelmann, Schormair et al. 2007). Two other variants were found within intronic regions of MEIS1 and MAP2K5/LBXCOR1 (Winkelmann, Schormair et al. 2007). Individuals who carry one risk-allele have a $50 \%$ increased risk of developing RLS (Stefansson, Rye et al. 2007). Coincidently, in the same year a similar study was carried out in the Icelandic and US population, and an association was found to the identical variant in BTBD9 (RLS6) (Stefansson, Rye et al. 2007). In this study the presence of PLM was used to support the diagnosis of RLS and the association of BTBD9 was based on individuals being PLM-positive. However, none of these genes are located in any of the previously described loci (RLS1-RLS5). In 2008, an association study which included 2458 RLS cases and 4749 controls focused on the RLS3 linkage region, on chromosome 9p. Two independent signals within splice variants expressed in the
central nervous system of PTPRD showed genome-wide significant association to RLS. PTPRD located on chromosome 9p23-24 is the fourth locus associated with RLS (Schormair, Kemlink et al. 2008). Also in 2008, a further association study in two Caucasian RLS samples of 918 independent cases and controls focused on the RLS1 linkage region. A total of 1536 SNPs in 366 genes within the 21 Mb region of RLS1 were analysed. Association was observed with SNP rs7977109 which is in the neuronal nitric oxide synthase (NOS1) gene, and therefore, suggests the involvement of the NO/arginine pathway in the pathogenesis of RLS (Winkelmann, Lichtner et al. 2008). These molecular findings suggest substantial genetic and clinical heterogeneity of RLS.

Table 3.1 lists the previously known RLS loci and associated regions, the ancestry, the number of generations or number of cases/controls, the method used to locate the region, the chromosomal location and locus name.

Table 3.1:Known RLS loci and associated regions.

| Study | Ancestry | No. Of generations (No. of members) or No. of cases/controls | Method | Chromosomal location | Locus |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | Irish | 2 (29) | Linkage analysis | 5q |  |
| $\begin{aligned} & \hline \begin{array}{l} \text { Desautels et al. } \\ (2001) \end{array} \end{aligned}$ | FrenchCanadian | 1 (25) | Linkage analysis | 12q12-q21 | RLS1 |
| $\begin{array}{\|l} \hline \begin{array}{l} \text { Bonati } \\ (2003) \end{array} \end{array} \text { et al. }$ | Italian | 1 (30) | Linkage analysis | 14q13-q31 | RLS2 |
| Chen et al. (2004) | United States | 2 (23) | Linkage analysis | 9p24-22 | RLS3 |
| $\begin{array}{\|lll} \hline \begin{array}{l} \text { Pichler } \\ (2006) \end{array} & \text { et } & \text { al. } \end{array}$ | South Tyrolean | Population Isolate $(530)$ | Linkage analysis | 2q33 | RLS4 |
| Levchenko et al. (2006) | FrenchCanadian | 1 (59) | Linkage analysis | 20p13 | RLS5 |
| Winkelmann et al (2006) | German | Unknown | Linkage analysis | 4 q |  |
| Winkelmann et al (2006) | German | Unknown | Linkage analysis | 17p |  |
| Winkelmann et al (2007) | German and Canadian case/controls | 1600 cases and 2000 controls | Association study | 6p21 | RLS6 |
| Stefansson et al (2007) | Icelandic | 429 cases and 16,866 controls | Association studies | 6p21 | RLS6 |
| Winkelmann et al (2007) | German and Canadian case/controls | 1600 cases and 2000 controls | Association studies | 2p14 | RLS7 |
| Winkelmann et al (2007) | German and Canadian case/controls | 1600 cases and 2000 controls | Association studies | 15q |  |
| $\begin{aligned} & \hline \begin{array}{l} \text { Schormair et al } \\ \text { (2008) } \end{array} \end{aligned}$ | German, Austrian, Czech, Canadian | 2458 RLS cases and 4749 controls | Association studies | 9p23-24 |  |
| Winkelmann et al (2008) | Caucasian | 918 independent cases | A threestage association study | 12 q |  |
| Levchenko et al (2008) | FrenchCanadian | 4 (46) | Linkage analysis | 16p |  |
| $\begin{array}{\|lll} \hline \begin{array}{l} \text { Kemlink } \\ (2008) \end{array} & \text { et } \quad \text { al. } \end{array}$ | Italian | 3 (12) | Linkage analysis | 19p |  |

The newly recruited Irish family RLS3002 had been previously examined for linkage to known loci. At the time of the initial research there were only four known loci: chromosome $12 \mathrm{q}, 14 \mathrm{q}, 9 \mathrm{p}$ and 5 q and hence only these loci were examined. The research successfully excluded the entire chromosome 14 locus. However, although the other three loci were excluded for linkage to RLS in this pedigree by haplotype analysis, gaps still remained which required further investigation.

Therefore, the aim of part one of this study was to completely exclude linkage from the three loci already examined, and to examine the newly identified loci and associated regions.

### 3.2 Materials and methods

## DNA extraction

The same QIAamp Blood Kit from Qiagen described in section 2.2 was used to extract DNA from peripheral venous blood samples.

## Primer selection

Polymorphic markers from the regions of RLS loci and associated SNPs were selected. Primer information was accessible from the UCSC genome browser (www.genome.ucsc.edu) and from the NCBI website (www.ncbi.nlm.nih.gov). Primers were ordered from biomers.net and the forward primer of each pair was fluorescently tagged to allow detection by the ABI 3130 system. The microsatellite markers for each of the RLS loci and associated SNPs examined are outlined in the tables below.

## Polymerase Chain Reaction (PCR)

For this section of the study and for the purpose of genotyping, Polymerase chain reaction was carried out in $15 \mu \mathrm{l}$ reaction volumes. Table 3.2 provides details of the reagents used.

Table 3.2: Reagents used for PCR reaction

| Reagent | $[$ Stock $]$ | $[$ Final $]$ | 1 sample $(\boldsymbol{\mu l})$ |
| :--- | :--- | :--- | :--- |
| 10 x buffer | 10 x | 1 x | 1.5 |
| $\mathrm{MgCl}_{2}$ | 25 mM | 1.5 mM | 0.9 |
| dNTP | 10 mM | 0.2 mM | 0.3 |
| Forward Primer | $100 \mathrm{ng} / \mu \mathrm{l}$ | 100 ng | 0.5 |
| Reverse Primer | $100 \mathrm{ng} / \mu \mathrm{l}$ | 100 ng | 0.5 |
| Taq polymerase | $5 \mathrm{U} / \mu \mathrm{l}$ | 0.05 U | 0.1 |
| $\mathrm{H}_{2} \mathrm{O}$ |  | 6.2 |  |
| DNA |  | $50-100 \mathrm{ng}$ | 5.0 |
| Total Volume |  |  | 15.0 |

Once the samples were prepared, each $15 \mu 1$ reaction was placed in a 0.2 ml capped PCR tube and placed on the GStorm Thermal Cycler PCR machine. A temperature gradient was initially used to discover the optimal annealing temperature for each individual marker (as outlined in section 2.3.1). The success of PCR was verified by agarose gel electrophoresis (as outlined in section 2.4).

Genotyping and Linkage analysis were successfully performed for all markers under investigation (as per section 2.5 to 2.7 ). Figure 3.1 below is an example of ABI analysis reading of genotyping for two parents and two children for marker D13S218.


Figure 3.1: An example of ABI genotyping of related individuals
Marker D13S218 using samples PATH046 (mother), PATH047 (father), PATH83 (child1) and PATH199 (child 2). The red peaks refer to the internal size standard (ROX) used to determine allele sizes. These size standards are labelled for PATH083, at $139 \mathrm{bp}, 150 \mathrm{bp}$ and 160 bp respectively. The alleles are shown as blue peaks. The colour of the peaks correspond to the colour of the fluorescent label, which in this example is 6-FAM. The mother (PATH046) is homozygous for this marker (one peak at 147 bp ) and the father (PATH047) is heterozygous (two peaks at 147 bp and 153 bp ). The positions of the peaks corresponds to the size of the allele which in this case is 141-153 bp. Child 1 (PATH083) is heterozygous and the alleles can be traced through the generations. He receives the 147 bp allele from his mother and the 153 bp allele from his father. Subsequently child 2 (PATH199) is homozygous for this marker (one single peak) with the inheritance of one 147 bp allele from the mother and another 147 bp from the father.

The allele fragment sizes obtained from genotyping were correlated with the allele numbers according to the CEPH database (www.cephb.fr). LOD scores were generated and distances excluded were determined. In addition, when gaps remained to be excluded in a locus, haplotype analysis was performed.

Chromosomes 5q, 12q, and 9p had been analysed previously and needed some further work; therefore, they were examined first. The Linkage analysis result of each locus and associated region is described separately.

### 3.3 Results

### 3.3.1 Chromosome 5 locus

A novel locus for autosomal dominant RLS was previously mapped to chromosome 5q. Based on linkage analysis and haplotype analysis of the large Irish family, the locus was placed in an 11.50 cM region between D5S2507 and D5S2042. Marker D5S2507 is located at 66.81 cM and marker D5S2042 is located at 78.31 cM . This locus had been previously examined for linkage for our RLS3002 Irish pedigree; however, linkage was not excluded from the entire locus. These previous results almost excluded the chromosome 5 locus but a small 3.08 cM gap remained between 72.53 cM and 75.61 cM . When this initial study was being carried out, only 11 family members were available. A number of other family members became available at a later date; therefore, it was necessary to genotype these members for the markers already examined. This was done in the hope that the additional family members would give more power to the study, giving a greater LOD score and thereby reduce or remove the remaining gap. Table 3.3 lists all the markers examined and outlines their positions (cM), the maximum LOD scores obtained at theta equal to zero and the corresponding maximum excluded distances in cM . Five of the six markers had been previously examined (Abdulrahim 2008). These markers are shown in blue. The results of the original data is shown in italics and the updated data is shown in regular text underneath.

Table 3.3: Markers used to examine chromosome 5 locus.
$\left.\begin{array}{|l|l|l|l|l|l|}\hline \text { Marker } & \text { Position (cM) } & \begin{array}{l}\text { Max } \\ \mathbf{( \theta = 0 )}\end{array} & \text { LOD } & \begin{array}{l}\text { Excluded } \\ (\mathbf{c M})\end{array} & \begin{array}{l}\text { Area } \\ \text { Excluded } \\ \text { from (cM) }\end{array}\end{array} \begin{array}{l}\text { Area } \\ \text { Excluded } \\ \text { (cM) }\end{array}\right]$

As mentioned, five markers which had been examined previously were genotyped for the remaining members of the RLS3002 family (PATH185, PATH186, PATH187, PATH199, PATH200, PATH207 and PATH208) which had been unavailable at the initial investigation of this locus. An additional marker, D5S418 was also examined for this locus. Following investigation with the additional family members the 3.08 cM gap was removed. Figure 3.2 illustrates the positions of the markers, and the corresponding regions of exclusion on chromosome 5. The total area excluded spans from 44.92 cM to 83.23 cM .


Figure 3.2: Illustration of areas of exclusion for chromosome 5 locus
Black arrow with scale represents a portion of chromosome 5. The black bar to the left of chromosome 5 represents the RLS locus spanning 11.50 cM . The regions excluded by the markers D5S418, D2S2500 and D2S2003 are represented by the coloured bars to the right of chromosome 5 . The dark green bar on the far left illustrates the total area excluded combining results from all three markers.

### 3.3.2 Chromosome 12 locus (RLS1; MIM 102300)

Chromosome 12 locus is flanked by markers D12S1044 and D12S78, located at 97.16 cM and 111.87 cM respectively. The distance between these markers is 14.17 cM (Desautels, Turecki et al. 2001). This locus was already examined for family RLS3002; however these results did not totally exclude the locus. There was a gap of 0.10 cM which remained to be excluded. Seven markers were previously examined for the chromosome 12 locus. During this study an additional four markers were examined at this locus. Table 3.4 lists all the markers examined during the initial study (blue text) and the four additional markers examined, the LOD scores obtained at theta equal to zero, the distance of excluded regions, and the corresponding region of exclusion. Marker D12S1300 was repeated for the seven additional markers, similar to the chromosome 5 markers. The results of the original data is shown in italics and the updated data is shown in regular text underneath.

Table 3.4: Markers used to examine chromosome 12 RLS1 locus. (Original data is shown in italics and updated data is shown in regular text.)

| Marker | Position <br> $(\mathbf{c M})$ | Max <br> $\mathbf{( \theta = 0} \mathbf{~ L O D ~}$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from (cM) | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D12S1052 | 83.19 | -3.50 | 0.00 | 83.19 | 83.19 |
| D12S326 | 86.40 | -6.75 | 3.20 | 83.20 | 89.60 |
| D12S1064 | 95.03 | -3.22 | 1.20 | 93.83 | 96.23 |
| D12S351 | 95.56 | -4.51 | 9.72 | 85.84 | 105.28 |
| D12S1044 | 103.90 | -6.10 | 4.92 | 98.98 | 108.82 |
| D12S1300 | 104.12 | -1.70 | 0.00 | - | - |
| D12S346 | 104.65 | -10.90 | 11.82 | 92.83 | 116.47 |
| D12S1030 | 109.47 | 0.01 | 0.00 | - | - |
| D12S318 | 109.47 | -2.78 | 0.90 | 108.57 | 110.37 |
| D12S78 | 111.87 | -2.78 | -1.40 | 105.67 | 118.07 |

In the previous study five markers obtained statistically significant LOD scores and excluded various regions of the chromosome 12 locus. The marker D12S1052 showed a significant LOD score however it was only significant at $\theta=0.00$. Hence there was no area of exclusion except at the position of the marker itself. Four additional markers D12S326, D12S351, D12S1300 and D12S346 were examined for this locus, to attempt to exclude the remaining 0.10 cM gap which was positioned between 110.37 cM and 110.47 cM . Marker D12S346 was successful at excluding this gap, excluding from 92.83 cM to 116.47 cM . Figure 3.3 illustrates the positions of the markers, and the corresponding regions of exclusion on chromosome 12. The total area excluded expands from to 83.19 cM to 118.07 cM .


Figure 3.3: Illustration of areas of exclusion on chromosome 12 locus
Black arrow with scales represents a portion of chromosome 12. The black bar to the left of chromosome 12 represents the chromosome 12 locus spanning 14.17 cM . The regions excluded by the markers D12S1064, D12S351, D12S1044, D12S1300 D12S346, D12S318, and D12S78 are represented by the coloured bars to the right of chromosome 12. The dark green bar on the far left illustrates the total area excluded combining results from all markers.

### 3.3.3 Chromosome 9 locus (RLS3; MIM 610438)

The chromosome 9 RLS locus is the largest found to date, and is located on the small arm of chromosome 9. The candidate region is defined by markers D9S1779 (0.00 $\mathrm{cM})$ and D9S162 ( 34.42 cM ) in a region covering a 34.42 cM . Five markers had been previously examined, of which four obtained a statistically significant LOD score. However, two gaps remained to be excluded on the chromosome 9 locus. The first gap was located at the telomeric end of chromosome 9 and was 15.00 cM in length, and the second was located between 21.36 cM and $24.81 \mathrm{cM}, 3.4 \mathrm{cM}$ in length. Two new markers, D9S1813 and D9S1810, located at the telomeric end were ordered for the 15.00 cM gap. Marker D9S274 was re-examined with the remaining members of the RLS3002 family which had been unavailable at the initial investigation of this locus. Table 3.5 lists the markers investigated and summarizes, their positions, the LOD scores obtained at theta equal to zero, the distance of excluded regions, and the corresponding region of exclusion.

Table 3.5: Markers used to examine chromosome 9 locus.
(Original data is shown in italics and updated data is shown in regular text.)

| Marker | Position <br> $(\mathbf{c M})$ | Max <br> $\mathbf{( \theta = 0 )}$ | LOD <br> (cMcluded | Area <br> Excluded <br> from (cM) | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D9S917 | 0.00 | -0.11 | 0.00 | - | - |
| D9S1779 | 0.00 | -2.20 | 0.00 | - | - |
| D9S1871 | 8.37 | 0.43 | 0.00 | - | - |
| D9S1813 | 9.83 | -5.80 | 6.30 | 3.50 | 16.16 |
| D9S288 | 9.83 | -8.35 | 7.45 | 2.38 | 17.28 |
| D9S1810 | 12.78 | -3.4 | 4.90 | 7.88 | 17.68 |
| D9S286 | 18.06 | -3.23 | 3.30 | - | - |
| D9S274 | 28.42 | -3.10 | 3.61 | - | - |
| D9S162 | 34.42 | -5.60 | 8.89 | 5.02 | - |

The results for each marker on the initial examination are highlighted in blue in table 3.5 above. The results which were achieved after genotyping the additional family members are in the unshaded lines below their corresponding marker. The two additional markers examined are included in this family. Following investigation with the additional family members the 3.40 cM gap located between 21.36 cM and 24.81 cM was removed. The 14.76 cM gap was partially removed; however a 2.38 cM gap remains at the top of chromosome 9. Figure 3.4 illustrates the positions of the markers, and the corresponding regions of exclusion on chromosome 9 .
Total area
cM


Figure 3.4: Illustration of areas of exclusion on chromosome 9 locus
Black arrow with scales represents chromosome 9 (scales in cM ). The black bar to the left of chromosome 9 represents the chromosome 9 locus spanning 34.42 cM between markers D9S1779 and D9S162. The regions excluded by the markers D9S1813, D9S1810, D9S286, D9S274 and D9S162 are represented by the light blue, purple, dark blue, green and pink bars to the right of chromosome 9 . The green bar on the far left illustrates the total area excludeds.

The results almost exclude the chromosome 9 locus, however a small 2.38 cM gap remains to be excluded at 0.00 cM to 2.38 cM . A haplotype analysis was carried out at this locus. It is clear from this analysis that there is no haplotype that is present in all the affected individuals and not present in the unaffected individuals. Therefore, due to the fact that the gap is small and that the haplotype analysis confirms exclusion of the locus, it can be assumed that the chromosome 9 locus is not linked to RLS in the pedigree.


Figure 3.5: Haplotype analysis of RLS3002 pedigree for the chromosome 4 locus
A list of the examined markers is present on the left hand side. The alleles for each individual obtained from genotyping using these markers are listed below each individual. Each haplotype formed from the series of alleles is given a coloured bar so it can be recognised easily. All the coloured haplotypes are present in both affected (filled squares and circles) and unaffected (squares and circles containing the letter N ) individuals. Hence there is no one shared haplotype between all the affected individuals that is absent in normal individuals.

This data, coupled with the LOD score results provides evidence that there is no linkage between RLS and the chromosome 9 locus in this family.

### 3.3.4 Chromosome 2 locus (RLS4; MIM 610439)

The chromosome 2 RLS locus is located on 2 q 33 . The candidate region is located between markers D2S311 (196.85 cM) and D2S2208 (205.06 cM), with a distance of 8.21 cM between them. At this locus four markers were examined. Each of the markers showed negative LOD scores of $<-2$, all of which excluded variable distances. Table 3.6 lists the markers investigated and summarises their relative positions, the LOD scores obtained at theta equal to zero, the distance of excluded regions, and the corresponding region of exclusion.

Table 3.6: Markers used to examine chromosome 2 locus.

| Marker | Position (cM) | Max LOD <br> $(\boldsymbol{\theta}=\mathbf{0})$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from (cM) | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D2S311 | 196.85 | -5.6 | 2.20 | 194.65 | 199.05 |
| D2S2289 | 199.18 | -3.4 | 2.60 | 196.58 | 201.78 |
| D2S2358 | 203.46 | -3.2 | 2.60 | 200.86 | 206.06 |
| D2S2208 | 205.06 | -5.6 | 9.90 | 195.16 | 214.96 |

Four markers were examined within and surrounding the chromosome 2 locus, each of which obtained statistically significant LOD scores and excluded distinct regions of the RLS4 locus. Figure 3.4 illustrates the positions of the markers, and the corresponding regions of exclusion on chromosome 2.


Figure 3.6: Illustration of areas of exclusion on chromosome 2 locus
Black arrow with scales represents a portion of chromosome 2 . The black bar to the left of chromosome 2 represents the RLS4 locus spanning 8.21 cM between markers D2S311 and D2S2208. The regions excluded by the markers D2S311, D2S2289, D2S2358, and D2S2208 are represented by the coloured bars to the right of chromosome 2. The green bar on the far left illustrates the total area excluded combining all four markers.

### 3.3.5 Chromosome 20 locus (RLS5; MIM 611242)

The chromosome 20 locus, referred to as RLS5, is located at 20p13. The region is flanked by the telomeric end of chromosome 20p (above marker D20S1155 at 0.00 cM ) and by a 1.07 cM interval between markers D20S849 (13.98 cM) and D20S835 ( 15.05 cM ). According to the Marshfield map the candidate region spans approximately 15.05 cM (http://research.marshfieldclinic.org/genetics/home/index.asp). It is important to note that the RLS5 locus paper estimates a locus of 16.00 cM (Levchenko, Provost et al. 2006). However, Levchenko and associates used the Decode map to estimate the positions of the microsatellite markers (http://www.decode.com).

Six markers were examined at this locus, all of which showed negative LOD scores of <-2. Each excluded varying distances on the chromosome 20 locus. Table 3.7 lists all of the six markers investigated and describes, their positions, the LOD scores obtained at theta equal to zero, the distance of excluded regions, and the corresponding region of exclusion.

Table 3.7: Markers used to examine chromosome 20 locus.

| Marker | Position (cM) | Max LOD <br> $\mathbf{( \theta = 0 )}$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from $(\mathbf{c M})$ | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D20S199 | 6.25 | -5.75 | 7.66 | 0.00 | 13.91 |
| D20S842 | 8.97 | -5.61 | 9.33 | 2.79 | 21.45 |
| D20S181 | 9.53 | -3.02 | 0.20 | 9.33 | 9.73 |
| D20S116 | 11.2 | -2.8 | 2.20 | 9.00 | 13.40 |
| D20S849 | 13.98 | -3.08 | 0.70 | 13.28 | 14.68 |
| D20S882 | 15.05 | -3.28 | 3.10 | 11.95 | 18.15 |

Six markers obtained statistically significant LOD scores and excluded various regions of the chromosome 20 locus, RLS5. The marker D20S842 showed a significant LOD score, -5.61 at $\theta=0.00$, and excluded from 0.00 cM to 21.45 cM , thereby excluding the entire chromosome 20 locus. Figure 3.7 illustrates the positions of the markers, and the corresponding regions of exclusion on chromosome 20.


Total area excluded

Figure 3.7: Illustration of areas of exclusion on chromosome 20 locus
Black arrow with scales represents a portion of chromosome 20. The black bar to the left of chromosome 20 represents the chromosome 20 locus spanning 8.20 cM between markers D20S311 and D20S2208. The regions excluded by the markers D20S199, D20S842, D20S181, D20S116, D20S849, and D20S882 are represented by the coloured bars to the right of chromosome 20 . The green bar on the far left illustrates the total area excluded combining results of all markers.

### 3.3.6 Chromosome 16p locus

The chromosome 16 locus is flanked by markers $16 \mathrm{p} \_$m30 $(43.21 \mathrm{cM})$ and 16p_m11 ( 44.36 cM ), and 16p_m31 ( 47.01 cM ) and D16S3068 ( 48.53 cM ). This locus is split into two linked regions of $\sim 830$ and $\sim 350 \mathrm{~Kb}$. In genetic distance the locus is split into two regions; the first which is 1.16 cM , spanning from 43.21 cM to 44.36 cM , and the second which is 0.83 cM spanning from 47.01 cM to 47.84 cM . Three markers (D16S3046, D16S403 and D16S3068), which were used in the initial study by Levchenko and associates, were examined for our pedigree (Levchenko, Montplaisir et al. 2008). Table 3.8 lists the three markers examined and outlines their positions, the LOD scores obtained at $\theta=0.00$ and the equivalent excluded regions for the statistically significant scores.

Table 3.8: Markers used to examine chromosome 2 locus.

| Marker | Position (cM) | Max LOD <br> $\mathbf{( \theta = 0 )}$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from $(\mathbf{c M})$ | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D16S3046 | 40.65 | -8.37 | 6.03 | 34.62 | 46.68 |
| D16S403 | 43.89 | -2.05 | 0.00 | - | - |
| D16S3068 | 48.53 | -8.35 | 10.66 | 37.87 | 59.19 |

All three markers obtained statistically significant LOD scores. Markers D16S3046 and D16S3068 both excluded various regions of the chromosome 16 locus. The marker D16S403 showed a significant LOD score, however it was only significant at $\theta=0.00$. Therefore, there was no distance of exclusion except at the position of the marker. Figure 3.8 illustrates the positions of the markers, and the corresponding regions of exclusion on chromosome 16. The total area excluded spans from 34.62 cM to 59.19 cM .


Figure 3.8: Illustration of areas of exclusion for the chromosome 16 locus
Black arrow with scales represents a portion of chromosome 16. The two black bars to the left of chromosome 16 represent the chromosome 16 locus spanning 1.16 cM and 0.83 cM respectively. The regions excluded by the markers D16S3046 and D16S3068 are represented by the coloured bars to the right of chromosome 16. The green bar on the far left illustrates the total area excluded.

### 3.3.7 Chromosome 19p locus

Suggestive evidence for linkage on chromosome 19 p has been reported. The locus is flanked by SNP markers rs754292 and rs273265, and was further mapped within a $1.90 \mathrm{cM}(0.30 \mathrm{Mb})$ interval between microsatellite markers D19S429 and D19S915. The locus is positioned at 45.38 cM to 47.31 cM on chromosome 19. Three markers were examined at this locus, and two of these markers, D19S226 and D19S414, were used in the initial study (Kemlink, Plazzi et al. 2008). All three markers showed negative LOD scores of <-2, each excluding variable distances. Table 3.9 lists the markers used to examine the chromosome 19 locus, their positions ( cM ), maximum obtained LOD scores at theta equal to zero, and the corresponding maximum excluded distances (cM).

Table 3.9: Markers used to examine the chromosome 19 locus.

| Marker | Position (cM) | Max LOD <br> $\mathbf{( \theta = 0 )}$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from (cM) | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D19S226 | 42.28 | -7.44 | 1.80 | 40.48 | 44.08 |
| D19S410 | 45.48 | -5.02 | 2.40 | 43.08 | 47.88 |
| D19S414 | 53.81 | -7.66 | 8.89 | 44.92 | 62.70 |

All three markers examined obtained statistically significant LOD scores and excluded various regions of the chromosome 19p locus. The marker D19S414, which had been used in the initial study, showed a significant LOD score of -7.66 at $\theta=0.00$, and excluded from 44.92 cM to 62.70 cM , thus excluding the entire chromosome 19p locus. Similarly for marker D19S410, it excluded the 1.90 cM locus as it excludes from 43.08 cM to 47.88 cM .

Figure 3.9 illustrates the positions of the three markers and the corresponding regions of exclusion on chromosome 19p.


Figure 3.9: Illustration of areas of exclusion the chromosome 19 locus
Black arrow with scales represents a portion of chromosome 19. The black bar to the left of chromosome 19 represents the chromosome 19 locus spanning 1.90 cM . The regions excluded by the markers D19S226, D19S410, and D19S414 are represented by the coloured bars to the right of chromosome 19. The green bar on the far left illustrates the total area excluded combining all three markers.

### 3.3.8 Chromosome 4q locus

The Chromosome 4 q locus is flanked by markers D4S406 and D4S402, located at 117.06 cM and 124.45 cM respectively. Three markers were examined at this locus, and all three markers showed negative LOD scores of <-2, each excluding variable distances.Table 3.10 lists the three markers examined and outlines, their positions, the LOD scores obtained at $\theta=0.00$ and the equivalent excluded regions for the statistically significant scores.

Table 3.10: Markers used to examine chromosome 4 locus.

| Marker | Position (cM) | Max LOD <br> $(\boldsymbol{\theta}=\mathbf{0})$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from $(\mathbf{c M})$ | Area <br> Excluded <br> fom to (cM) |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D4S2989 | 117.06 | -8.38 | 3.61 | 113.45 | 120.67 |
| D4S407 | 117.06 | -8.68 | 4.51 | 112.55 | 121.57 |
| D4S1575 | 132.05 | -11.17 | 8.87 | 123.18 | 140.92 |

All three markers obtained statistically significant LOD scores. Markers D4S2989 and D4S407 excluded various regions of the chromosome 4 locus from 113.45 cM to 121.57 cM . Marker D4S1575 excluded from 123.18 cM to 140.92 cM . Therefore, a 1.62 cM gap remains to be excluded from the chromosome 4 locus. Figure 3.10 illustrates the positions of the markers, and the corresponding regions of exclusion on chromosome.


Figure 3.10: Illustration of areas of exclusion on chromosome 4 locus
Black arrow with scales represents a portion chromosome 4. The black bar to the left of chromosome 4 represents the chromosome 4 locus spanning 7.39 cM between markers D4S406 and D4S402. The regions excluded by the markers D4S2989, D4S407 and D4S1575 are represented by the coloured bars to the right of chromosome 4. The green bar on the far left illustrates the total area excluded combining all four markers.

The results almost exclude the chromosome 4 locus, however a small 1.62 cM gap remains to be excluded. A haplotype analysis was carried out at this locus. It is clear from this analysis that there is no haplotype that is present in all the affected individuals and not present in the unaffected individuals. Therefore, due to the fact that the gap is small and that the haplotype analysis confirms exclusion of the locus, it can be assumed that the chromosome 4 locus is not linked to RLS in the pedigree.


Figure 3.11: Haplotype analysis of RLS3002 pedigree for the chromosome 4 locus
A list of the examined markers is present on the left hand side. The alleles for each individual obtained from genotyping using these markers are listed below each individual. Each haplotype formed from the series of alleles is given a coloured bar so it can be recognised easily. Unfortunately PATH187, PATH207 and PATH208 could not be assigned a second bar as only one marker defined the two bars for PATH185 (their mother), and that marker information was not available for the three children. All the coloured haplotypes are present in both affected (filled squares and circles) and unaffected (squares and circles containing the letter N ) individuals. Hence there is no one shared haplotype between all the affected individuals that is absent in normal individuals. It is therefore possible to conclude that there is no linkage between RLS and the chromosome 4 locus in this family.

### 3.3.9 Chromosome 17p locus

The chromosome 17p RLS locus extends from D17S1857 (43.01 cM) to D17S1294 ( 50.74 cM ) spanning a distance of 7.73 cM (Winkelmann J 2006). Four markers were examined at this locus. Three of the four markers showed a negative LOD score of <-2, each excluding variable distances. Table 3.11 lists the markers that were examined, the LOD scores obtained at $\theta=0.00$ and the corresponding excluded regions.

Table 3.11: Markers used to examine chromosome 17 locus.

| Marker | Position (cM) | Max LOD <br> $\mathbf{( \theta = 0 )}$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from (cM) | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D17S1857 | 43.01 | -1.51 | 0.00 | - | - |
| D17S2196 | 44.62 | -8.52 | 7.76 | 36.86 | 52.38 |
| D17S1294 | 50.74 | -6.01 | 6.03 | 44.71 | 56.77 |
| D17S798 | 53.41 | -6.50 | 5.52 | 47.89 | 58.93 |

The area excluded by the three markers, D17S2196, D17S1294 and D17S798, extends from 36.86 cM to 58.93 cM . The chromosome 17 p locus extends from 43.01 cM to 50.74 cM ; therefore, it is possible to conclude that there is no linkage between RLS and the chromosome 17 p locus in this family. Figure 3.12 illustrates the position of the three markers that obtained significant negative LOD scores and the corresponding regions of exclusion.


Figure 3.12: Illustration of areas of exclusion on chromosome 17 locus
Black arrow with scales represents a portion of chromosome 17. The black bar to the left of chromosome 17 represents the chromosome 17 locus spanning 7.73 cM between markers D17S1857 and D17S1294. The regions excluded by the markers D17S2196, D17S1294 and D17S798 are represented by the coloured bars to the right of chromosome 17. The green bar on the far left illustrates the total area excluded .

### 3.3.10 Association within the BTBD9 gene (RLS6; MIM 611185)

Association with several SNPs in the BTBD9 gene has been confirmed by a number of independent studies since 2007. Three SNPs in particular; rs9296249, rs9357271, and rs3922809, have been reported to be associated with susceptibility to RLS. These SNPs are located at $53.80-53.82 \mathrm{cM}$ on chromosome 6 . Two microsatellite markers were examined in this area; D6S1610 and D6S1607. Both showed significant LOD scores of <-2, excluding varied distances. Table 3.12 lists the two markers in questions, showing the LOD score obtained at $\theta=0.00$ and the corresponding excluded regions.

Table 3.12: Markers used to examine SNPs within the BTBD9 gene.

| Marker | Position (cM) | Max LOD <br> $\mathbf{( \theta = 0 )}$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from $(\mathbf{c M})$ | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D6S1610 | 53.81 | -2.71 | 1.20 | 52.61 | 55.01 |
| D6S1607 | 59.34 | -8.54 | 6.64 | 52.70 | 65.98 |

The two markers examined for this region both obtained significantly significant LOD scores, and excluded the area containing the associated SNPs. The markers D6S1610 showed a significant LOD score of -2.71 at $\theta=0.00$, excluding 1.20 cM either side. The marker D6S1607 showed a significant LOD score of -8.54 at $\theta=0.00$, excluding 6.64 cM either side, thereby overlapping with the area excluded by marker D6S1607. Figure 3.13 illustrates the positions of the two markers and the regions excluded on chromosome 6.


Figure 3.13: Illustration of areas of exclusion for variants within the BTBD9 gene
Black arrow with scales represents a portion of chromosome 2. The black arrow at approximately 53.80 cM represents the area where the SNPs are located. The regions excluded by the markers D6S1610 and D6S1607 are represented by the coloured bars to the right of chromosome 6 . The green bar on the far left illustrates the total area excluded combining the two markers.

### 3.3.11 Association within the MEIS1 gene (RLS7; MIM 612853)

A significant association was found between RLS and rs2300478 in the MEIS1 gene (601739) on the short arm of chromosome 2; 2p14-p13. This SNP is located at approximately 84.42 cM . Seven markers were selected and examined in this region. Six of the seven markers showed negative LOD scores of <-2. However, only three of these markers excluded variable distances. Table 3.13 lists all of the markers investigated and shows their positions $(\mathrm{cM})$, the LOD scores obtained at $\theta=0.00$ and the regions excluded for the markers with statistically significant LOD scores.

Table 3.13: Markers used to examine SNP within the MEIS1 gene.

| Marker | Position (cM) | $\begin{array}{\|l} \hline \operatorname{Max} \\ (\boldsymbol{\theta}=0) \end{array}$ | LOD | Excluded (cM) | Area <br> Excluded <br> from (cM) | Area Excluded to (cM) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D2S2739 | 73.61 | -5.59 |  | 7.56 | 66.05 | 81.17 |
| D2S337 | 80.60 | -5.59 |  | 3.00 | 77.60 | 83.60 |
| D2S290 | 84.42 | -2.17 |  | 0.00 | - | - |
| D2S166 | 84.42 | -3.23 |  | 0.00 | - | - |
| D2S285 | 86.02 | -2.41 |  | 0.00 | - | - |
| D2S2113 | 88.15 | 0.22 |  | 0.00 | - | - |
| D2S2110 | 90.82 | -5.12 |  | 8.27 | 82.55 | 99.09 |

Seven markers were examined in this region, however only three of these obtained a statistically significant LOD score, and excluded various regions surrounding the SNP rs2300478. The markers D2S290, D2S166, and D2S285 showed a significant LOD score of $<-2$, however it was only significant at $\theta=0.00$. Therefore, there was no distance of exclusion aside from the position of the marker itself. The marker D2S2113 did not show a significant LOD score, and yielded an inconclusive LOD score of 0.22 at $\theta=0.00$.

Figure 3.14 illustrates the positions of the markers, and the corresponding regions of exclusion on chromosome 2. The total area excluded spans from 66.05 cM to 99.09 cM .


Figure 3.14: Illustration of areas of exclusion for the SNP within the MEIS1 gene
Black arrow with scales represents a portion of chromosome 2. The black arrow at approximately 84.42 cM to the left of chromosome 2 represents the region which the SNP is located. The regions excluded by the markers D2S273, D2S337, and D2S211 are represented by the coloured bars to the right of chromosome 2. The dark green bar on the far left illustrates the total area excluded.

### 3.3.12 Association between RLS and variants in the MAP2K5/LBXCOR1 gene

Highly significant associations were found between RLS and a number of variants in a locus containing the genes MAP2K5 and LBXCOR1 on the long arm of chromosome 15. These associations were located at approximately 66.90 cM . Four markers were selected and examined in this region. All four of the markers showed negative LOD scores of $<-2$, each excluding variable distances, depending on the LOD scores obtained. Table 3.14 displays the markers investigated and outlines their positions (cM), the LOD score obtained at $\theta=0$, and the corresponding excluded regions.

Table 3.14: Markers used to examine variants within the MAP2K5 and LBXCOR1 genes.

| Marker | Position (cM) | Max LOD <br> $\mathbf{( \theta = 0 )}$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from (cM) | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D15S1015 | 66.90 | -3.91 | 1.10 | 65.80 | 68.00 |
| D15S153 | 68.10 | -5.51 | 3.71 | 64.39 | 71.81 |
| D15S131 | 71.28 | -9.82 | 12.03 | 59.25 | 83.31 |
| D15S211 | 75.85 | -5.92 | 7.45 | 68.40 | 83.30 |

Four markers were examined in this region, all of which obtained significantly significant LOD scores, each excluding varying distances on chromosome 15. The marker D15S131 obtained a LOD score of -9.82 at $\theta=0.00$, excluding a large distance of 12.03 cM either side of the marker, thus overlapping with the regions excluded by the other three markers. Figure 3.15 illustrates the positions of the markers, and the corresponding regions of exclusion on chromosome 15. The total area excluded spans from 59.25 cM to 83.31 cM .


Figure 3.15: Illustration of areas of exclusion for variants within the MAP2K5/LBXCOR1 genes
Black arrow with scales represents a portion of chromosome 15 . The black arrow at approximately 66.90 cM to the left of chromosome 15 represents the region which the SNP is located. The regions excluded by the markers D15S1011, D15S153, D15S211, and D15S131 are represented by the coloured bars to the right of chromosome 15. The dark green bar on the far left illustrates the total area excluded combining the four markers.

### 3.3.13 Protein tyrosine phosphatase, receptor type, D (PTPRD)

Protein tyrosine phosphatase, receptor type, D (PTPRD) is a member of the protein tyrosine phosphatase (PTP) family. A significant association was found between RLS and two independent SNPs, rs1975917 and rs4626664, in the PTPRD gene (5789) on the short arm of chromosome 9; 9p23-p24.3 (Schormair, Kemlink et al. 2008). Two markers were selected and examined in this region. Both of these markers showed negative LOD scores of <-2, thereby excluding variable distances. Table 3.15 lists all of the markers investigated and shows their positions, the LOD scores obtained at $\theta=0.00$ and the regions excluded for the markers with statistically significant LOD scores.

Table 3.15: Markers used to examine SNPs within the PTPRD gene.

| Marker | Position (cM) | Max <br> $\mathbf{( \theta = 0 )}$ | LOD | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from | Area <br> Excluded to |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| D9S286 | 18.06 | -5.06 | 7.56 | 10.50 | 25.62 |  |
| D9S274 | 28.42 | -5.61 | 3.00 | 19.53 | 37.31 |  |

Two markers were examined in this region, and both excluded the region surrounding the SNPs rs 1975917 and rs4626664. Figure 3.16 illustrates the positions of the markers, and the corresponding regions of exclusion on chromosome 9. The total area excluded spans from 10.50 cM to 37.31 cM .


Figure 3.16: Illustration of areas of exclusion for SNPs within the PTPRD gene Black arrow with scales represents a portion of chromosome 9. The two black arrows at approximately 19.17 cM and 20.12 cM to the left of chromosome 9 represent the regions which the SNPs are located. The regions excluded by the markers D9S286 and D9S274 are represented by the coloured bars to the right of chromosome 9. The dark green bar on the far left illustrates the total area excluded combining all three markers.

### 3.3.14 Association of variants in the NOS1 gene and RLS

Highly significant associations were found between RLS and a number of variants in the neuronal nitric oxide synthase (NOS1) gene on the long arm of chromosome 12. Association was observed with SNP rs7977109 which is located at a genetic distance of 129.30 cM on chromosome 12 (Winkelmann, Lichtner et al. 2008). Two markers were selected and examined in this region. The two markers, D12S2082 and D12S366, both showed negative LOD scores of <-2, each excluding variable distances, depending on the LOD scores obtained. Table 3.16 displays the markers investigated and outlines their positions $(\mathrm{cM})$, the LOD score obtained at $\theta=0.00$ and the corresponding excluded regions.

Table 3.16: Markers used to examine variants within the NOS1 gene.

| Marker | Position (cM) | $\begin{aligned} & \text { Max } \\ & (\boldsymbol{\theta}=\mathbf{0}) \end{aligned}$ | LOD | Excluded (cM) | Area <br> Excluded <br> from (cM) | Area Excluded to (cM) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D12S2082 | 130.94 | -6.63 |  | 2.20 | 128.74 | 133.14 |
| D12S366 | 133.33 | -8.56 |  | 2.20 | 131.13 | 135.53 |

Two markers were examined in this region, both of which obtained significant LOD scores, each excluding varying distances on chromosome 12. Marker D12S2082 obtained a LOD score of -6.63 at $\theta=0.00$ and marker D12S366 obtained a LOD score of -8.56 at $\theta=0.00$, however both excluded 2.20 cM either side of the position in which it is located. Figure 3.17 illustrates the positions of the markers, and the corresponding regions of exclusion on chromosome 12. The total area excluded spans from 128.74 cM to 135.53 cM .


Figure 3.17: Illustration of areas of exclusion for variants within the NOS1 gene Black arrow with scales represents a portion of chromosome 12. The black arrow at 129.30 cM to the left of chromosome 12 represents the region which the SNP rs7977109 is located. The regions excluded by the markers D12S2082 and D12S366 are represented by the orange and blue bars to the right of chromosome 12 . The dark green bar on the far left illustrates the total area excluded.

### 3.4 Conclusion

A new Irish family RLS3002 which consisted of 18 members had been previously recruited (Figure 2.1). This is a three generation family. Unfortunately the affected grandmother and unaffected grandfather were deceased at the time of the study, and DNA samples were unavailable. In the second and third generation, there are 11 affected individuals, three unaffected spouses and four unaffected children. Although the chromosome 12 q locus mapped by Desautels and associates in a FrenchCanadian family displayed autosomal recessive inheritance (Desautels, Turecki et al. 2001), this Irish pedigree displayed and autosomal dominant pattern of inheritance as reported in all the other RLS loci. This is evident as all the affected individuals have only one parent who is affected.

At the time the previous study was carried out only four loci had been described, chromosome 12q, 14p, 9p and 5q. RLS linkage was successfully excluded for the entire RLS2 locus (chromosome 14q) in this family. Due to time constraints the other three loci were not fully excluded; therefore, further work had to be carried out for these loci. A further six RLS loci and five associated regions had been identified since the initial study was performed. It was therefore necessary to investigate if pedigree RLS3002 was linked to any of these regions.

When the previous study was carried out only 11 family members were available to participate in this study. However, over the years following, physical examination and questionnaires were administered for the remaining seven individuals, and subsequently these additional blood sample were collected. The addition of these seven members would add considerable power to the study. Therefore, this study began by re-investigating the markers used for the chromosome 5 p, $12 q$ and 9 p loci,
and genotyping was performed for any individuals who hadn't been previously available. This was in the hope that the addition of the genotypes would increase the power of the study and generate stronger LOD scores for specific markers and therefore, exclude any gaps remaining in the loci.

The chromosome 5 locus was examined first. A 1.10 cM gap remained in this locus which was not excluded. Following investigation with the additional family member the LOD score of marker D5S2500 decreased from -5.51 at $\theta=0.00$ to -6.89 at $\theta=0.00$. Previously it had been excluded from 65.93 cM to 72.53 cM , however it now excluded from 62.29 cM to 76.17 cM , thereby excluding the gap which was positioned between 72.53 cM to 73.61 cM . The chromosome 5 locus was successfully excluded for RLS3002.

Chromosome 12 locus (RLS1) was next examined. A small gap of 0.10 cM remained to be excluded for this locus. Four additional markers were examined at this locus and marker D12S346 which gave a LOD score of 10.90 at $\theta=0.00$ was successful at excluding the gap which was positioned between 110.37 cM and 110.47 cM . The chromosome 12 locus was successfully excluded for RLS3002.

The chromosome 9 locus, which was found in an Italian population, is a very large locus, and two gaps remained to be investigated for pedigree RLS3002. The two gaps spanned 14.76 cM at the telomeric end of chromosome 9 and the other 3.40 cM gap was located between 21.36 cM and 24.81 cM . Following investigation with the additional family members and marker D9S274, which was used in the previous study, the 3.40 cM gap was removed. Two additional markers were investigated at the telomeric end, D9S1813 and D9S1810. The 14.76 cM gap was partially excluded for linkage, however there is still a small area where the markers failed to cover and
a 3.53 cM gap remains. The haplotye analysis provides evidence to confirm exclusion of linkage to this locus. Examination of further markers in the region of the area not excluded would confirm this further.

The chromosome 2 locus (RLS4) was next examined for linkage in this Irish pedigree. Four evenly spaced markers were chosen and examined, each of which obtained statistically significant LOD scores and successfully excluded the RLS4 locus for linkage to this pedigree. The markers D2S311 and D2S2208 which flank the locus and were used in the initial study by Pichler and associates were used in this study also. It is interesting that marker D2S2208 could solely exclude this locus for linkage to our Irish pedigree. It excluded 9.90 cM either side of where it was positioned, the locus only being 8.20 cM in length.

The RLS5 locus which was located in a French-Canadian population is located on the short arm of chromosome 20. Six markers were examined for this locus and each one excluded varying distances on the chromosome 20 locus, successfully excluding the entire chromosome 20 locus.

The chromosome 16p locus which was also found in a French-Canadian family is unusual as it is split into two linked regions of $\sim 830$ and $\sim 350 \mathrm{~Kb}$ (Levchenko, Montplaisir et al. 2008). It is unusual to see a locus split into two linked regions caused by an unusual and rare double recombination in just one family member. Genotyping errors and DNA contamination was ruled out, so therefore the tight double recombination was either a consequence of a recombination event without crossover interference or due to the fact a very rare event occurred. Three markers were examined for this locus. All three gave a statistically significant LOD score,
however only two of the markers excluded various regions of the chromosome 16 locus. These markers were sufficient to fully exclude linkage for pedigree RLS3002.

There was suggestive linkage to chromosome 19p13. Three markers were examined at this locus, two of which were used in the initial study by Kemlink and associates (Kemlink, Plazzi et al. 2008). All three markers examined obtained a statistically significant LOD score, and RLS linkage was successfully excluded for the chromosome 19p13 locus in the family.

A relatively new approach used to find regions associated with RLS is the genomewide association study or GWAS; this is a high-throughput approach which scans the entire genome in an unbiased manner, using statistical methods to determine associations between chromosomal loci and a given phenotype. It requires a large number of cases and a large number of controls which is often difficult to collect. Association studies don't always have to scan the entire genome and they can be used for specific regions, such as the explorative study carried out by Winkelmann and associates in a 21 Mb region encompassing the RLS1 critical region on chromosome 12 (Winkelmann, Lichtner et al. 2008).

Two recent studies used a large-scale high-density genome-wide approach. In the first study which was conducted in the German and Canadian population, three genomic regions were identified encoding the genes MEIS1, BTBD9 and a third region encoding the genes MAP2K5 and LBXCORI (Winkelmann, Schormair et al. 2007). Coincidently a similar study was carried out in the Icelandic and US population, and an association was found in the identical variant in BTBD9 (Stefansson, Rye et al. 2007).

Three SNPs were found to be associated in the BTBD9 gene, RLS6. Two markers were examined for linkage in this area on chromosome 6 , both excluding varying distances, and specifically the region in which the variants are located. Therefore the SNPs associated with the BTBD9 gene are not linked to our family and can be excluded.

The MEIS1 gene is located on chromosome 2 p 14 . Seven genes needed to be examined for this region. Four of the seven genes did not give any exclusion; therefore it was necessary to examine a further three markers. These markers excluded varying overlapping distances and subsequently excluded the region containing the associated SNP.

Highly significant associations were found between RLS and a number of variants in the locus containing the genes encoding MAP2K5 and LBXCOR1 on chromosome $15 q$. Four markers in this region were chosen and examined for linkage to RLS3002. All four markers obtained significant LOD scores, enabling the exclusion of this region.

Two independent signals within splice variants expressed in the CNS of PTPRD showed genome-wide significant association to RLS (Schormair, Kemlink et al. 2008). Two markers were chosen in this region and linkage analysis was performed for family RLS3002. Markers were successful in excluding the associated region for linkage to the family.

The association of variants in the NOS1 gene and RLS implies an involvement of the NO/arginine pathway in the pathogenesis of RLS. Two markers were chosen in this region and following examination of our family with these markers, the associated region was excluded for linkage to our family.

Our analysis shows that the disease is not linked to any of the known RLS loci or risk-associated genes. Hence it can be assumed that members of the family may carry a gene mutation in a novel genetic locus. Therefore a new unidentified locus awaits discovery and provides further evidence for the genetic heterogeneity of RLS. In order to identify a novel-locus a genome-wide scan is required.

## Chapter 4

## Genome-wide Scan

### 4.1 Introduction

Initial work showed that pedigree RLS3002 is not linked to any of the known RLS loci or risk associated genes. Therefore it can be assumed that a new locus awaits identification in this pedigree, a locus which carries a gene mutation. In order to look for this novel locus a genome-wide scan was performed. This approach tests for linkage of the trait to anonymous polymorphic markers spread throughout the genome. A good genome-wide scan will attempt to cover the entire human genome using a set of markers evenly spaced across a given genetic map. The polymorphic markers used are generally chosen for their ease of genotyping and their position within a genetic map. An advantage of doing such a scan is that no previous knowledge of the function of any genes is required. Another huge advantage of this method, particularly for RLS, is that knowledge of the biology of the trait or disease in question is not a requirement. Genome-wide scans rely on a standard set of markers which are generally termed a "mapping set." For this study, two mapping set were used.

The first mapping set used was the Research Genetics Inc. mapping set (Appendix I). It comprised of 146 microsatellite markers, which were spaced at approximately 20 cM intervals throughout the genome. The markers were predominantly tetranucleotide repeats, although the set did contain some dinucleotide and trinucleotide markers also. The markers were fluorescently labelled with 6-FAM (blue), HEX (green) or TET (yellow) labels.

The second mapping set used was the Applied Biosystems Linkage mapping set version 2.5 (Appendix II). This mapping set comprises of 28 panels of fluorescently labeled primer pairs selected to amplify informative microsatellite loci from the

Généthon human linkage map. All markers in this set are dinucleotide repeats, and are fluorescently labeled with either 6-FAM (blue), VIC (green) or NED (yellow). There is exactly 400 microsatellite markers in this mapping set and the set has a resolution of 10 cM . Each of the 28 panels consists of a group of markers that can be loaded into one capillary or into one lane in a gel. Therefore no one marker in any panel has the same fluorescent dye or is in the same size range as another marker in that panel. Panels sometimes contain markers from more than one chromosome in order to gain the most efficiency. The allele size range for this mapping set is based on the range of sizes seen for a particular marker in the CEPH database. However, Applied Biosystems optimised and tailed the markers, and the primers used for this set are often different to those recommended by the standard databases e.g. NCBI and Ensembl. Therefore it is often necessary to correct the genotyping results by adding/subtracting values up to 150 bp in order to adjust them to CEPH values.

It was important to find the total length of each chromosome before starting the genome-wide scan. By knowing the total genetic length of a chromosome it was possible to tell whether or not the telomere at the end of the chromosome was totally excluded or if another marker was required to exclude this area. The total sex averaged genetic length of the 22 autosomes is 3488 cM (Broman, Murray et al. 1998). Table 4.1 lists the genetic length of each individual chromosome Each of the 22 autosomes and its corresponding genetic distance (in cM ) is given.

Table 4.1: The genetic length of each chromosome

| Chromosome | Length $(\mathbf{c M})$ | Chromosome | Length $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- |
| 1 | 289.66 | 12 | 168.79 |
| 2 | 269.07 | 13 | 114.99 |
| 3 | 228.14 | 14 | 138.19 |
| 4 | 211.66 | 15 | 122.15 |
| 5 | 197.54 | 16 | 134.13 |
| 6 | 193.14 | 17 | 126.46 |
| 7 | 181.97 | 18 | 126.00 |
| 8 | 167.91 | 19 | 105.02 |
| 9 | 168.98 | 20 | 101.22 |
| 10 | 173.13 | 21 | 57.78 |
| 11 | 147.78 | 22 | 62.32 |

As previously discussed (section 1.12) LOD scores of 3.00 (1000:1 odds in favour of linkage) or more are evidence of linkage and -2.00 or less are indicative of nonlinkage/exclusion (Haines J.L 1998). Values between -2.00 and 3.00 are inconclusive and require further examination. Power calculation for linkage analysis based on family RLS3002 was performed using SLINK (http:/linkage.rockefeller.edu/soft/slink.html). SLINK simulation analysis indicated that the maximum LOD score achievable for this family is 3.41 at $\theta=0.01$. This value is very informative in that it allows the investigator to gain insight into the full information content of the available pedigree material. The true maximum LOD score is the highest potential LOD score when all meiosis are fully informative under the assumed model and allele frequencies (Haines J.L 1998). It is important to consider that when carrying out two-point linkage analysis the maximum LOD score may not be attained even if there is linkage. When considering the significance of a particular LOD score it is important to note whether all the available linkage information has been captured. The observed maximum LOD score may not match the attainable maximum LOD score for several reasons, including lack of informativeness of key individuals or a difference in allele frequencies from
simulated results. Therefore when performing linkage analysis for RLS3002 any microsatellite marker which resulted in a maximum LOD score of approximately 1.00 or more was investigated further.

### 4.2 Materials and Methods

## DNA extraction

The QIAamp Blood Kit from Qiagen described in section 2.2 was used to extract DNA from peripheral venous blood samples.

## Polymerase Chain Reaction (PCR)

For this section of the study, two different recipes for PCR were used, depending on the mapping set in question.

## Research Genetics Inc. mapping set

The first mapping set used was the Research Genetics Inc. mapping set, and PCR was carried out in $15 \mu \mathrm{l}$ reaction volumes. Table 4.2 provides details of the reagents used. The table contains the name of the reagent, the stock concentration, the concentration used, and the amount of each reagent in a $15 \mu \mathrm{l}$ reaction volume.

Table 4.2 Reagents used for PCR in $15 \mu \mathrm{l}$ reaction volumes.

| Reagent | $[$ Stock $]$ | $[$ Final $]$ | 1 sample $(\boldsymbol{\mu l})$ |
| :--- | :--- | :--- | :--- |
| 5 x buffer Promega | 5 x | 1 x | 1.50 |
| $\mathrm{MgCl}_{2}$ Promega | 25 mM | 1.5 mM | 0.90 |
| dNTP | 10 mM | 0.2 mM | 0.30 |
| Forward Primer | $100 \mathrm{ng} / \mu \mathrm{l}$ | 100 ng | 0.50 |
| Reverse Primer | $100 \mathrm{ng} / \mu \mathrm{l}$ | 100 ng | 0.50 |
| Taq polymerase <br> Promega | $5 \mathrm{U} / \mu \mathrm{l}$ | 0.05 U | 0.10 |
| $\mathrm{H}_{2} \mathrm{O}$ |  | $50-100 \mathrm{ng}$ | 5.00 |
| DNA |  |  | 15.0 |
| Total Volume |  |  |  |

Once the samples were prepared, each $15 \mu 1$ reaction was placed in a 0.2 ml capped PCR tube and placed on the GStorm Thermal Cycler PCR machine. A temperature gradient was initially used to discover the optimal annealing temperature for each individual marker (as outlined earlier in section 3.2). The success of PCR was verified by agarose gel electrophoresis (as outlined in section 2.4).

## Applied Biosystems Linkage mapping set version 2.5

The second mapping set, the Applied Biosystems Linkage mapping set version 2.5, required a different recipe for PCR. For this mapping set True Allele $\circledR$ PCR Premix was used. This is a PCR master mix that contains AmpliTaq Gold® DNA polymerase, dNTPs, $\mathrm{MgCl}_{2}$, and buffer. True Allele ${ }^{\circledR}$ PCR Premix had been optimized for strong, specific PCR amplification of microsatellite markers in the ABI PRISM® Linkage Mapping Set. The reaction consisted of premix, primers, DNA and water and was performed in $15 \mu \mathrm{l}$ reaction volumes. Table 4.3 provides details of the reagents used. The table contains the name of the reagent, the concentration used, and the amount of each reagent in a $15 \mu \mathrm{l}$ reaction volume.

Table 4.3. Reagents used for PCR using the PCR premix from Applied Biosystems.

| Reagent | [Final $]$ | 1 sample ( $\boldsymbol{\mu} \mathbf{I})$ |
| :--- | :--- | :--- |
| True Allele® PCR Premix |  | 9.00 |
| Primer mix | 5 pmol | 0.60 |
| $\mathrm{H}_{2} \mathrm{O}$ |  | 3.00 |
| DNA | $50-100 \mathrm{ng}$ | 2.40 |
| Total Volume |  | 15.00 |

PCR was carried out on a GStorm Thermal cycler, under temperature control using the following program:
$95^{\circ} \mathrm{C}$ for 12 min (Denaturation)
$94^{\circ} \mathrm{C}$ for 15 s
$55^{\circ} \mathrm{C}$ for 15 s
$72^{\circ} \mathrm{C}$ for 30 s
10 cycles
$89^{\circ} \mathrm{C}$ for 15 s
$55^{\circ} \mathrm{C}$ for 15 s
$72^{\circ} \mathrm{C}$ for 30 s
32 cycles
$72^{\circ} \mathrm{C}$ for 10 min (Extension)
The success of PCR was verified by agarose gel electrophoresis (as outlined in section 2.4).

Genotyping and Linkage analysis were successfully performed for all markers under investigation (as per section 2.5 to 2.7 ). Figure 4.2 below is an example of ABI analysis reading of genotyping for two parents and two children. The marker used in this example is D10S597, a marker which was used in the genome-wide scan, and part of the Applied Biosystems Linkage mapping set version 2.5.


Figure 4.1: An example of ABI genotyping of related individuals
Marker D10S597 using samples PATH046 (mother), PATH047 (father), PATH048 (child1) and PATH049 (child 2). The red peaks refer to the internal size standard used to determine allele sizes. The alleles are shown as green peaks. The colour of the peak corresponds to the colour of the fluorescent label, which in this example is HEX. The positions of the peaks correspond to the size of the allele which in this case is 273-297 bp. The mother (PATH046) is homozygous for this marker (one peak), and carries two copies of the 292 bp allele. The father (PATH047) is heterozygous (two peaks) and carries a copy of the 292 bp allele and a copy of the 294 bp allele. Child 1 (PATH048) is heterozygous and the alleles can be traced through the generations. The 292 bp allele is passed from the mother and the 294 bp allele is passed from the father. Child 2 (PATH049) is homozygous for this marker (one single peak) with the inheritance of one 292 bp allele from the mother and another 292 bp from the father.

### 4.3 Results

The results for each chromosome will be given individually, combining the results from the two mapping sets. Markers which had been previously ordered for examination of RLS loci and associated regions, and which have been previously discussed in chapter 3, have been included in these results also in order to identify regions that have been excluded.

### 4.3.1 Chromosome 1

The Research Genetics Inc. mapping set contained eight markers for chromosome one, five of which were tetranucleotide repeats and the remaining three were trinucleotide. The Applied Biosystems mapping set contained 31 markers for chromosome 1. Table 4.4 lists the markers that were examined from both mapping sets, their positions, the LOD scores obtained at $\theta=0.00$ the corresponding maximum excluded distances in cM, and the regions excluded. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set.

Table 4.4: Markers used to examine chromosome 1.

| Marker | Position (cM) | $\begin{aligned} & \text { Max LOD } \\ & (\theta=0) \end{aligned}$ | Excluded (cM) | Area Excluded from (cM) | Area <br> Excluded to $(\mathbf{c M})$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| D1S468 | 4.22 | -5.52 | 1.90 | 2.32 | 6.12 |
| D1S214 | 14.04 | -5.31 | 2.40 | 11.64 | 16.44 |
| D1S450 | 20.61 | -6.79 | 3.61 | 17.00 | 24.22 |
| D1S2667 | 24.68 | -5.49 | 1.70 | 22.98 | 26.38 |
| D1S2697 | 37.05 | -5.55 | 6.33 | 30.72 | 43.38 |
| D1S199 | 45.33 | -8.21 | 2.00 | 43.33 | 47.33 |
| D1S234 | 55.10 | -8.24 | 9.00 | 46.10 | 64.10 |
| D1S1622 | 56.74 | -5.31 | 2.50 | 54.24 | 59.24 |
| D1S255 | 65.47 | -5.09 | 0.40 | 65.07 | 65.87 |
| D1S2797 | 75.66 | -2.08 | 0.00 | - | - |
| D1S2890 | 85.68 | -6.61 | 3.10 | 82.57 | 88.78 |
| D1S3728 | 89.49 | -5.43 | 1.60 | 87.89 | 91.09 |
| D1S230 | 95.31 | -6.58 | 2.40 | 92.91 | 97.71 |
| D1S2841 | 106.45 | -8.36 | 12.13 | 94.32 | 118.58 |
| D1S1728 | 109.04 | -2.55 | 0.40 | 108.64 | 109.44 |
| D1S2868 | 126.16 | 0.60 | 0.00 | - | - |
| D1S206 | 134.20 | -4.25 | 4.31 | 129.89 | 138.51 |
| D1S3723 | 140.39 | -5.59 | 7.35 | 133.04 | 147.74 |
| D1S2726 | 144.28 | -5.08 | 5.32 | 138.96 | 149.6 |
| D1S498 | 155.89 | -7.22 | 1.90 | 153.99 | 157.79 |
| D1S484 | 169.69 | 1.69 | 0.00 | - | - |
| D1S2878 | 177.86 | -10.62 | 9.51 | 168.35 | 187.37 |
| D1S196 | 181.49 | -7.37 | 5.62 | 175.87 | 187.11 |
| D1S1589 | 192.05 | -3.24 | 3.10 | 188.95 | 195.15 |
| D1S413 | 212.42 | -3.34 | 4.81 | 207.61 | 217.23 |
| D1S249 | 222.65 | -1.86 | 0.00 | - | - |
| GATA124F08 | 226.16 | -8.57 | 10.14 | 216.02 | 236.30 |
| D1S425 | 231.11 | -1.79 | 0.00 | - | - |
| D1S213 | 242.23 | -7.65 | 9.31 | 232.92 | 251.54 |
| D1S3462 | 247.23 | -5.27 | 1.30 | 245.93 | 248.53 |
| D1S2800 | 252.12 | -7.64 | 7.45 | 244.67 | 259.57 |
| D1S2785 | 266.27 | -5.88 | 1.50 | 264.77 | 267.77 |
| D1S547 | 267.51 | 0.34 | 0.00 | - | - |
| D1S2842 | 273.96 | -1.79 | 0.00 | - | - |
| D1S2836 | 285.75 | -5.24 | 1.50 | 284.25 | 287.25 |

Thirty-five markers from the two mapping sets were examined for chromosome 1.
Twenty-eight of these markers obtained a statistically significant LOD score, and excluded various regions on the chromosome. Marker D1S2797 showed a significant LOD score of $<-2$, however it was only significant at $\theta=0.00$. Therefore, there was
no distance of exclusion aside from the position of the marker itself. Markers D1S2868, D1S484, D1S249, D1S425, D1S547 and D1S2842 did not show significant LOD scores, and yielded inconclusive LOD scores of 0.60 at $\theta=0.00,1.69$ at $\theta=0.00,-1.86$ at $\theta=0.00,-1.79$ at $\theta=0.00,0.34$ at $\theta=0.00$ and -1.79 at $\theta=0.00$ respectively. Figure 4.1 illustrates the positions of the markers that obtained significant negative LOD scores and the corresponding regions of exclusion on chromosome 1.


Figure 4.2: Illustration of areas of exclusion for Chromosome 1
Black vertical line with scales represents chromosome 1 (scale in cM ). The red bars on the right illustrates the total areas excluded combining all markers from the two linkage sets. The marker(s) which contributed to these exclusion values are also indicated.

The results of the genome-wide scan exclude a substantial area of chromosome 1. A number of gaps remain on chromosome 1 however the majority of these gaps are small. The largest gap is seen between 65.87 cM and 82.58 cM . A total of 196.14 cM of chromosome 1 was excluded, which equates to $67.7 \%$ of the entire chromosome. The highest LOD score seen for this chromosome was for D1S484 at 169.69 cM , which presented with a LOD score of 1.69 at $\theta=0.00$. Although this LOD score was less than the +3 required for evidence of linkage, it was one of the highest LOD scores observed, therefore it required further investigation (section 4.4.1).

### 4.3.2 Chromosome 2

The Research Genetics Inc. mapping set contained eleven markers for chromosome 2, nine of which were tetranucleotide repeats. The Applied Biosystems mapping set contained 30 markers for chromosome 2. Table 4.5 lists the markers that were examined from both mapping sets, their positions, the LOD scores obtained at $\theta=0.00$, the corresponding maximum excluded distances in cM , and the regions excluded. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set.

Eight markers from the Research Genetics Inc mapping set were completed for chromosome two and eighteen makers from the Applied Biosystems Mapping set were completed. Eleven additional markers on chromosome 2 had been ordered previously to examine the chromosome 2 locus (Section 3.3.4) and to examine the association between RLS and a variation within the MEIS1 gene (section 3.3.11). Figure 4.2 illustrates the total areas excluded from chromosome 2 when markers from both mapping sets and previously examined markers have been combined.

Table 4.5: Markers used to examine chromosome 2.

| Marker | Position (cM) | $\begin{aligned} & \text { Max LOD } \\ & (\theta=0) \end{aligned}$ | Excluded (cM) | Area <br> Excluded <br> from (cM) | Area <br> Excluded to (cM) |
| :---: | :---: | :---: | :---: | :---: | :---: |
| D2S2211 | 15.61 | 0.03 | 0.00 | - | - |
| D2S2952 | 17.88 | -3.08 | 6.84 | 11.04 | 24.72 |
| D2S168 | 27.6 | -5.56 | 3.10 | 23.96 | 30.16 |
| D2S405 | 47.97 | -1.78 | 0.00 | - | - |
| D2S367 | 54.96 | -8.28 | 11.71 | 43.25 | 66.67 |
| D2S2259 | 64.29 | -8.11 | 8.48 | 55.81 | 72.77 |
| D2S1356 | 64.29 | -5.86 | 3.10 | 61.19 | 67.39 |
| D2S391 | 70.31 | -7.05 | 8.89 | 61.42 | 79.20 |
| D2S2739 | 73.61 | -5.59 | 7.56 | 66.05 | 81.17 |
| D2S337 | 80.6 | -5.59 | 3.00 | 77.6 | 83.6 |
| D2S290 | 84.42 | -2.17 | 0.00 | - | - |
| D2S166 | 82.42 | -3.23 | 0.00 | - | - |
| D2S285 | 86.02 | -2.41 | 0.00 | - | - |
| D2S327 | 88.15 | -5.17 | 1.90 | 86.25 | 90.05 |
| D2S2113 | 88.15 | 0.22 | 0.00 | - | - |
| D2S2110 | 90.82 | -5.12 | 8.27 | 82.55 | 99.09 |
| D2S286 | 94.05 | -6.07 | 10.55 | 83.50 | 104.60 |
| D2S1777 | 99.41 | 0.22 | 0.00 | - | - |
| D2S329 | 101.02 | -2.79 | 2.20 | 98.82 | 103.22 |
| D2S2333 | 103.16 | -8.18 | 6.54 | 96.62 | 109.70 |
| D2S160 | 122.96 | -4.69 | 4.11 | 118.85 | 127.07 |
| D2S112 | 141.62 | -8.54 | 1.20 | 140.42 | 142.82 |
| D2S1334 | 145.08 | -5.62 | 9.93 | 135.15 | 155.01 |
| D2S151 | 152.04 | -8.36 | 7.35 | 144.69 | 159.39 |
| D2S142 | 161.26 | -6.51 | 1.80 | 159.46 | 163.06 |
| D2S117 | 194.45 | -7.66 | 6.64 | 187.81 | 201.09 |
| D2S311 | 196.90 | -5.61 | 4.40 | 192.50 | 201.30 |
| D2S2289 | 199.20 | -3.43 | 4.00 | 195.20 | 203.20 |
| D2S2358 | 203.50 | -3.16 | 5.20 | 198.30 | 208.70 |
| D2S325 | 204.53 | -5.49 | 7.97 | 196.56 | 212.50 |
| D2S2208 | 205.10 | -5.55 | 9.90 | 196.56 | 212.50 |
| D2S2382 | 213.50 | -7.67 | 5.22 | 208.28 | 218.72 |
| D2S434 | 215.73 | -3.57 | 0.60 | 215.13 | 216.33 |
| D2S126 | 221.13 | 0.48 | 0.00 | - | - |
| D2S206 | 240.79 | -0.22 | 0.00 | - | - |
| D2S2968 | 251.94 | 0.52 | 0.00 | - | - |
| D2S125 | 260.63 | -5.97 | 7.97 | 252.66 | 268.60 |



Figure 4.3: Illustration of areas of exclusion for Chromosome 2
Black vertical line with scales represents chromosome 2 (scales in cM ). The red bars on the right illustrates the total areas excluded combining all markers from the two linkage set. The marker(s) which contributed to these exclusion values are also indicated.

The results of the genome-wide scan exclude a substantial area on chromosome 2. A number of gaps remain on chromosome 2 ; the largest gap present is between 218.72 cM and 252.66 cM . A total of 168.55 cM of chromosome 2 was excluded, which equates to $62.6 \%$ of the entire chromosome. The highest LOD score seen for this chromosome was for D1S2968 at 251.94 cM , which presented with a LOD score of 0.52 at $\theta=0.00$. This LOD score was not high enough to require further investigation.

### 4.3.3 Chromosome 3

The Research Genetics Inc. mapping set contained nine markers for chromosome 3, five of which were tetranucleotide repaeats and the remaining three were trinucleotide. The Applied Biosystems mapping set contained 16 markers for chromosome one. Table 4.6 lists the markers that were examined from both mapping sets, their positions, the LOD scores obtained at $\theta=0.00$, the corresponding maximum excluded distances in cM , and the regions excluded. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set. Markers highlighted in green represent markers which were found in both mapping sets.

Table 4.6: Markers used to examine chromosome 3.

| Marker | Position (cM) | $\begin{aligned} & \text { Max } L O D \\ & (\theta=0) \end{aligned}$ | Excluded (cM) | Area Excluded from (cM) | Area Excluded to (cM) |
| :---: | :---: | :---: | :---: | :---: | :---: |
| D3S1304 | 22.33 | -2.07 | 0.00 | - | - |
| D3S1263 | 36.10 | -8.58 | 8.89 | 27.21 | 44.99 |
| D3S2338 | 42.10 | -3.69 | 0.20 | 41.90 | 42.30 |
| D3S3038 | 44.81 | -5.64 | 9.00 | 35.81 | 53.81 |
| D3S1266 | 52.60 | -3.12 | 0.20 | 52.40 | 52.80 |
| D3S1277 | 61.52 | 0.49 | 0.00 | - | - |
| D3S1289 | 71.41 | -11.08 | 7.15 | 64.26 | 78.56 |
| D3S1300 | 80.32 | -6.91 | 3.10 | 77.22 | 83.42 |
| D3S1285 | 91.18 | -6.34 | 3.91 | 87.27 | 95.09 |
| D3S2406 | 102.64 | -5.45 | 2.70 | 99.94 | 105.34 |
| D3S1271 | 117.76 | -3.98 | 1.30 | 116.46 | 119.06 |
| D3S3045 | 124.16 | -5.75 | 2.70 | 121.46 | 126.86 |
| D3S1278 | 129.73 | -8.21 | 6.84 | 122.89 | 136.57 |
| D3S1303 | 136.32 | -5.84 | 3.40 | 132.92 | 139.72 |
| D3S1267 | 139.12 | -7.92 | 10.55 | 128.57 | 149.67 |
| D3S1292 | 146.60 | -4.08 | 11.18 | 135.42 | 157.78 |
| D3S1569 | 158.38 | -5.85 | 10.45 | 147.93 | 168.83 |
| D3S3053 | 181.87 | -8.54 | 11.30 | 170.57 | 193.17 |
| D3S1565 | 186.04 | -6.02 | 1.80 | 184.24 | 187.84 |
| D3S1262 | 201.14 | -3.04 | 1.00 | 200.14 | 202.14 |
| D3S1580 | 207.73 | -4.96 | 9.10 | 198.63 | 216.83 |
| D3S3023 | (deCode) | 0.26 | 0.00 | - | - |

Twenty-two markers from the two mapping sets were examined for chromosome 3. Nineteen of these markers obtained a statistically significant LOD score, and excluded various regions on the chromosome. Marker D3S1304 showed a significant LOD score of $<-2$, however it was only significant at $\theta=0.00$. Therefore there was no distance of exclusion aside from the position of the marker itself. Markers D3S1277 and D3S3023 did not show significant LOD scores, and yielded inconclusive LOD scores of 0.49 at $\theta=0.00$ and 0.26 at $\theta=0.00$ respectively. Marker D3S3023 was part of the Research Genetics Inc mapping set, however the position of the marker on the Marshfield map was not available. A genetic position was only available from the deCODE map (www.decode.com/). The marker did not give a significant LOD score and therefore the position on the Marshfield map was not needed per se. Figure 4.3 illustrates the positions of the markers that obtained significant negative LOD scores and the corresponding regions of exclusion on chromosome 3 .


Figure 4.4: Illustration of areas of exclusion for Chromosome 3
Black vertical line with scales represents chromosome 3 (scales in cM ). The red bars on the right illustrates the total areas excluded combining all markers from the two linkage set. The marker(s) which contributed to these exclusion values are also indicated.

These results exclude a large area of chromosome 3. A number of gaps remain on chromosome 3, the largest gap present at the telomere between 0.00 cM and 27.21 cM. A total of 154.25 cM of chromosome three was excluded, which equates to $67.6 \%$ of the entire chromosome. The highest LOD score seen for this chromosome was for D 3 S 1277 at 61.52 cM , which presented with a LOD score of 0.49 at $\theta=0.00$. This LOD score was not high enough to deserve further investigation.

### 4.3.4 Chromosome 4

The Research Genetics Inc. mapping set contained eight markers for chromosome 4, six of which were tetranucleotide repeats. The Applied Biosystems mapping set contained 29 markers for chromosome four. Table 4.7 lists the markers that were examined from both mapping sets. The positions (cM), maximum obtained LOD scores at theta equal to zero, the corresponding maximum excluded distances in cM , and the regions excluded are listed here. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set. Markers highlighted in green represent markers which were found in both mapping sets. Markers which have not been highlighted were ordered previously for investigation of linkage to the chromosome 4 locus (section 3.3.7).

Table 4.7: Markers used to examine chromosome 4.

| Marker | Position <br> $(\mathbf{c M})$ | Max LOD <br> $\mathbf{( \theta = 0 )}$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from (cM) | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D4S412 | 4.74 | 0.33 | 0.00 | - | - |
| D4S403 | 25.9 | -8.64 | 12.24 | 13.66 | 38.14 |
| D4S2632 | 50.53 | -5.61 | 10.35 | 40.18 | 60.88 |
| D4S3248 | 72.52 | -3.21 | 0.90 | 71.62 | 73.42 |
| D4S3243 | 88.35 | -1.75 | 0.00 | - | - |
| D4S1534 | 95.09 | -5.94 | 6.13 | 88.96 | 101.22 |
| D4S414 | 100.75 | -8.57 | 3.41 | 97.34 | 104.16 |
| D4S1647 | 104.94 | -5.08 | 3.00 | 101.94 | 107.94 |
| D4S1572 | 107.95 | -1.78 | 0.00 | - | - |
| D4S406 | 117.06 | -8.38 | 2.90 | 114.16 | 119.96 |
| D4S2989 | 117.06 | -8.38 | 3.61 | 113.45 | 120.67 |
| D4S407 | 117.06 | -8.68 | 4.51 | 112.55 | 121.57 |
| D4S402 | 124.45 | -5.69 | 0.00 | - | - |
| D4S1575 | 132.05 | -11.18 | 8.87 | 123.18 | 140.92 |
| D4S424 | 144.56 | -5.79 | 7.45 | 137.11 | 152.01 |
| D4S1629 | 157.99 | -5.31 | 3.00 | 154.99 | 160.99 |
| D4S413 | 157.99 | -5.32 | 0.20 | 157.79 | 158.19 |
| D4S1539 | 176.19 | -2.51 | 0.80 | 175.39 | 176.99 |
| D4S415 | 181.36 | -5.85 | 4.31 | 177.05 | 185.67 |
| D4S2417 | 181.93 | -2.06 | 0.00 | - | - |
| D4S408 | 195.06 | -2.05 | 0.70 | 194.36 | 195.76 |
| D4S1535 | 195.06 | -2.85 | 0.20 | 194.86 | 195.26 |
| D4S426 | 206.98 | -10.98 | 13.63 | 193.35 | 220.61 |
|  |  |  |  |  |  |

Twenty-one markers from the two mapping sets were examined for chromosome 4.
Two markers had been ordered previously for investigation of the chromosome 4 locus (included in the table above). Eighteen of the markers from the mapping sets obtained a statistically significant LOD score, and excluded various regions on the chromosome. Markers D4S402 and D4S2417 showed significant LOD scores of <-2, however they were only significant at $\theta=0.00$. There was no distance of exclusion aside from the position of the marker itself. Markers D4S412, D4S3243 and D4S1572 did not show significant LOD scores, and yielded inconclusive LOD scores of 0.33 at $\theta=0.00,-1.75$ at $\theta=0.00$ and -1.78 at $\theta=0.00$ respectively.

Figure 4.4 illustrates the positions of the markers that obtained significant negative LOD scores and the corresponding regions of exclusion on chromosome 4.


Figure 4.5: Illustration of areas of exclusion for Chromosome 4
Black vertical line with scales represents chromosome 4 (scales in cM ). The red bars on the right illustrates the total areas excluded combining all markers from the two linkage set. The marker(s) which contributed to these exclusion values are also indicated.

These results exclude a large area of chromosome 4. A number of gaps remain on chromosome 4, the largest gap present at the telomere between 0.00 cM and 13.66 cM. A total of 147.29 cM of chromosome four was excluded, which equates to $69.6 \%$ of the entire chromosome. The highest LOD score seen for this chromosome was for D4S412 at 4.74 cM , which presented with a LOD score of 0.33 at $\theta=0.00$. This LOD score was not high enough to deserve further investigation.

### 4.3.5 Chromosome 5

The Research Genetics Inc. mapping set contained seven markers for chromosome 5, five of which were tetranucleotide repeats. The Applied Biosystems mapping set contained 22 markers for chromosome 5. Table 4.8 lists the markers that were examined from both mapping sets, their positions (cM), maximum obtained LOD scores at theta equal to zero, the corresponding maximum excluded distances in cM , and the regions excluded. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set. Markers which have not been highlighted were ordered previously for investigation of linkage to the chromosome 5 locus (section 3.3.1).

Table 4.8: Markers used to examine chromosome 5.

| Marker | Position <br> $(\mathbf{c M})$ | Max LOD <br> $\mathbf{( \theta = 0 )}$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from (cM) | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D5S1981 | 1.72 | -8.37 | 8.38 | 0.00 | 10.10 |
| D5S406 | 11.85 | -5.53 | 2.10 | 9.75 | 13.95 |
| D5S630 | 19.67 | -5.59 | 6.13 | 13.94 | 25.80 |
| D5S817 | 22.88 | -2.87 | 0.20 | 22.68 | 23.08 |
| D5S416 | 28.76 | -5.32 | 3.61 | 25.15 | 32.37 |
| D5S419 | 39.99 | -7.19 | 10.55 | 29.44 | 50.54 |
| D5S1470 | 45.34 | -5.43 | 5.02 | 40.32 | 50.36 |
| D5S426 | 51.99 | -13.46 | 10.55 | 41.44 | 62.54 |
| D5S418 | 58.55 | -11.04 | 13.63 | 44.92 | 72.18 |
| D5S2500 | 69.23 | -6.86 | 6.94 | 62.29 | 76.17 |
| D5S2122 | 78.31 | -5.23 | 2.70 | 75.60 | 81.01 |
| D5S2003 | 78.31 | -5.44 | 4.92 | 73.39 | 83.23 |
| D5S424 | 81.95 | -4.09 | 1.50 | 80.45 | 83.45 |
| D5S641 | 92.38 | -11.19 | 4.41 | 87.97 | 96.79 |
| D5S428 | 95.40 | -11.18 | 3.61 | 91.79 | 99.01 |
| D5S1725 | 97.82 | -3.31 | 6.74 | 91.08 | 104.56 |
| D5S644 | 104.76 | -8.35 | 11.29 | 93.47 | 116.05 |
| D5S2027 | 119.50 | -6.11 | 2.60 | 116.90 | 122.10 |
| D5S1505 | 129.83 | -5.63 | 4.31 | 125.52 | 134.14 |
| D5S471 | 129.83 | -7.76 | 7.66 | 122.17 | 137.49 |
| D5S2115 | 138.64 | -10.70 | 7.35 | 131.29 | 145.99 |
| D5S436 | 147.49 | -8.37 | 12.03 | 135.46 | 159.52 |
| D5S1480 | 147.49 | -3.16 | 4.31 | 143.18 | 151.80 |
| D5S410 | 156.47 | -5.09 | 4.51 | 151.96 | 160.98 |
| D5S400 | 174.80 | -7.91 | 4.11 | 170.69 | 178.91 |
| D5S211 | 182.89 | -5.78 | 4.41 | 178.48 | 187.30 |
| D5S408 | 195.49 | -1.51 | 0.00 | - | - |
|  |  |  |  |  |  |
|  |  | 0 |  |  |  |

Twenty-five markers from the two mapping sets were examined for chromosome 5.
Two additional markers, which have been included in table 4.8, had been ordered previously for investigation to the chromosome 5 locus. Twenty-four of the markers from the mapping sets obtained a statistically significant LOD score, and excluded various regions on the chromosome. Marker D5S408 did not show a significant LOD score, and presented with an inconclusive LOD score of -1.51 at $\theta=0.00$.

Figure 4.5 illustrates the positions of the markers that obtained significant negative LOD scores and the corresponding regions of exclusion on chromosome 5.


Figure 4.6: Illustration of areas of exclusion for Chromosome five
Black vertical line with scales represents chromosome 5 (scales in cM ). The red bars on the right illustrates the total areas excluded combining all markers from the two linkage set. The marker(s) which contributed to these exclusion values are also indicated.

These results exclude a large area of chromosome 5. Only four gaps remain on chromosome five. These are positioned at 83.23 cM to $87.97 \mathrm{cM}, 116.05 \mathrm{cM}$ to $116.90 \mathrm{cM}, 160.98 \mathrm{cM}$ to 170.69 cM and 187.30 cM to 197.54 cM . A total of 172 cM of chromosome five was excluded, which equates to $87.1 \%$ of the entire chromosome. The highest LOD score seen for this chromosome was for D5S408 at 195.49 cM , which presented with a LOD score of -1.51 at $\theta=0.00$. This LOD score did not need any warrant further investigation.

### 4.3.6 Chromosome 6

The Research Genetics Inc. mapping set contained five markers for chromosome 6, five which were tetranucleotide repeats and the remaining one a trinucleotide. The Applied Biosystems mapping set contained 20 markers for chromosome six. Table 4.9 lists the markers that were examined from both mapping sets, the LOD scores obtained at $\theta=0.00$ and the corresponding excluded regions. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set. Markers which have not been highlighted were ordered previously for investigation of linkage to the chromosome 6 locus (section 3.3.10).

Table 4.9: Markers used to examine chromosome 6.

| Marker | Position <br> $(\mathbf{c M})$ | Max LOD <br> $\mathbf{(}=\mathbf{0})$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from $(\mathbf{c M})$ | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D6S1574 | 9.81 | -6.02 | 3.41 | 6.40 | 13.22 |
| D6S470 | 18.22 | -11.02 | 11.71 | 6.51 | 29.93 |
| D6S2434 | 25.08 | -5.32 | 1.80 | 23.28 | 26.88 |
| D6S289 | 29.93 | -8.10 | 6.64 | 23.29 | 36.57 |
| D6S422 | 35.66 | -8.64 | 9.72 | 25.94 | 45.38 |
| D6S2439 | 42.27 | -2.05 | 0.08 | 42.19 | 42.35 |
| D6S1051 | 50.75 | -3.12 | 0.20 | 50.55 | 50.95 |
| D6S1610 | 53.81 | -3.69 | 3.71 | 50.10 | 57.52 |
| D6S1607 | 59.34 | -8.54 | 6.64 | 52.70 | 65.98 |
| D6S2410 | 73.13 | -2.77 | 0.00 | - | - |
| D6S257 | 79.92 | -8.27 | 1.80 | 78.12 | 81.72 |
| D6S460 | 89.93 | -5.01 | 0.70 | 89.13 | 90.53 |
| D6S462 | 99.01 | -5.32 | 1.60 | 97.41 | 100.61 |
| D6S287 | 121.97 | -8.62 | 10.66 | 111.31 | 132.63 |
| D6S1040 | 128.93 | -8.54 | 3.81 | 125.12 | 132.74 |
| D6S262 | 130.00 | -5.43 | 3.20 | 126.80 | 133.20 |
| D6S292 | 136.97 | -6.40 | 4.71 | 132.26 | 141.68 |
| D6S308 | 144.46 | 0.35 | 0.00 | - | - |
| D6S441 | 154.1 | -6.23 | 1.00 | 153.10 | 155.10 |
| D6S2436 | 154.64 | -5.84 | 5.22 | 149.42 | 159.86 |
| D6S1581 | 164.78 | 0.29 | 0.00 | - | - |
| D6S264 | 179.07 | -5.96 | 5.62 | 173.45 | 184.69 |
| D6S446 | 189.00 | 0.16 | 0.00 | - | - |
| D6S281 | 190.14 | -4.19 | 0.10 | 190.04 | 190.24 |
|  |  |  |  |  |  |

Twenty-two markers from the two mapping sets were examined for chromosome 6 .
Two additional markers, which have been included in table 4.8, had been ordered previously for investigation to the chromosome 6 locus. Eighteen of the markers from the mapping sets obtained a statistically significant LOD score, and excluded various regions on the chromosome. Marker D6S2410 showed a significant LOD scores of $<-2$, however it was only significant at $\theta=0.00$. Therefore there was no
distance of exclusion aside from the position of the marker itself. Markers D6S308, D6S1581, and D6S446 did not show significant LOD scores, and produced inconclusive LOD scores of 0.35 at $\theta=0.00,0.29$ at $\theta=0.00$ and 0.16 at $\theta=0.00$ respectively. Figure 4.6 illustrates the positions of the markers that obtained significant negative LOD scores and the corresponding regions of exclusion on chromosome 6.


Figure 4.7: Illustration of areas of exclusion for Chromosome 6
Black vertical line with scales represents chromosome 6 (scales in cM ). The red bars on the right illustrates the total areas excluded combining all markers from the two linkage set. The marker(s) which contributed to these exclusion values are also indicated.

These results exclude a large area of chromosome 6 . A number of gaps remain on chromosome 6 , the largest gap of 13.59 cM present between 159.86 cM and 173.45 cM. A total of 115.31 cM of chromosome 6 was excluded, which equates to $59.7 \%$ of the entire chromosome. The highest LOD score seen for this chromosome was for D6S308 at 144.46 cM , which presented with a LOD score of 0.35 at $\theta=0.00$. This LOD score was not high enough to require further investigation.

### 4.3.7 Chromosome 7

The Research Genetics Inc. mapping set contained eight markers for chromosome 7, five of which were tetranucleotide repeats. The Applied Biosystems mapping set contained 22 markers for chromosome 7. Table 4.10 lists the markers that were examined from both mapping sets, the LOD scores obtained at $\theta=0.00$ and the corresponding excluded regions. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set. Markers highlighted in green represent markers which were found in both mapping sets.

Table 4.10: Markers used to examine chromosome 7.

| Marker | Position <br> $(\mathbf{c M})$ | Max <br> $(\boldsymbol{\theta}=\mathbf{0})$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from (cM) | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D7S517 | 7.44 | -5.61 | 1.20 | 6.24 | 8.64 |
| D7S513 | 17.74 | -5.59 | 7.54 | 10.20 | 25.28 |
| D7S3051 | 29.28 | -3.47 | 0.50 | 28.78 | 29.78 |
| D7S493 | 34.69 | -8.38 | 3.81 | 30.88 | 38.50 |
| D7S516 | 41.69 | -1.79 | 0.00 | - | - |
| D7S484 | 53.70 | -1.92 | 0.00 | - | - |
| D7S2846 | 57.59 | -3.31 | 1.10 | 56.49 | 58.69 |
| D7S519 | 69.03 | -5.77 | 7.56 | 61.47 | 76.59 |
| D7S3046 | 78.65 | -2.69 | 0.10 | 78.55 | 78.75 |
| D7S669 | 90.42 | -3.51 | 2.90 | 87.52 | 93.32 |
| D7S821 | 109.12 | -5.61 | 11.30 | 97.82 | 120.42 |
| D7S530 | 134.55 | -6.41 | 6.74 | 127.81 | 141.29 |
| D7S1804 | 136.95 | -1.31 | 0.00 | - | - |
| D7S640 | 137.83 | -7.21 | 8.07 | 129.76 | 145.90 |
| D7S684 | 147.22 | -5.43 | 4.71 | 142.51 | 151.93 |
| D7S661 | 155.10 | -8.01 | 9.31 | 145.79 | 164.41 |
| D7S1823/ | 173.71 | -5.35 | 0.40 | 173.31 | 174.11 |
| D7S3058 |  |  |  |  |  |

Seventeen markers from the two mapping sets were examined for chromosome 7.
Fourteen of the markers from the mapping sets obtained a statistically significant LOD score, and excluded various regions on the chromosome. Markers D7S516, D7S484, and D7S1804 did not show significant LOD scores, and produced inconclusive LOD scores of -1.79 at $\theta=0.00,-1.92$ at $\theta=0.00$ and -1.31 at $\theta=0.00$ respectively. Figure 4.7 illustrates the positions of the markers that obtained significant negative LOD scores and the corresponding regions of exclusion on chromosome 7.


## Figure 4.8: Illustration of areas of exclusion for Chromosome 7

Black vertical line with scales represents chromosome 7 (scales in cM ). A number of gaps remain on chromosome 7 , the largest which is seen between 38.50 cM and 56.49 cM . The red bars on the right illustrates the total areas excluded combining all markers from the two linkage set. The marker(s) which contributed to these exclusion values are also indicated.

These results of the genome-wide scan exclude a large area of chromosome 7. A total of 109.42 cM of chromosome 7 was excluded, which equals $60.1 \%$ of the entire chromosome. There were three markers which resulted in an inconclusive LOD score none of which were high enough to require further investigation and the marker(s) which contributed to these exclusion values.

### 4.3.8 Chromosome 8

The Research Genetics Inc. mapping set contained ten markers for chromosome 8, five of which were tetranucleotide repeats. The Applied Biosystems mapping set contained 14 markers for chromosome 8 . Table 4.11 lists the markers that were examined from both mapping sets, the LOD scores obtained at $\theta=0.00$ and the corresponding excluded regions. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set. Markers highlighted in green represent markers which were found in both mapping sets.

Table 4.11: Markers used to examine chromosome 8.

| Marker | Position <br> $(\mathbf{c M})$ | Max <br> $(\boldsymbol{\theta}=\mathbf{0})$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from (cM) | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D8S264 | 0.73 | -0.25 | 0.00 | - | - |
| D8S1130 | 22.41 | -3.24 | 5.83 | 16.58 | 28.24 |
| D8S549 | 31.73 | -4.37 | 5.42 | 26.31 | 37.15 |
| D8S258 | 41.55 | -6.19 | 4.51 | 37.04 | 46.06 |
| D8S136 | 43.96 | 0.58 | 0.00 | - | - |
| D8S1771 | 50.05 | -6.62 | 5.93 | 44.12 | 55.98 |
| D8S505 | 60.87 | -4.67 | 5.42 | 55.45 | 66.29 |
| D8S532 | 64.75 | -8.19 | 10.35 | 54.40 | 75.10 |
| D8S285 | 71.00 | -5.69 | 2.80 | 68.20 | 73.80 |
| D8S260 | 79.36 | -4.57 | 7.25 | 72.11 | 86.61 |
| D8S2324 | 94.08 | -5.91 | 11.08 | 83.00 | 105.16 |
| GAAT1A4 | 110.20 | 0.33 | 0.00 | - | - |
| D8S1784 | 118.15 | 0.37 | 0.00 | - | - |
| D8S514 | 130.00 | -5.95 | 0.40 | 129.6 | 130.4 |
| D8S1179 | 135.08 | -5.89 | 3.81 | 131.27 | 138.89 |
| D8S284 | 143.82 | -6.03 | 3.61 | 140.21 | 147.43 |
| D8S256 | 148.12 | -0.02 | 0.00 | - | - |
| D8S272 | 154.02 | -6.21 | 4.71 | 149.31 | 158.73 |

Eighteen markers from the two mapping sets were examined for chromosome 8 . Two of these markers were found in both mapping sets, D8S185 and D8S260. Thirteen of the markers from the mapping sets obtained a statistically significant LOD score, and excluded various regions on the chromosome. Markers D8S264, D8S136, GAAT1A4, D8S1784 and D8S256 did not show significant LOD scores, and produced inconclusive LOD scores of -0.25 at $\theta=0.00,0.58$ at $\theta=0.00,0.33$ at $\theta=0.00,0.37$ at $\theta=0.00$ and -0.02 at $\theta=0.00$ respectively. Figure 4.8 illustrates the positions of the markers that obtained significant negative LOD scores and the corresponding regions of exclusion on chromosome 8.


Figure 4.9: Illustration of areas of exclusion for Chromosome 8
Black vertical line with scales represents chromosome 8 (scales in cM ). The red bars on the right illustrates the total areas excluded combining all markers from the two linkage set. The marker(s) which contributed to these exclusion values are also indicated.

The results of the genome-wide scan exclude a substantial area of chromosome 8 . The largest gap which remains is between 105.16 cM and 129.60 cM . A large area of exclusion was observed between 16.58 cM and 105.16 cM . A total of 113.62 cM of chromosome 8 was excluded, which equates to $67.7 \%$ of the entire chromosome. The highest LOD score seen for this chromosome was for D8S136 at 43.96 cM , which presented with a LOD score of 0.58 at $\theta=0.00$. This LOD score was not high enough to deserve further investigation and the marker(s) which contributed to these exclusion values.

### 4.3.9 Chromosome 9

The Research Genetics Inc. mapping set contained seven markers for chromosome 9, five of which were tetranucleotide repeats, one a trinucleotide, and the remaining two markers were dinucleotide. The Applied Biosystems mapping set contained 20 markers for chromosome nine. Table 4.12 lists the markers that were examined from both mapping sets, the LOD scores obtained at $\theta=0.00$ and the corresponding excluded regions. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set. Markers which have not been highlighted were ordered previously for investigation of linkage to the chromosome 9 locus (section 3.3.3).

Table 4.12: Markers used to examine chromosome 9.

| Marker | Position (cM) | $\begin{aligned} & \text { Max LOD } \\ & (\theta=0) \end{aligned}$ | Excluded (cM) | Area <br> Excluded <br> from (cM) | Area <br> Excluded to (cM) |
| :---: | :---: | :---: | :---: | :---: | :---: |
| D9S917 | 0 | -0.11 | 0.00 | - | - |
| D9S1779 | 0 | -2.20 | 0.00 | - | - |
| D9S1871 | 8.37 | 0.43 | 0.00 | - | - |
| D9S288 | 9.83 | -8.35 | 7.45 | 2.38 | 17.28 |
| D9S1813 | 9.83 | -5.81 | 6.33 | 3.50 | 16.16 |
| D9S1810 | 12.78 | -3.39 | 4.90 | 7.88 | 17.68 |
| D9S286 | 18.06 | -3.23 | 3.30 | 14.76 | 21.36 |
| D9S274 | 28.42 | -5.61 | 8.89 | 19.53 | 37.31 |
| D9S285 | 29.52 | -8.37 | 9.72 | 19.80 | 39.24 |
| D9S157 | 32.24 | -8.37 | 12.24 | 20.00 | 44.48 |
| D9S162 | 34.42 | -3.32 | 5.02 | 29.40 | 39.44 |
| D9S171 | 42.73 | -3.35 | 4.31 | 38.42 | 47.04 |
| D9S1679 | 44.28 | -7.66 | 9.72 | 34.56 | 54.00 |
| D9S1121 | 44.28 | -0.05 | 0.00 | - | - |
| D9S169 | 49.20 | -7.65 | 6.94 | 42.26 | 56.14 |
| D9S161 | 51.87 | -5.72 | 6.94 | 44.93 | 58.81 |
| D9S1118 | 58.26 | -5.31 | 2.70 | 55.56 | 60.96 |
| D9S273 | 65.79 | -2.06 | 0.70 | 65.09 | 66.49 |
| D9S175 | 70.33 | -5.31 | 2.70 | 67.63 | 73.03 |
| D9S1122 | 75.88 | 0.32 | 0.00 | - | - |
| D9S167 | 83.41 | -4.98 | 9.51 | 73.90 | 92.92 |
| D9S257 | 91.87 | 0.00 | 0.00 | - | - |
| D9S287 | 103.42 | -8.37 | 9.82 | 93.60 | 113.24 |
| D9S1690 | 106.63 | -7.79 | 6.23 | 100.40 | 112.60 |
| D9S1677 | 117.37 | -5.42 | 0.60 | 116.77 | 117.97 |
| D9S1776 | 123.33 | -5.43 | 4.21 | 119.12 | 127.54 |
| D9S1682 | 132.09 | -1.77 | 0.00 | - | - |
| D9S282 | 136.47 | -1.79 | 0.00 | - | - |
| D9S290 | 140.86 | -3.49 | 2.80 | 138.06 | 143.66 |
| D9S1826 | 159.61 | -5.84 | 0.20 | 159.41 | 159.81 |

Twenty-one markers from the two mapping sets were examined for chromosome 9 .
Nine additional markers, which have been included in table 4.12, had been ordered previously for investigation to the chromosome 9 locus. Sixteen of the markers from
the mapping sets obtained a statistically significant LOD score, and excluded various regions on the chromosome. Markers D9S1121, D9S1122, D9S257, D9S1682 and D9S282 did not show significant LOD scores, and yielded inconclusive LOD scores of -0.05 at $\theta=0.00,0.32$ at $\theta=0.00,0.00$ at $\theta=0.00,-1.77$ at $\theta=0.00$ and -1.79 at $\theta=0.00$ respectively. Figure 4.9 illustrates the positions of the markers that obtained significant negative LOD scores and the corresponding regions of exclusion on chromosome 9.


Figure 4.10: Illustration of areas of exclusion for Chromosome 9
Black vertical line with scales represents chromosome 9 (scales in cM ). The red bars on the right illustrates the total areas excluded combining all markers from the two linkage set. The marker(s) which contributed to these exclusion values are also indicated.

These results exclude a large area of chromosome 9. A number of gaps remain on chromosome 9, the largest gap of 15.75 cM present between 143.66 cM and 159.41 cM. A total of 122.27 cM of chromosome 9 was excluded, which equates to $72.4 \%$ of the entire chromosome. The highest LOD score seen for this chromosome was for D9S1122 at 75.88 cM , which presented with a LOD score of 0.32 at $\theta=0.00$. This LOD score was not high enough to warrant further investigation.

### 4.3.10 Chromosome 10

The Research Genetics Inc. mapping set contained eight markers for chromosome 10 , four of which were tetranucleotide repeats, two were trinucleotide repeats, and the remaining two markers were dinucleotide. The Applied Biosystems mapping set contained 19 markers for chromosome 10 . Table 4.13 lists the markers that were examined from both mapping sets, the LOD scores obtained at $\theta=0.00$ and the corresponding excluded regions. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set. Markers highlighted in green represent markers which were found in both mapping sets.

Table 4.13: Markers used to examine chromosome 10.

| Marker | Position <br> $(\mathbf{c M})$ | Max <br> LOD <br> $(\boldsymbol{\theta}=\mathbf{0})$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from (cM) | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D10S249 | 2.13 | -5.39 | 0.20 | 1.93 | 2.33 |
| D10S189 | 19.00 | -5.61 | 11.18 | 7.82 | 30.18 |
| D10S547 | 29.15 | -5.56 | 6.54 | 22.61 | 35.69 |
| D10S1653 | 40.36 | -5.84 | 0.30 | 40.06 | 40.66 |
| D10S548 | 45.70 | 1.71 | 0.00 | - | - |
| D10S1423 | 46.23 | -5.67 | 6.84 | 39.39 | 53.07 |
| D10S197 | 52.10 | -7.18 | 3.81 | 48.29 | 55.91 |
| D10S1208 | 63.30 | 0.45 | 0.00 | - | - |
| D10S196 | 70.23 | -6.82 | 6.84 | 63.39 | 77.07 |
| GATA121A08 | 88.41 | -3.11 | 4.31 | 84.10 | 92.72 |
| D10S537 | 91.13 | -5.92 | 7.90 | 83.23 | 99.03 |
| D10S2327 | 100.92 | -2.86 | 1.70 | 99.22 | 102.62 |
| D10S1686 | 105.04 | -7.71 | 6.23 | 98.81 | 111.27 |
| D10S185 | 116.34 | -6.65 | 2.50 | 113.84 | 118.84 |
| D10S192 | 124.27 | 0.24 | 0.00 | - | - |
| D10S1239 | 125.41 | -2.61 | 6.13 | 119.28 | 131.54 |
| D10S597 | 128.73 | -5.98 | 1.73 | 127 | 130.46 |
| D10S1693 | 137.39 | -5.57 | 4.21 | 133.18 | 141.6 |
| D10S1230 | 142.78 | -5.86 | 5.83 | 136.95 | 148.61 |
| D10S587 | 147.57 | -5.97 | 8.69 | 138.88 | 156.26 |
| D10S217 | 157.89 | -5.71 | 0.20 | 157.69 | 158.09 |
| D10S1651 | 168.77 | -3.94 | 2.40 | 166.37 | 171.17 |
| D10S212 | 170.94 | -11.34 | 14.71 | 156.23 | 185.65 |
|  |  |  | -27 |  |  |

Twenty-three markers from the two mapping sets were examined for chromosome 10. Twenty of the markers from the mapping sets obtained a statistically significant LOD score, and excluded various regions on the chromosome. Markers D10S548, D10S1208 and D10S192 did not show significant LOD scores, and yielded inconclusive LOD scores of 1.71 at $\theta=0.00,0.48$ at $\theta=0.00$ and 0.24 at $\theta=0.00$ respectively. Figure 4.10 illustrates the positions of the markers that obtained
significant negative LOD scores and the corresponding regions of exclusion on chromosome 10.


Figure 4.11: Illustration of areas of exclusion for Chromosome 10
Black vertical line with scales represents chromosome 10 (scales in cM ). The red bars on the right illustrates the total areas excluded combining all markers from the two linkage set. The marker(s) which contributed to these exclusion values are also indicated.

These results exclude a substantial area of chromosome 10. A number of small gaps remain on chromosome 10, the largest gap only 7.48 cM , located between 55.91 cM and 63.39 cM . A total of 143.66 cM of chromosome 10 was excluded, which equates to $83 \%$ of the entire chromosome. The highest LOD score seen for this chromosome was for D10S548 at 45.70 cM , which presented with a LOD score of 1.71 at $\theta=0.00$. This LOD score required further investigation (section 4.4.2).

### 4.3.11 Chromosome 11

The Research Genetics Inc. mapping set contained seven markers for chromosome 11, five of which were tetranucleotide repeats, one a trinucleotide, and the remaining two markers were dinucleotide. The Applied Biosystems mapping set contained 20 markers for chromosome 11. Table 4.14 lists the markers that were examined from both mapping sets, the LOD scores obtained at $\theta=0.00$ and the corresponding excluded regions. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set. Markers highlighted in green represent markers which were found in both mapping sets.

Table 4.14: Markers used to examine chromosome 11.

| Marker | Position <br> $(\mathbf{c M})$ | Max LOD <br> $(\boldsymbol{\theta = 0})$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from $(\mathbf{c M})$ | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D11S4046 | 2.79 | -6.22 | 8.48 | 0.00 | 11.27 |
| D11S1338 | 12.92 | -5.57 | 5.22 | 7.70 | 18.14 |
| D11S902 | 21.47 | -4.29 | 0.10 | 21.37 | 21.57 |
| D11S1981 | 21.47 | -1.46 | 0.00 | - | - |
| D11S904 | 33.57 | -7.08 | 3.41 | 30.16 | 36.98 |
| D11S935 | 45.94 | -5.54 | 5.11 | 40.83 | 51.05 |
| D11S905 | 51.95 | -1.77 | 0.00 | - | - |
| D11S4191 | 60.09 | -8.06 | 4.81 | 55.28 | 64.90 |
| D11S987 | 67.48 | -7.66 | 2.50 | 64.98 | 69.98 |
| D11S1314 | 73.64 | -2.06 | 0.00 | - | - |
| D11S2371 | 76.13 | -2.48 | 0.00 | - | - |
| D11S937 | 79.98 | -4.89 | 0.60 | 79.38 | 80.58 |
| D11S901 | 85.48 | -2.05 | 0.00 | - | - |
| D11S4175 | 91.47 | -6.56 | 0.60 | 90.87 | 92.07 |
| D11S898 | 98.98 | -5.83 | 3.10 | 95.88 | 102.08 |
| D11S2000 | 100.62 | -3.51 | 4.41 | 96.21 | 105.03 |
| D11S4090 | 105.74 | -5.42 | 2.90 | 102.84 | 108.64 |
| D11S908 | 108.59 | -5.31 | 5.62 | 102.97 | 114.21 |
| D11S1998 | 113.13 | -2.05 | 0.00 | - | - |
| D11S925 | 118.47 | -4.29 | 5.83 | 112.64 | 124.30 |
| D11S912 | 131.26 | -5.74 | 8.38 | 122.88 | 139.64 |
| D11S1320 | 141.91 | -4.42 | 3.61 | 138.30 | 145.52 |
| D11S968 | 147.77 | 0.44 | 0.00 | - | - |
|  |  |  |  |  |  |
|  |  |  |  |  |  |

Twenty-three markers from the two mapping sets were examined for chromosome 11. Sixteen of the markers from the mapping sets obtained a statistically significant LOD score, and excluded various regions on the chromosome. Markers D11S1314, D11S2371, D11S901 and D11S1998 showed significant LOD scores of <-2, however they were only significant at $\theta=0.00$. Therefore there was no distance of exclusion aside from the position of the marker itself. Markers D11S1981, D11S905 and D11S968 did not show significant LOD scores, and yielded inconclusive LOD
scores of -1.46 at $\theta=0.00,-1.77$ at $\theta=0.00$ and 0.44 at $\theta=0.00$ respectively. Figure 4.11 illustrates the positions of the markers that obtained significant negative LOD scores and the corresponding regions of exclusion on chromosome 11.


Figure 4.12: Illustration of areas of exclusion for Chromosome 11
Black vertical line with scales represents chromosome eleven (scales in cM). The red bars on the right illustrates the total areas excluded combining all markers from the two linkage set. The marker(s) which contributed to these exclusion values are also indicated.

These results exclude a considerable area of chromosome 11. A number of gaps remain on chromosome 11, the largest gap being 10.29 cM , located between 80.58 cM and 90.87 cM . A total of 101.52 cM of chromosome 11 was excluded, which equates to $68.7 \%$ of the entire chromosome. The highest LOD score seen for this chromosome was for marker D11S968 located at 147.77 cM , which presented with a LOD score of 0.44 at $\theta=0.00$. This LOD score is inconclusive and is not high enough to require further investigation.

### 4.3.12 Chromosome 12

The Research Genetics Inc. mapping set contained four markers for chromosome 12, three of which were tetranucleotide repeats, and the remaining marker a trinucleotide repeat. The Applied Biosystems mapping set contained 19 markers for chromosome 12. Table 4.15 lists the markers that were examined from both mapping sets, the LOD scores obtained at $\theta=0.00$ and the corresponding excluded regions. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set. Markers which have not been highlighted were ordered previously for investigation of linkage to the chromosome 12 locus (section 3.3.2) and the NOS1 associated region (section 3.3.14).

Table 4.15: Markers used to examine chromosome 12.

| Marker | Position <br> $(\mathbf{c M})$ | Max LOD <br> $(\boldsymbol{\theta}=\mathbf{0})$ | Excluded <br> (cM) | Area <br> Excluded <br> from $(\mathbf{c M})$ | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D12S352 | 0.00 | -1.38 | 0.00 | - | - |
| D12S99 | 12.60 | -1.75 | 0.00 | - | - |
| D12S1617 | 44.03 | -5.95 | 3.81 | 40.22 | 47.84 |
| D12S345 | 53.09 | -6.33 | 2.60 | 50.49 | 55.69 |
| D12S368 | 66.03 | -5.94 | 7.35 | 58.68 | 73.38 |
| D12S398 | 68.16 | -5.64 | 5.02 | 63.14 | 73.18 |
| D12S83 | 75.17 | -7.07 | 14.6 | 60.57 | 89.77 |
| D12S326 | 86.40 | -6.75 | 3.20 | 83.20 | 89.60 |
| D12S1064 | 95.03 | -3.22 | 1.20 | 93.83 | 96.23 |
| D12S351 | 95.56 | -4.51 | 9.72 | 85.84 | 105.28 |
| D12S1044 | 103.90 | -6.10 | 4.90 | 99.00 | 108.8 |
| D12S1300 | 104.12 | -2.82 | 2.20 | 101.92 | 106.32 |
| D12S346 | 104.65 | -10.91 | 11.82 | 92.83 | 116.47 |
| D12S318 | 109.40 | -2.80 | 0.90 | 108.50 | 110.3 |
| D12S78 | 111.80 | -6.65 | 6.20 | 105.67 | 118.07 |
| D12S2070 | 125.30 | 0.24 | 0.00 | - | - |
| D12S79 | 125.31 | -4.51 | 5.32 | 119.99 | 130.63 |
| D12S2082 | 130.94 | -7.58 | 2.20 | 128.74 | 133.14 |
| D12S366 | 133.33 | -6.63 | 2.20 | 131.13 | 135.53 |
| D13S86 | 134.54 | -11.22 | 9.93 | 124.61 | 144.47 |
| D12S324 | 149.17 | -8.67 | 6.54 | 142.63 | 155.71 |
| D12S2078 | 149.60 | -8.56 | 7.45 | 142.15 | 157.05 |
| D12S1659 | 155.94 | -7.76 | 5.93 | 150.01 | 161.87 |
| D12S1723 | 164.63 | -3.60 | 3.10 | 161.53 | 167.73 |
|  |  |  |  |  |  |

Nineteen markers from the two mapping sets were examined for chromosome 12. Five markers had been ordered and investigated previously for investigation of the chromosome 12 locus and associated region. These markers are included in the table above. Sixteen of the markers from the mapping sets obtained a statistically significant LOD score, and excluded various regions on the chromosome. Markers D12S352, D12S99 and D12S2070 did not show significant LOD scores, and yielded inconclusive LOD scores of -1.38 at $\theta=0.00,-1.75$ at $\theta=0.00$ and 0.24 at $\theta=0.00$
respectively. Figure 4.12 illustrates the positions of the markers that obtained significant negative LOD scores and the corresponding regions of exclusion on chromosome 12.


Figure 4.13: Illustration of areas of exclusion for Chromosome 12
Black vertical line with scales represents chromosome 12 (scales in cM ). The red bars on the right illustrates the total areas excluded combining all markers from the two linkage set. The marker(s) which contributed to these exclusion values are also indicated.

These results exclude a considerable area of chromosome 12. A number of gaps remain on chromosome 12 , the most substantial being that at the telomeric end of chromosome 12 where a gap of 40.22 cM remains between 0.00 cM and 40.22 cM . A total of 119.95 cM of chromosome ten was excluded, which equates to $71.1 \%$ of the entire chromosome. The highest LOD score seen for this chromosome was for marker D12S2070 located at 125.30 cM , which presented with a LOD score of 0.24 at $\theta=0.00$. This LOD score is inconclusive and is not high enough to require further investigation.

### 4.3.13 Chromosome 13

The Research Genetics Inc. mapping set contained four markers for chromosome 13, three of which were tetranucleotide repeats and the remaining one a trinucleotide repeat. The Applied Biosystems mapping set contained 14 markers for chromosome 13. Table 4.16 lists the markers that were examined from both mapping sets, their positions (cM), the LOD scores obtained at $\theta=0.00$, the corresponding maximum excluded distances in cM, and the regions excluded. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set.

Table 4.16: Markers used to examine chromosome 13.

| Marker | Position <br> $(\mathbf{c M})$ | Max LOD <br> $(\mathbf{\theta}=\mathbf{0})$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from $(\mathbf{c M})$ | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D13S175 | 6.03 | -7.03 | 5.52 | 0.51 | 11.55 |
| D13S217 | 17.21 | -5.38 | 10.40 | 6.81 | 27.61 |
| D13S171 | 25.08 | 0.38 | 0.00 | - | - |
| D13S1493 | 25.80 | -2.13 | 0.00 | - | - |
| D13S218 | 32.90 | 0.00 | 0.00 | - | - |
| D13S263 | 38.82 | -4.73 | 0.10 | 38.72 | 38.92 |
| D13S153 | 45.55 | -8.13 | 2.90 | 42.65 | 48.45 |
| D13S800 | 55.31 | -5.52 | 0.60 | 54.71 | 55.91 |
| D13S156 | 55.85 | -3.22 | 5.52 | 50.33 | 61.37 |
| D13S170 | 63.90 | -10.16 | 9.62 | 54.28 | 73.52 |
| D13S793 | 76.26 | -5.74 | 4.01 | 72.25 | 80.27 |
| D13S779 | 82.93 | -5.52 | 4.61 | 78.32 | 87.54 |
| D13S173 | 93.52 | -5.51 | 7.76 | 85.76 | 101.28 |
| D13S1265 | 98.82 | -7.09 | 10.24 | 88.58 | 109.06 |

Fourteen markers from the two mapping sets were examined for chromosome 13. Eleven of the markers from the mapping sets obtained a statistically significant LOD score, and excluded various regions on the chromosome. Marker D13S1493
presented with a significant LOD score of $<-2$, however it was only significant at $\theta=0.00$. Therefore there was no distance of exclusion aside from the position of the marker itself. Markers D13S171 and D13S218 did not show significant LOD scores, and yielded inconclusive LOD scores of 0.38 at $\theta=0.00$ and 0.00 at $\theta=0.00$ respectively. Figure 4.13 illustrates the positions of the markers that obtained significant negative LOD scores and the corresponding regions of exclusion on chromosome 13.


Figure 4.14: Illustration of areas of exclusion for Chromosome 13
Black vertical line with scales represents chromosome 13 (scales in cM ). The red bars on the right illustrates the total areas excluded combining all markers from the two linkage set. The marker(s) which contributed to these exclusion values are also indicated.

These results exclude a considerable area of chromosome 13. A number of small gaps remain on chromosome 13 , the largest being 11.11 cM located between 27.61 cM and 38.72 cM . A total of 91.83 cM of chromosome 13 was excluded, which equates to $79.9 \%$ of the entire chromosome. The highest LOD score seen for this chromosome was for marker D13S171 located at 25.08 cM , which presented with a LOD score of 0.38 at $\theta=0.00$. This LOD score is inconclusive and is not high enough to require further investigation.

### 4.3.14 Chromosome 14

The Research Genetics Inc. mapping set contained eleven markers for chromosome 14, six of which were tetranucleotide repeats, two trinucleotides, and the remaining markers were dinucleotide. The Applied Biosystems mapping set contained 14 markers for chromosome 14. Table 4.17 lists the markers that were examined from both mapping sets, their positions ( cM ), the LOD scores obtained at $\theta=0.00$, the corresponding maximum excluded distances in cM , and the regions excluded. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set. Markers which have not been highlighted were ordered previously for investigation of linkage to the chromosome 14 locus (Abdulrahim 2008).

Table 4.17: Markers used to examine chromosome 14

| Marker | Position <br> $(\mathbf{c M})$ | Max $\mathbf{L O D}$ <br> $(\boldsymbol{\theta = 0} \mathbf{~}$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from $(\mathbf{c M})$ | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D14S608 | 28.01 | 0.08 | 0.00 | - | - |
| D14S283 | 13.89 | -4.96 | 8.58 | 5.31 | 22.47 |
| D14S70 | 40.11 | -5.91 | 4.31 | 35.80 | 44.42 |
| D14S599 | 40.68 | 0.39 | 0.00 | - | - |
| D14S1049 | 40.94 | -3.44 | 5.93 | 35.01 | 46.87 |
| D14S1048 | 45.12 | -3.23 | 1.10 | 44.02 | 46.22 |
| D14S301 | 45.12 | -2.95 | 0.10 | 45.02 | 45.22 |
| D14S288 | 47.51 | -3.17 | 0.90 | 46.61 | 48.41 |
| D14S1013 | 47.51 | -6.07 | 4.51 | 43.00 | 52.02 |
| D14S1068 | 50.50 | -6.14 | 4.61 | 45.89 | 55.11 |
| D14S587 | 55.82 | -1.75 | 0.00 | - | - |
| D14S592 | 66.80 | -5.43 | 1.90 | 64.9 | 68.7 |
| D14S63 | 69.18 | -11.33 | 12.88 | 56.3 | 82.06 |
| D14S588 | 75.61 | -8.57 | 7.76 | 67.85 | 83.37 |
| D14S258 | 76.28 | -7.96 | 10.55 | 65.73 | 86.83 |
| D14S53 | 86.29 | -2.92 | 1.20 | 85.09 | 87.49 |
| D14S1279 | 95.89 | -5.61 | 1.70 | 94.19 | 97.59 |
| D14S65 | 117.30 | -6.35 | 4.31 | 112.99 | 121.61 |
| D14S985 | 126.61 | -3.72 | 3.00 | 123.61 | 129.61 |

Fourteen markers from the two mapping sets were examined for chromosome 14. Five markers had been ordered, investigated and previously discussed for the investigation of the chromosome 14 locus. These markers are included in the table above. Eleven of the markers from the mapping sets obtained a statistically significant LOD score, and excluded various regions on the chromosome. Markers D14S608, D14S599 and D14S587 did not show significant LOD scores, and yielded inconclusive LOD scores of 0.08 at $\theta=0.00,0.39$ at $\theta=0.00$ and -1.75 at $\theta=0.00$ respectively. Figure 4.12 illustrates the positions of the markers that obtained significant negative LOD scores and the corresponding regions of exclusion on chromosome 14.


Figure 4.15: Illustration of areas of exclusion for Chromosome 14
Black vertical line with scales represents chromosome 14 (scales in cM ). The red bars on the right illustrates the total areas excluded combining all markers from the two linkage set. The marker(s) which contributed to these exclusion values are also indicated.

These results exclude a substantial area of chromosome 14. A number of gaps remain on chromosome 14 , the largest being 15.40 cM located between 97.59 cM and 112.99 cM . A total of 86.47 cM of chromosome 14 was excluded, which equates to $62.6 \%$ of the entire chromosome. The highest LOD score seen for this chromosome was for marker D14S599 located at 40.68 cM , which presented with a LOD score of 0.39 at $\theta=0.00$. This LOD score is inconclusive and is not high enough to require further investigation.

### 4.3.15 Chromosome 15

The Research Genetics Inc. mapping set contained sixteen markers for chromosome 15; however three markers were only available at the time. Two of these markers were tetranucleotides, and the third marker a trinucleotide. The Applied Biosystems mapping set contained 13 markers for chromosome 15 . Table 4.18 lists the markers that were examined from both mapping sets, their positions (cM), maximum obtained LOD scores at theta equal to zero, the corresponding maximum excluded distances in cM , and the regions excluded. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set. The marker which has not been highlighted was ordered previously for investigation of linkage to the variants containing the genes encoding MAP2K5 and LBXCORI (section 3.3.12).

Table 4.18: Markers used to examine chromosome 15.

| Marker | Position <br> $(\mathbf{c M})$ | Max <br> $(\boldsymbol{\theta = 0})$ | LOD <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from $(\mathbf{c M})$ | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D15S128 | 6.11 | -4.95 | 1.30 | 4.81 | 7.41 |
| D15S1002 | 14.58 | -6.21 | 1.40 | 13.18 | 15.98 |
| D15S1007 | 25.86 | -10.98 | 12.77 | 13.09 | 38.63 |
| D15S994 | 40.25 | -10.84 | 8.69 | 31.56 | 48.94 |
| D15S978 | 45.62 | -11.22 | 9.51 | 36.11 | 55.13 |
| D15S117 | 51.21 | -11.11 | 12.45 | 38.76 | 63.66 |
| D15S1507 | 60.17 | -5.59 | 3.00 | 57.17 | 63.17 |
| D15S1015 | 66.9 | -3.91 | 1.10 | 65.80 | 68.00 |
| D15S153 | 68.1 | -5.50 | 3.71 | 64.39 | 71.81 |
| D15S131 | 71.28 | -9.82 | 12.03 | 59.25 | 83.31 |
| D15S211 | 75.85 | -5.92 | 7.45 | 68.40 | 83.30 |
| D15S205 | 78.92 | -6.50 | 4.41 | 74.51 | 83.33 |
| D15S127 | 86.81 | -8.08 | 8.58 | 78.23 | 95.39 |
| D15S816 | 100.59 | -3.28 | 0.40 | 100.19 | 100.99 |
| D15S130 | 100.59 | -0.05 | 0.00 | - | - |
| D15S120 | 112.58 | -1.15 | 0.00 | - | - |

Fifteen markers from the two mapping sets were examined for chromosome 15. A separate marker had been ordered, investigated and previously discussed for the investigation of variants within chromosome 15 . This marker is included in the table above. Thirteen of the markers from the mapping sets obtained a statistically significant LOD score, and excluded various regions on the chromosome. Markers D15S130 and D15S120 did not show significant LOD scores, and yielded inconclusive LOD scores of -0.05 at $\theta=0.00$ and -1.15 at $\theta=0.00$ respectively. Figure 4.12 illustrates the positions of the markers that obtained significant negative LOD scores and the corresponding regions of exclusion on chromosome 15.


Figure 4.16: Illustration of areas of exclusion for Chromosome 15
Black vertical line with scales represents chromosome 15 (scales in cM ). The red bars on the right illustrates the total areas excluded combining all markers from the two linkage set. The marker(s) which contributed to these exclusion values are also indicated.

These results exclude a large amount of chromosome 15. Only four gaps remain on chromosome 15. The largest and most significant of these gaps is seen between 100.99 cM and the telomere ( 122.15 cM ). A substantial area of exclusion can be observed between 13.18 cM and 95.39 cM . A total of 85.61 cM of chromosome thirteen was excluded, which equals $70.1 \%$ of the entire chromosome. There was no considerably high LOD score observed for any of the markers on chromosome 15.

### 4.3.16 Chromosome 16

The Research Genetics Inc. mapping set contained two markers for chromosome 16, one which was a tetranucleotide repeat, and the other a trinucleotide repeat. The Applied Biosystems mapping set contained 13 markers for chromosome 16. Table 4.19 lists the markers that were examined from both mapping sets, their positions $(\mathrm{cM})$, maximum obtained LOD scores at theta equal to zero, the corresponding maximum excluded distances in cM , and the regions excluded. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set.

Table 4.19: Markers used to examine chromosome 16.

| Marker | Position <br> $(\mathbf{c M})$ | Max LOD <br> $(\boldsymbol{\theta = 0})$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from (cM) | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D16S404 | 18.07 | -8.51 | 6.64 | 11.43 | 24.71 |
| D16S3075 | 23.28 | -6.21 | 1.80 | 21.48 | 25.08 |
| D16S3046 | 40.65 | -8.37 | 6.03 | 34.62 | 46.68 |
| D16S403 | 43.89 | -2.05 | 0.00 | - | - |
| D16S3068 | 48.53 | -8.36 | 10.66 | 37.87 | 59.19 |
| D16S3136 | 62.11 | -0.22 | 0.00 | - | - |
| D16S415 | 67.62 | -7.33 | 2.70 | 64.92 | 70.32 |
| D16S3253 | 71.77 | -0.96 | 0.00 | - | - |
| D16S503 | 83.55 | 0.19 | 0.00 | - | - |
| D16S515 | 92.1 | -1.35 | 0.00 | - | - |
| D16S516 | 100.39 | -1.77 | 0.00 | - | - |
| D16S3091 | 111.12 | -5.71 | 2.00 | 109.12 | 113.12 |
| D16S520 | 125.82 | -2.98 | 0.60 | 125.22 | 126.42 |

Thirteen markers from the two mapping sets were examined for chromosome 16. Only seven of these markers from the mapping sets obtained a statistically significant LOD score, and excluded various regions on the chromosome. Marker D16S403 presented with a significant LOD scores of <-2, however it was only significant at $\theta=0.00$. Therefore there was no distance of exclusion aside from the
position of the marker itself. Markers D16S3253, D16S503, D16S515 and D16S516 did not show significant LOD scores, and yielded inconclusive LOD scores of -0.96 at $\theta=0.00,0.19$ at $\theta=0.00,-1.35$ at $\theta=0.00$ and -1.77 at $\theta=0.00$ respectively. Figure 4.16 illustrates the positions of the markers that obtained significant negative LOD scores and the corresponding regions of exclusion on chromosome 16.


Figure 4.17: Illustration of areas of exclusion for Chromosome 16
Black vertical line with scales represents chromosome 16 (scales in cM ). The red bars on the right illustrates the total areas excluded combining all markers from the two linkage set. The marker(s) which contributed to these exclusion values are also indicated.

This result for the scan of chromosome 16 was not as successful as the other chromosomes. A large number of substantial gaps remain on chromosome 16. The largest gap is 38.80 cM and located between 70.32 cM and 109.12 cM . Five of the markers obtained an inconclusive LOD score and the other markers showed quite small areas of exclusion. Therefore a large number of gaps remain to be investigated further. A total of 48.82 cM of chromosome 16 was excluded, which equals $36.4 \%$ of the entire chromosome. There was no considerably high LOD score observed for any of the markers on chromosome 16.

### 4.3.17 Chromosome 17

The Research Genetics Inc. mapping set contained six markers for chromosome 17, two which were tetranucleotide repeats, two which were trinucleotide repeats, and the remaining two markers were dinucleotide. The Applied Biosystems mapping set contained 15 markers for chromosome 17. Table 4.20 lists the markers that were examined from both mapping sets. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set. Markers highlighted in green represent markers which were found in both mapping sets. The marker which has not been highlighted was ordered previously for investigation of linkage to the chromosome seventeen locus (section 3.3.9).

Table 4.20: Markers used to examine chromosome 17.

| Marker | Position <br> $(\mathbf{c M})$ | Max LOD <br> $(\boldsymbol{\theta}=\mathbf{0})$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from (cM) | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D17S849 | 0.63 | -8.49 | 8.48 | -7.85 | 9.11 |
| D17S831 | 6.60 | -5.96 | 5.52 | 1.08 | 12.12 |
| D17S938 | 14.69 | -7.93 | 5.42 | 9.27 | 20.11 |
| D17S974 | 22.24 | -5.61 | 6.23 | 16.01 | 28.47 |
| D17S1852 | 22.24 | -6.34 | 7.97 | 14.27 | 30.21 |
| D17S799 | 31.96 | -5.41 | 3.10 | 28.86 | 35.06 |
| D17S921 | 36.14 | -4.11 | 1.80 | 34.34 | 37.94 |
| D17S1857 | 43.01 | -1.51 | 0.00 | - | - |
| D17S2196 | 44.62 | -8.52 | 7.76 | 36.86 | 52.38 |
| D17S1294 | 50.74 | -6.01 | 6.03 | 44.71 | 56.77 |
| D17S798 | 53.41 | -6.50 | 5.52 | 47.89 | 58.93 |
| D17S1868 | 64.16 | -5.83 | 9.62 | 54.54 | 73.78 |
| D17S2180 | 66.85 | 0.44 | 0.00 | - | - |
| D17S787 | 74.99 | -9.59 | 4.61 | 70.38 | 79.60 |
| D17S944 | 82.56 | 1.36 | 0.00 | - | - |
| D17S2193 | 89.32 | -3.78 | 0.00 | - | - |
| D17S949 | 93.27 | -5.86 | 0.40 | 92.87 | 93.67 |
| D17S785 | 103.53 | -8.91 | 8.48 | 95.05 | 112.01 |
| D17S784 | 116.86 | -3.27 | 3.30 | 113.56 | 120.16 |
| D17S928 | 126.46 | -1.19 | 0.00 | - | - |
|  |  |  |  |  |  |

Nineteen markers from the two mapping sets were examined for chromosome 17. A separate marker had been ordered, investigated and previously discussed for the investigation of the chromosome 17 RLS locus. This marker is included in the table above. Fifteen of the markers from the mapping sets obtained a statistically significant LOD score, and excluded various regions on the chromosome. Marker D17S2193 presented with a significant LOD scores of <-2, however it was only significant at $\theta=0.00$. Therefore there was no distance of exclusion aside from the position of the marker itself. Markers D17S1857, D17S2180, D17S944 and D17S928 did not show significant LOD scores, and yielded inconclusive LOD scores of -1.51 at $\theta=0.00,0.44$ at $\theta=0.00,1.36$ at $\theta=0.00$ and -1.19 at $\theta=0.00$ respectively. Figure 4.17 illustrates the positions of the markers that obtained significant negative LOD scores and the corresponding regions of exclusion on chromosome 17.


Figure 4.18: Illustration of areas of exclusion for Chromosome 17
Black vertical line with scales represents chromosome 17 (scales in cM ). The red bars on the right illustrates the total areas excluded combining all markers from the two linkage set. The marker(s) which contributed to these exclusion values are also indicated.

These results exclude a large amount of chromosome 17. The largest gap which remains on chromosome 17 is located between 79.60 cM and 92.87 cM . A substantial area of exclusion can be observed between 0.00 cM and 79.60 cM . A total of 103.96 cM of chromosome seventeen was excluded, which equals $82.2 \%$ of the entire chromosome. The highest LOD score seen for this chromosome was for D17S944 at 82.56 cM , which presented with a LOD score of 1.36 at $\theta=0.00$. Although this LOD score was less than the +3.00 required for evidence of linkage, it was one of the highest LOD scores observed, therefore it required further investigation (section 4.4.2).

### 4.3.18 Chromosome 18

The Research Genetics Inc. mapping set contained five markers for chromosome 18, three of which were tetranucleotide repeats, and the remaining two markers were dinucleotide. The Applied Biosystems mapping set contained 20 markers for chromosome 18. Table 4.21 lists the markers that were examined from both mapping sets, their positions (cM), maximum obtained LOD scores at theta equal to zero, the corresponding maximum excluded distances in cM , and the regions excluded. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set. Markers highlighted in green represent markers which were found in both mapping sets. The marker which has not been highlighted was ordered previously for investigation of linkage to the chromosome 18 locus (section 3.3.9).

Table 4.21: Markers used to examine chromosome 18.

| Marker | Position <br> $(\mathbf{c M})$ | Max LOD <br> $\mathbf{( \theta = 0 )}$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from $(\mathbf{c M})$ | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D18S59 | 0.00 | -5.63 | 0.60 | -0.60 | 0.60 |
| D18S63 | 8.30 | -5.57 | 1.50 | 6.80 | 9.80 |
| D18S976 | 12.88 | -5.81 | 4.11 | 8.77 | 16.99 |
| D18S452 | 18.70 | -2.07 | 0.00 | - | - |
| D18S464 | 31.17 | -4.81 | 7.45 | 23.72 | 38.62 |
| D18S53 | 41.24 | -1.79 | 0.00 | - | - |
| D18S542 | 41.24 | -1.82 | 0.00 | - | - |
| D18S877 | 54.40 | -1.49 | 0.00 | - | - |
| D18S1102 | 62.84 | -6.95 | 4.01 | 58.83 | 66.85 |
| D18S64 | 84.80 | -6.01 | 3.91 | 80.89 | 88.71 |
| D18S1357 | 88.62 | 0.68 | 0.00 | - | - |
| D18S68 | 96.48 | -4.57 | 1.32 | 95.16 | 97.80 |
| D18S61 | 105.03 | -8.38 | 6.74 | 98.29 | 111.77 |
| ATA82B04 | 106.81 | -1.49 | 0.00 | - | - |
| D18S1161 | 114.26 | -8.60 | 6.94 | 107.32 | 121.20 |
| D18S462 | 120.05 | -8.58 | 10.66 | 109.39 | 130.71 |
| D18S70 | 126.00 | -5.79 | 8.27 | 117.73 | 134.27 |

Seventeen markers from the two mapping sets were examined for chromosome 18. Twelve of the markers from the mapping sets obtained a statistically significant LOD score, and excluded various regions on the chromosome. Markers D18S452 presented with a significant LOD scores of <-2, however it was only significant at $\theta=0.00$. Therefore there was no distance of exclusion aside from the position of the marker itself. Marker D18S53, D18S542, D18S877, D18S1357 and ATA82B04 did not show significant LOD scores, and yielded inconclusive LOD scores of -1.79 at $\theta=0.00-1.82$ at $\theta=0.00,-1.49$ at $\theta=0.00,0.68$ at $\theta=0.00$ and -1.49 at $\theta=0.00$. Figure 4.18 illustrates the positions of the markers that obtained significant negative LOD scores and the corresponding regions of exclusion on chromosome 18.


Figure 4.19: Illustration of areas of exclusion for Chromosome 18
Black vertical line with scales represents chromosome 18 (scales in cM ). The red bars on the right illustrates the total areas excluded combining all markers from the two linkage set. The marker(s) which contributed to these exclusion values are also indicated.

These results exclude a substantial area of chromosome 18. A number of small gaps remain on chromosome 18, the largest gap only 7.48 cM , located between 38.62 cM and 58.83 cM . A total of 72.38 cM of chromosome 18 was excluded, which equates to $57.4 \%$ of the entire chromosome. The highest LOD score seen for this chromosome was for D18S1357 at 57.44 cM , which presented with a LOD score of 0.68 at $\theta=0.00$. Although this LOD score was less than the +3.00 required for evidence of linkage, it was one of the highest LOD scores observed, therefore it required further investigation.

### 4.3.19 Chromosome 19

The Research Genetics Inc. mapping set contained seven markers for chromosome 19 , five of which were tetranucleotide repeats, one a trinucleotide, and the remaining two markers were dinucleotide. The Applied Biosystems mapping set contained 20 markers for chromosome 19. Table 4.22 lists the markers that were examined from both mapping sets, their positions (cM), maximum obtained LOD scores at theta equal to zero, the corresponding maximum excluded distances in cM , and the regions excluded. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set. The marker which has not been highlighted was ordered previously for investigation of linkage to the chromosome 19 locus (section 3.3.6).

Table 4.22: Markers used to examine chromosome 19.

| Marker | Position <br> $(\mathbf{c M})$ | Max LOD <br> $(\boldsymbol{\theta = 0})$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from (cM) | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D19S209 | 10.97 | -0.71 | 0.00 | - | - |
| D19S216 | 20.01 | -2.62 | 0.00 | - | - |
| D19S1034 | 20.75 | -5.95 | 1.00 | 19.75 | 21.75 |
| D19S884 | 26.37 | -4.88 | 0.20 | 26.17 | 26.57 |
| D19S586 | 32.94 | -2.84 | 0.00 | - | - |
| D19S221 | 36.22 | 0.48 | 0.00 | - | - |
| D19S226 | 42.28 | -7.44 | 1.80 | 40.48 | 44.08 |
| D19S410 | 45.48 | -5.03 | 2.40 | 43.08 | 47.88 |
| D19S414 | 54.01 | -5.44 | 8.89 | 45.12 | 62.9 |
| D19S220 | 62.03 | -5.68 | 9.10 | 52.93 | 71.13 |
| D19S420 | 66.30 | -8.36 | 14.06 | 52.24 | 80.36 |
| D19S178 | 68.08 | -5.59 | 6.64 | 61.44 | 74.72 |
| D19S902 | 72.72 | -9.16 | 7.86 | 64.86 | 80.58 |
| D19S589 | 87.66 | -2.56 | 0.40 | 87.26 | 88.06 |
| D19S210 | 100.01 | -6.406 | 6.60 | 93.41 | 106.61 |

Fourteen markers from the two mapping sets were examined for chromosome 19. A separate marker had been ordered, investigated and previously discussed for the
investigation of the chromosome 19 RLS locus. This marker is included in the table above. Ten of the markers from the mapping sets obtained a statistically significant LOD score, and excluded various regions on the chromosome. Markers D19S216 and D19S586 presented with a significant LOD scores of <-2; however they were only significant at $\theta=0.00$. Therefore there was no distance of exclusion aside from the position of the marker itself. Marker D19S209 and D19S221 did not show significant LOD scores, and yielded inconclusive LOD scores of -0.71 at $\theta=0.00$ and 0.47 at $\theta=0.00$. Figure 4.19 illustrates the positions of the markers that obtained significant negative LOD scores and the corresponding regions of exclusion on chromosome 19.


Figure 4.20: Illustration of areas of exclusion for Chromosome 19
Black vertical line with scales represents chromosome 19 (scales in cM ). The red bars on the right illustrates the total areas excluded combining all markers from the two linkage set. The marker(s) which contributed to these exclusion values are also indicated.

These results exclude substantial areas on chromosome 19. A number of gaps remain on chromosome 19 , the largest gap located between 0.00 cM and 19.75 cM . A total of 56.50 cM of chromosome 19 was excluded, which equates to $53.8 \%$ of the entire chromosome. The highest LOD score seen for this chromosome was for D19S209 at 10.97 cM , which presented with a LOD score of 2.01 at $\theta=0.67$. Although this LOD score was less than the +3.00 required for evidence of linkage, it was the highest LOD score seen during the process of the genome-wide search and therefore further investigation of the region surrounding marker D19S209 was required.

### 4.3.20 Chromosome 20

The Research Genetics Inc. mapping set contained two markers for chromosome 20, one which was a tetranucleotide repeat, and one which was a dinucleotide repeat. The Applied Biosystems mapping set contained 20 markers for chromosome 20. Table 4.23 lists the markers that were examined from both mapping sets, their positions (cM), maximum obtained LOD scores at theta equal to zero, the corresponding maximum excluded distances in cM , and the regions excluded. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set. The markers which have not been highlighted were ordered previously for investigation of linkage to the Chromosome 20 locus (section 3.3.5).

Table 4.23: Markers used to examine chromosome 20.

| Marker | Position <br> $(\mathbf{c M})$ | Max <br> $(\boldsymbol{\theta = 0} \mathbf{~ L O D ~}$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from $(\mathbf{c M})$ | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D20S864 | 0.00 | -1.75 | 0.00 | - | - |
| D20S117 | 2.83 | -1.87 | 0.00 | - | - |
| D20S199 | 6.25 | -5.72 | 7.66 | -1.41 | 13.91 |
| D20S113 | 8.97 | 0.14 | 0.00 | - | - |
| D20S842 | 8.97 | -5.61 | 9.33 | -0.36 | 18.30 |
| D20S181 | 9.53 | -3.02 | 0.20 | 9.33 | 9.73 |
| D20S193 | 9.53 | 0.00 | 0.00 | - | - |
| D20S116 | 11.20 | -2.80 | 2.20 | 9.00 | 13.40 |
| D20S889 | 11.20 | -8.20 | 11.60 | 0.00 | 22.80 |
| D20S482 | 12.12 | -5.61 | 9.33 | 2.79 | 21.45 |
| D20S849 | 13.98 | -3.08 | 0.70 | 13.28 | 14.68 |
| D20S895 | 13.98 | 0.00 | 0.00 | - | - |
| D20S882 | 15.05 | -3.28 | 3.10 | 11.95 | 18.15 |
| D20S115 | 21.15 | -6.19 | 4.41 | 16.74 | 25.56 |
| D20S851 | 24.70 | -3.12 | 0.30 | 24.4 | 25.00 |
| D20S186 | 32.30 | -8.52 | 10.24 | 22.06 | 42.54 |
| D20S112 | 39.25 | -8.63 | 8.07 | 31.18 | 47.32 |
| D20S107 | 55.74 | -5.68 | 7.76 | 47.98 | 63.50 |
| D20S119 | 61.77 | -8.53 | 8.17 | 53.6 | 69.94 |
| D20S178 | 66.16 | -6.19 | 4.01 | 62.15 | 70.17 |
| D20S196 | 75.01 | -11.33 | 8.07 | 66.94 | 83.08 |
| D20S100 | 84.78 | -2.07 | 0.00 | - | - |
| D20S171 | 95.70 | -8.97 | 5.62 | 90.08 | 101.32 |
| D20S173 | 98.09 | -2.99 | 1.40 | 96.69 | 99.49 |
|  |  |  |  |  |  |

Fourteen markers from the two mapping sets were examined for chromosome 20.
Ten separate markers had been ordered, investigated and previously discussed for the investigation of the chromosome twenty RLS locus. These markers are included in the table above. Twelve of the markers from the mapping sets obtained a statistically significant LOD score, and excluded various regions on the chromosome. Marker D20S100 presented with a significant LOD scores of <-2, however it was only significant at $\theta=0.00$. Therefore there was no distance of exclusion aside from the position of the marker itself. Marker D20S928 did not show significant LOD scores, and yielded an inconclusive LOD scores of -1.87 at $\theta=0.00$. Figure 4.20 illustrates
the positions of the markers that obtained significant negative LOD scores and the corresponding regions of exclusion on chromosome 20.


Figure 4.21: Illustration of areas of exclusion for Chromosome 20
Black vertical line with scales represents chromosome 20 (scales in cM ). The red bars on the right illustrates the total areas excluded combining all markers from the two linkage set. The marker(s) which contributed to these exclusion values are also indicated.

The results of the genome-wide scan exclude a large amount of chromosome 20. Only two small gaps remain on the chromosome and these are positioned from 47.32 cM to 47.98 cM and from 83.08 cM to 101.32 cM . A total of 93.56 cM of chromosome 20 was excluded, which equals $92.4 \%$ of the entire chromosome. The highest LOD score seen for this chromosome was for D20S113 at 8.97 cM , which presented with a LOD score of 0.14 at $\theta=0.00$. This LOD score was not high enough to require further investigation.

### 4.3.21 Chromosome 21

The Research Genetics Inc. mapping set contained only one marker for chromosome 21, which was a GATA tetranucleotide repeat. The Applied Biosystems mapping set contained five markers for chromosome 21. Table 4.24 lists the markers that were examined from both mapping sets, their positions (cM), maximum obtained LOD scores at theta equal to zero, the corresponding maximum excluded distances in cM , and the regions exclude. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set.

Table 4.24: Markers used to examine chromosome 21.

| Marker | Position <br> $(\mathbf{c M})$ | Max LOD <br> $(\boldsymbol{\theta = 0})$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from (cM) | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D21S1914 | 19.39 | -8.38 | 11.50 | 7.89 | 30.89 |
| D21S263 | 27.40 | -8.68 | 6.84 | 20.56 | 34.24 |
| D21S1252 | 35.45 | -8.68 | 9.93 | 25.52 | 45.38 |
| D21S2055 | 40.49 | -5.23 | 5.62 | 34.87 | 46.11 |
| D21S266 | 45.87 | -5.49 | 4.01 | 41.86 | 49.88 |

Five markers were examined for chromosome 21. All five markers from the two mapping sets obtained statistically significant LOD scores. Figure 4.21 illustrates the positions of the markers that obtained significant negative LOD scores and the corresponding regions of exclusion on chromosome 21.


Figure 4.22: Illustration of areas of exclusion for Chromosome 21
Black vertical line with scales represents chromosome 21 (scales in cM ). The red bars on the right illustrates the total areas excluded combining all markers from the two linkage set. The marker(s) which contributed to these exclusion values are also indicated.

The results of the genome-wide scan excluded a large section of chromosome 21, between 7.89 cM and 49.88 cM . A 7.89 cM gap remains at the telomeric end of the short arm of chromosome 21. A 7.90 cM gap remains at the opposite end of chromosome 21 also. A total of 41.99 cM of chromosome 21 was excluded, which equates to $72.7 \%$ of the entire chromosome.

### 4.3.22 Chromosome 22

The Research Genetics Inc. mapping set contained only one marker for chromosome 22, which was a GCT trinucleotide repeat. The Applied Biosystems mapping set contained seven markers for chromosome 22. Table 4.25 lists the markers that were examined from both mapping sets, their positions (cM), maximum obtained LOD scores at theta equal to zero, the corresponding maximum excluded distances in cM , and the regions excluded are listed here. Markers highlighted in blue represent markers from the Research Genetics Inc. mapping set. Markers highlighted in pink represent markers from the Applied Biosystems mapping set.

Table 4.25: Markers used to examine chromosome 22.

| Marker | Position <br> $(\mathbf{c M})$ | Max LOD <br> $(\boldsymbol{\theta = 0})$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from $(\mathbf{c M})$ | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D22S420 | 4.06 | -8.68 | 8.38 | -4.32 | 12.44 |
| D22S539 | 14.44 | 0.19 | 0.00 | - | - |
| GCT10C10 | 18.10 | -0.66 | 0.00 | - | - |
| D22S315 | 21.47 | -4.79 | 6.33 | 15.14 | 27.80 |
| D22S280 | 31.30 | -1.48 | 0.00 | - | - |
| D22S283 | 38.62 | -7.39 | 9.10 | 29.52 | 47.72 |
| D22S423 | 46.42 | -7.55 | 9.10 | 37.32 | 55.52 |
| D22S274 | 51.54 | -1.47 | 0.00 | - | - |

Four out of the eight markers from the two mapping sets obtained statistically significant LOD scores. Figure 4.22 illustrates the positions of the markers that obtained significant negative LOD scores and the corresponding regions of exclusion on chromosome 22.


Figure 4.23: Illustration of areas of exclusion for Chromosome 22
Black vertical line with scales represents chromosome 22 (scales in cM ). The red bars on the right illustrates the total areas excluded combining all markers from the two linkage set. The marker(s) which contributed to these exclusion values are also indicated.

These results exclude a large area of chromosome 22. Three gaps are present on the chromosome and are positioned between: 12.44 cM and $15.14 \mathrm{cM} ; 27.80 \mathrm{cM}$ and 29.52 cM ; and 55.52 cM and 62.32 cM . A total of 55.10 cM of chromosome 22 was excluded, which equates to $82 \%$ of the entire chromosome. The highest LOD score seen for this locus was for D22S539, which presented with a LOD score of 0.19 at $\theta=0.00$. This LOD score was not high enough to require further investigation.

### 4.4 Areas of further Investigation

### 4.4.1 Chromosome 1

Marker D1S484 (169.69 cM) which was part of the Applied biosystems mapping set obtained a positive LOD score of 1.69 at $\theta=0.00$. Although this LOD score is inconclusive, it was one of the highest LOD scores seen throughout the genomewide scan; therefore it was necessary to investigate the region further. Marker D1S2878 ( 177.86 cM ) which was also part of the Applied biosysystems mapping set is positioned 8.17 cM away from D1S484. Marker D1S2878 obtained a LOD score of -10.62 at $\theta=0.00$, and excluded linkage from 168.35 cM to 187.37 cM . Therefore the region where D1S484 is positioned was excluded for linkage to RLS. Nevertheless to ensure that RLS is not linked to this region a haplotye analysis was carried out. The haplotype analysis was carried out using three other markers from the mapping sets. One marker was positioned distal to D1S484 and the other two were positioned further along chromosome 1. From looking at the haplotype analysis (figure 4.23), it becomes clear that there is no haplotype that is present in all the affected patients and not present in the unaffected individuals. So due to the fact that there is exclusion of the region and that haplotype analysis confirms exclusion of this region on chromosome 1 , it can be safely assumed that the region surrounding marker D1S484 is not linked to RLS in this family.


Figure 4.24: Haplotype analysis of RLS3002 pedigree for chromosome 1 region A list of the examined markers is present on the left hand side. The alleles for each individual obtained from genotyping using these markers are listed below each individual. Each haplotype formed from the series of alleles is given a coloured bar so it can be recognised easily. All the coloured haplotypes are present in both affected (filled squares and circles) and unaffected (squares and circles containing the letter N) individuals. Hence there is no one shared haplotype between all the affected individuals that is absent in normal individuals. It is therefore possible to conclude that there is no linkage between RLS and the region surrounding marker D1S484 on chromosome 1.

### 4.4.2 Chromosome 10

Marker D10S548 ( 45.70 cM ) which was part of the Applied biosystems mapping set showed a LOD score of 1.71 at $\theta=0.00$. This LOD score was inconclusive however it was necessary to investigate it further as it was close to the value which would suggest linkage. Marker D10S548 is positioned at 45.70 cM on chromosome 10. This region was later excluded by marker D10S1423, which was also part of the Applied Biosystems mapping set. Marker D10S1423 ( 46.23 cM ) is positioned only 0.53 cM away from marker D10S548, and showed a LOD score of -5.67 at $\theta=0.00$, excluding from 39.39 cM to 53.07 cM . Therefore the position at which D10S548 is situated was excluded for linkage. To ensure that RLS is not linked to this region a haplotye analysis was carried out. Looking at the haplotype analysis (figure 4.24), it becomes clear that there is no haplotype that is present in all the affected patients and not present in the normal individuals. So due to the fact that there is exclusion of the region and that haplotype analysis confirms exclusion of this region on chromosome 10 , it can be safely assumed that the region surrounding marker D10S548 is not linked to RLS in this family.


Figure 4.25: Haplotype analysis of RLS3002 pedigree for chromosome 10 region A list of the examined markers is present on the left hand side. The alleles for each individual obtained from genotyping using these markers are listed below each individual. Each haplotype formed from the series of alleles is given a coloured bar so it can be recognised easily. All the coloured haplotypes are present in both affected (filled squares and circles) and unaffected (squares and circles containing the letter N) individuals. Hence there is no one shared haplotype between all the affected individuals that is absent in normal individuals. It is therefore possible to conclude that there is no linkage between RLS and the region surrounding marker D10S548 on chromosome 10.

### 4.4.3 Chromosome 17

Marker D17S944 ( 83.56 cM ) which was part of the Applied Biosystems mapping set yielded a LOD score of 1.36 at $\theta=0.00$. This area was not excluded by any other marker in the mapping sets. To ensure that RLS is not linked to this region a haplotye analysis was carried out. From looking at the haplotype analysis (figure 4.25), it becomes clear that there is no haplotype that is present in all the affected patients and not present in the normal individuals. Therefore, due to the fact that haplotype analysis confirms exclusion of this region on chromosome 17, it can be safely assumed that the region surrounding marker D17S944 is not linked to RLS in this family.


Figure 4.26: Haplotype analysis of RLS3002 pedigree for chromosome 17 region A list of the examined markers is present on the left hand side. The alleles for each individual obtained from genotyping using these markers are listed below each individual. Each haplotype formed from the series of alleles is given a coloured bar so it can be recognised easily. All the coloured haplotypes are present in both affected (filled squares and circles) and unaffected (squares and circles containing the letter N ) individuals. Therefore, there is no one shared haplotype between all the affected individuals that is absent in normal individuals.

### 4.4.4 Chromosome 18

Marker D18S1357 (88.62 cM) which was part of the Research Inc. mapping set yielded a LOD score of 0.68 at $\theta=0.00$. This area was not excluded by any other marker in the mapping sets; therefore it was necessary to investigate this area further. Four additional markers in the area were chosen and ordered. Table 4.26 lists the markers that were ordered. Their positions (cM), size (bp), and the fluorescent tags with which they were labelled are listed here.

Table 4.26: Markers used to examine the area surrounding D18S1357.

| Marker | Position (cM) | Size (bp) | Fluorescent label |
| :--- | :--- | :--- | :--- |
| D18S1134 | 88.62 | $204-232$ | 6-FAM |
| D18S1148 | 90.60 | $132-154$ | 6-FAM |
| D18S1147 | 90.60 | $207-219$ | HEX |
| D18S465 | 100.11 | $232-251$ | 6-FAM |

The four microsatellite markers surrounding marker D18S1357 were examined. Two of the four markers did not yield a statistically significant LOD score (i.e. <-2 or >3). Table 4.27 lists the markers that were examined, the LOD scores obtained at $\theta=0.00$ and the corresponding excluded regions. Their positions (cM), maximum obtained LOD scores at theta equal to zero, the corresponding maximum excluded distances in cM , and the regions excluded are listed here.

Table 4.27: Markers used to examine the area surrounding D18S1357.

| Marker | Position <br> $(\mathbf{c M})$ | Max LOD <br> $\mathbf{(}=\mathbf{0})$ | Excluded <br> (cM) | Area <br> Excluded <br> from $(\mathbf{c M})$ | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D18S1134 | 88.62 | -2.04 | 0.10 | 88.52 | 88.72 |
| D18S1148 | 90.60 | -4.96 | 0.00 | - | - |
| D18S1147 | 90.60 | -1.49 | 0.00 | - | - |
| D18S465 | 100.11 | -1.76 | 0.00 | - | - |

The markers were not successful in excluding the area in question. The marker D18S1134 (88.62 cM) showed a significant LOD score of -2.04 at $\theta=0.00$ and excluded 0.10 cM either side. The marker D18S1148 showed a significant score however it was only significant at $\theta=0.00$. Hence there was no distance of exclusion except at the position of the marker. Therefore the region in question has not been excluded by the extra markers ordered in this area. However by looking at the haplotype analysis (figure 4.26), it becomes clear that there is no haplotype that is present in all the affected patients and not present in the normal individuals. So due to the fact that there is exclusion of D18S1148 at the exact position it is located and that haplotype analysis confirms exclusion of this region on chromosome 18, it can be safely assumed that the region surrounding marker D18S1357 is not linked to RLS in this family.


Figure 4.27: Haplotype analysis of RLS3002 pedigree for chromosome 18 region A list of the examined markers is present on the left hand side. The alleles for each individual obtained from genotyping using these markers are listed below each individual. Each haplotype formed from the series of alleles is given a coloured bar so it can be recognised easily. All the coloured haplotypes are present in both affected (filled squares and circles) and unaffected (squares and circles containing the letter N ) individuals. There is no one shared haplotype between all the affected individuals that is absent in normal individuals. Therefore, there is no linkage between RLS and the region surrounding marker D18S1357 on chromosome 18.

### 4.4.5 Chromosome 19

Marker D19S209 (10.97 cM) on chromosome 19, which was part of the Applied Biosystems mapping set yielded a LOD score of 2.01 at $\theta=0.67$. This was the highest LOD score seen during the process of the genome-wide search and suggested linkage of RLS to the region. The nearest marker D19S216 (20.01 cM) was positioned almost 10.00 cM away and although it showed a significant LOD score of -2.62 at $\theta=0.00$, it was only significant at this point. Hence, there was no distance of exclusion except at the position of the marker. The area surrounding D19S209 was not excluded by any other marker; therefore it was necessary to investigate this area further.

The highest LOD score for D19S209 was observed at $\theta=0.67$. This indicates that the area of interest is approximately 6.00 cM away from the marker, which is positioned at 10.97 cM . Therefore our area of interest was at approximately 5.00 cM or at 17.00 cM . This was important information for ordering additional markers. Four additional markers in the area were chosen; the first positioned at 6.57 cM , the second positioned at 15.55 cM , a third positioned close to D 19 S 209 at 9.84 cM and a final marker positioned at the telomere of chromosome 19. Table 4.28 lists the markers that were ordered. Their positions (cM), size (bp), and the fluorescent tags with which they were labelled are also listed.

Table 4.28: Markers used to examine the area surrounding marker D19S209.

| Marker | Position (cM) | Size (bp) | Fluorescent label |
| :--- | :--- | :--- | :--- |
| D19S886 | 0.00 | $134-158$ | 6-FAM |
| D19S878 | 6.57 | $208-230$ | 6-FAM |
| D19S591 | 9.84 | $96-112$ | 6-FAM |
| D19S894 | 15.55 | $144-168$ | HEX |

The four markers surrounding marker D18S1357 were examined. Only one of the four markers yielded a statistically significant LOD score (i.e. <-2 or >3). Table 4.29 lists the markers that were examined Their positions (cM), maximum obtained LOD scores at theta equal to zero, the corresponding maximum excluded distances in cM , and the regions excluded.

Table 4.29: Markers used to examine the area surrounding D18S1357.

| Marker | Position <br> $(\mathbf{c M})$ | Max LOD <br> $(\boldsymbol{\theta}=\mathbf{0})$ | Excluded <br> $(\mathbf{c M})$ | Area <br> Excluded <br> from $(\mathbf{c M})$ | Area <br> Excluded to <br> $(\mathbf{c M})$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| D19S886 | 0.00 | 1.87 | 0.00 | - | - |
| D19S878 | 6.57 | 3.59 | 0.00 | - | - |
| D19S591 | 9.84 | 1.81 | 0.00 | - | - |
| D19S894 | 15.55 | -5.44 | 0.60 | 14.95 | 16.15 |

These results provide evidence of linkage to chromosome 19. Marker D19S878 yielded a maximum LOD score of 3.59 at $\theta=0.00$. A LOD score of $>3$ is required to be statistically significant. It was proposed that the locus would be positioned approximately 6.00 cM away from marker D19S209, which is located at 10.97 cM . Marker D19S878 is positioned 4.40 cM from D19S209 which is in the approximate location estimated. The marker D19S894 chosen to be 6.00 cM in the other direction away from D19S209, yielded a LOD score of -5.44 and excluded 0.60 cM either side of the position of the marker. The other two markers examined did not show statistically significant LOD scores, however both had LOD scores of approximately 1.80 at $\theta=0.00$, which would have been questioned if found independently. Furthermore, by looking at the haplotype analysis (figure 4.27), it becomes obvious that there is one haplotype (red bar) that is present in all the affected individuals and not present in the unaffected individuals. Therefore following further investigation, it can be concluded that this region on chromosome 19 is linked to RLS in our Irish RLS3002 pedigree.


Figure 4.28: Haplotype analysis of RLS3002 pedigree for chromosome 19 region A list of the examined markers is present on the left hand side. The alleles for each individual obtained from genotyping using these markers are listed below each individual. Each haplotype formed from the series of alleles is given a coloured bar so it can be recognised easily. The red bar (haplotype) is in all affected individuals (filled squares and circles) and a green or blue bar (haplotype) is present in the unaffected (squares and circles containing the letter N ) individuals. Hence there is a shared haplotype between all the affected individuals that is absent in normal individuals. It is therefore possible to conclude that there is linkage between RLS and the region on chromosome 19.

### 4.5 Discussion

Following exclusion of pedigree RLS3002 to all known RLS loci and associated regions it was necessary to conduct a genome-wide search. The aim of this approach was to find out the rough location of the causative gene relative to a microsatellite marker, which has it position already known. The first mapping set used was the Research Genetics Inc. mapping set. This mapping set consisted of microsatellite markers spaced approximately every 20 cM along the entire genome. The majority of the markers in this set were tetranucleotide. Following completion of this mapping set, no positive LOD score of $>3.00$ was observed. Therefore it was necessary to continue the genome-wide search.

For the second mapping set, it was decided that a mapping set with greater coverage was necessary. Therefore the Applied Biosystems mapping set version 2.5-MD10 was used. This mapping set has approximately 400 microsatellite markers spaced at 10 cM intervals along the genome. This mapping set also had an average heterozygosity of 0.79 .

Subsequently four areas of interest were found and required further investigation. The first region of interest was observed on chromosome 1 where marker D1S484 showed a LOD score of 1.69 at $\theta=0.67$. This region was excluded for linkage by marker D1S2878, and a haplotype analysis confirmed that RLS3002 was not linked to this region.

The second area of interest was on chromosome 10, where a LOD score of 1.71 at $\theta=0.00$ was observed for marker D10S548. This region had also been excluded by
marker D10S1423, however a haplotype analysis confirmed pedigree RLS3002 was not linked to this region.

The third area which was investigated further was a region on chromosome 18. Marker D18S1357 showed a LOD score of 0.68 at $\theta=0.00$, and although this value was inconclusive, a large area surrounding the marker was not excluded for linkage and therefore required further investigation. Four additional markers surrounding D18S1357 were ordered and investigated. The markers were mostly inconclusive, however following haplotype analysis it was clear that pedigree RLS3002 was not linked to this.

A positive LOD score of 2.01 at $\theta=0.67$ obtained for marker D19S209 suggested linkage of RLS to the region on chromosome 19. This was the fourth area which required investigation. Four adjacent markers were ordered and examined. Linkage analysis of marker D19S878 yielded a LOD score of 3.59 at $\theta=0.00$, thereby providing evidence of linkage to this region. SLINK simulation analysis indicates that the maximum LOD score achievable with this pedigree is 3.41 at $\theta=0.01$. To confirm that this region on chromosome 19 is linked to RLS in pedigree RLS3002, a haplotype analysis was performed. For the haplotype analysis the four newly ordered markers and two markers from the Applied Biosystems mapping set were used. These markers were located between 0.00 cM and 20.01 cM on chromosome 19 . Haplotype analysis of the genotype data revealed a disease haplotype that was present in all 11 affected members. Therefore the haplotype analysis confirmed that this region was linked to RLS in our Irish pedigree.

## Chapter 5

## Fine-Mapping Chromosome 19

RLS locus

### 5.1 Introduction

Linkage to RLS in our Irish pedigree RLS3002 was confirmed to chromosome 19p. Marker D19S878 yielded a LOD score of 3.59 at $\theta=0.00$, thereby providing evidence of linkage to this region. Haplotype analysis of the region also confirmed linkage. Initial detection of a genetic locus generally does not yield much precision on its location, thus leaving too vast an area to explore for candidate genes. It is therefore necessary to refine the location of the genetic determinants. Localisation or finemapping is the process of refining the location. Studies which have an increased number of meiosis between individuals, narrowing the expected preserved segment lengths and thus increasing the resolution, are most advantageous. Fine-mapping of a region can be achieved in a number of ways, the most common being; increasing marker density within the chromosomal area of interest or by increasing the number of individuals for which phenotype information can be obtained. Since all known members of the RLS3002 have been recruited and used for this study, it was necessary to identify microsatellite markers that were very tightly linked to the targeted area and thereby used to further refine the susceptibility region.

The region in question was a marker poor region; therefore, it was necessary to identify novel microsatellite markers in the region. This was essential for generation of a more detailed haplotype. In order to design more microsatellite markers it was necessary to examine DNA sequences in databases for clusters of short tandemly repeated nucleotide bases. The areas which were targeted were areas in which no known microsatellite markers were present. For this study repeats which were greater than 10 units long were considered for fine-mapping. Following
identification of short tandem repeats in the desired area, primers were designed flanking the area of interest (see section 2.7 for primer design).

### 5.2 Materials and Methods

### 5.2.1 Microsatellite marker selection

The area on chromosome 19 which was found to be linked to RLS in this Irish family required further examination in order to refine the region.

## Known Microsatellite Markers

Following a search of genetic databases www.ncbi.nlm.nih.gov and www.genome.ucsc.org, only five other microsatellite markers in the region were identified. These microsatellite markers were D19S883, D19S565, D19S247, D19S120 and D19S424. The marker located nearest to the telomere was D19S883, which is located at 5.54 cM . There was no microsatellite marker in the databases between the telomere and 5.54 cM . In an attempt to refine this novel region it was necessary to design novel microsatellite markers.

## Novel Microsatellite Markers

This region is a marker poor region, and it was therefore necessary to create additional microsatellite markers in the region. The regions which were marker poor and had no documented microsatellite markers were identified. The main region was located between the telomere and marker D19S883 ( 5.54 cM ). Figure 5.1 shows this region (1-1,454,490 bp). The map shows genetic STS markers which have a red line beside their name, and radiation hybrid markers which have a black line beside their name. Only two genetic markers were observed in this map, and they were AFMA319WD9 (D19S886) and AFMA299YC1 (D19S883), which is highlighted in the figure. Microsatellite marker D19S886 had been previously used (chapter 4).

D19S886 is located at $998,664 \mathrm{bp}$ on chromosome 19 , therefore there was nearly a $1,000,000 \mathrm{bp}$ region which was devoid of microsatellite markers. The aim was to attempt to design novel microsatellite markers in this region.

# UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly 





Figure 5.1:. Chromosome 19p13.3. microsatellite poor regions
This figure shows chromosome 19 ideogram with a red box indicating the location of the area shown. The area shown is $1-1,454,490 \mathrm{bp}$. Below the ideogram is a list of all STS genetic marker (red) and radiation hybrids (black). The highlighted marker is AFMA299YC1, also known as D19S883. There is a microsatellite poor region between the telomere and marker AFMA310WD9 (D19S886). UCSC Genome Browser on Human, Feb 2009 Assembly (http://genome.ucsc.edu).

Furthermore, the highest LOD score to date was 3.59 at $\theta=0.00$ for Microsatellite marker D19S878 (section 4.4.2). A recombination event was observed in PATH053 between marker D19S878 and D19S209 (10.97 cM) (figure 4.27). Therefore it was necessary to investigate the region proximal to microsatellite marker D19S878 in the hope of reducing the locus further (Fig. 5.2).


Figure 5.2: Region proximal to marker D19S878 on Chromosome 19p13.3.
This figure shows chromosome 19 ideogram with a red box indicating the location of the area shown. The area shown is $12,290,900-2,768,109 \mathrm{bp}$. Below the ideogram is a list of all STS genetic markers (red) and radiation hybrids (black). Two microsatellite markers are observed, AFMA282VE9 (D19S878) and AFMA052WB9 (D19S565). It is the region proximal to D19S878 which requires further investigation. UCSC Genome Browser on Human, Feb 2009 Assembly (http://genome.ucsc.edu).

The sequence of the microsatellite poor regions of interest were copied and pasted into EditSeq and the sequence was searched for multiple GA, GATA, CA, and CAG etc repeats. If a repeat was located the number of repeats were counted and if it consisted of more than ten repeats, it was accepted. One could not be certain it these regions were polymorphic but it was assumed that if there were at least 10 repeat units there was some evidence that the region might be polymorphic. Primers were designed for these region as per primer design (section 2.8).

### 5.2.2 Calculation of Genetic position

A method was required to calculate the genetic position of microsatellite markers which were not found in the database and were designed by relying on the position of known microsatellite repeats in the region. Linear interpolation based on physical positions was used to estimate the genetic position (Levchenko, Montplaisir et al. 2008). Figure 5.3 represents the genetic map and the physical map where the new microsatellite marker (B) is located. Microsatellite markers A and C are known microsatellite markers for which their genetic and physical position is known. The physical location of marker B is known, however the genetic position requires calculation.


Figure 5.3: Genetic and physical maps required when determining the genetic distance of marker $B$
The black vertical lines represent a chromosome, with relative positions at markers A, B and C. A1, B1 and C1 represent the genetic positions of markers A, B and C indicated. A2, B2 and C 2 represent the physical positions of markers $\mathrm{A}, \mathrm{B}$ and C respectively. Marker B represents the microsatellite marker with an unknown genetic location. Using the known locations of the other markers and the known physical location of marker B, a formula is used to find the genetic position of marker B.

To find the genetic location of marker B, the following formula was used:

$$
\frac{B 2-A 2}{C 2-A 2}=\frac{x}{C 1-A 1}, \text { where } x=B 1-A 1
$$

The relative genetic distance ' $x$ ' is then added to the known genetic position of marker A1.

### 5.2.3 Genotyping

Genotyping of novel and known microsatellite markers to fine-map the chromosome 19 RLS locus was performed as per section 2.5.

### 5.2.4 Haplotype analysis

Haplotype analysis of the novel chromosome 19 locus was carried out according to section 2.7.

### 5.2.5 Multipoint Linkage Analysis

Multipoint linkage analysis is commonly used to evaluate linkage of a disease to multiple markers in a small region. It is an extension of two-point linkage analysis, where linkage of the disease trait is tested not to just a single microsatellite marker, but to a map of markers. The analysis usually involves three or more linked markers. In general multipoint linkage analysis is not used in the testing phase of a study, i.e. when using highly polymorphic markers to find linkage. Multipoint Linkage analysis is more beneficial when estimating the location of a linked disease gene.

In multipoint analysis, linkage analysis is carried out for a set of markers that are linked to each other against a disease. It is assumed that the marker order and the intermarker distances are known. The disease is then placed in each marker interval, as well as left and right of the leftmost and right most markers. Subsequently, the likelihood is computed for all possible positions of the trait locus in the considered chromosomal region. One can then estimate the position of the disease gene along a chromosomal region (Ziegler A 2010). Multipoint linkage analysis is more complex than two-point analysis. The order of the loci now matters. Furthermore the number of parameters, haplotypes, and genotypes increases drastically with the number of loci considered.

As before, the disease gene is an autosomal dominant disease with disease allele frequency of 0.01 and disease allele frequency of 0.95 . For this study, LINKMAP was used for multipoint linkage analysis (ftp://linkage.rockefeller.edu/software/linkage). LINKMAP performs the calculations by allowing the user to fix the order and spacing of two or more of the markers. Likelihoods of the pedigree data are then calculated allowing the locus
whose position is unknown to be placed in all possible intervals. The software MAPFUN was utilized to convert between recombination fractions and morgans. A graph is used to interpret the results. From the graph it is possible to estimate the location of the disease gene.

Multipoint Linkage analysis requires a number of essential steps:

1. A batch file is prepared using LCP.
2. Recompile the LINKMAP program if necessary. The constants may need to be modified to suit the problem which is under consideration.
3. The analysis is performed.
4. The program does not produce the actual multipoint LOD scores, therefore it is necessary to calculate the multipoint LOD scores. The program LINKLODS will perform this calculation.
5. Convert the recombination fractions to map distance using MAPFUN.
6. Graph the results. The $y$ axis is the multipoint lod score and the $x$ axis is the map distance.
7. The graph is used to interpret the location of the disease gene.

There are two major advantages to multipoint LOD score analysis. The first is that it offers an opportunity to impute the genotype information at an original uninformative locus via haplotype information. Consequently, the linkage results are less sensitive to the uninformative or missing genotype at any given marker. Secondly, multipoint linkage analysis can be extremely useful to pintpoint a disease gene location in the fine-mapping of a Mendelian disorder (Haines J.L 1998). This can be accomplished by evaluating many positions $Z(x)$ on a fixed map. At a certain location (x), any true recombinant will contribute a strongly negative LOD score and decrease the possibility that his location contains a candidate locus. Thus this will help to further define the candidate region. On the other hand, the position where the
maximum multipoint LOD score is observed can pinpoint the location of the causative gene and can be used as a guide for selecting candidate genes.

### 5.3 Results

### 5.3.1 Novel Microsatellite Markers

The newly designed primers, their location (bp), the forward and reverse primer used, the fluorescent tag used to label the forward primer, and the expected size of the product are detailed in Table 5.1. Their physical positions (bp), primers used for amplification, the fluorescent tag, and the annealing temperature are listed here.

Table 5.1. List of newly designed microsatellite markers used to fine-map the chromosome 19 RLS locus.

| Marker | Location <br> (bp) | Primers | Fluorescent <br> Tag | Product <br> Size <br> (bp | Annealing <br> Temp ${ }^{\circ} \mathbf{C}$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
| CA19S322F | 322,195 | agacgctttcaaaactcaccact | HEX | 326 | $*$ |
| CA19S322R |  | gggcacccacctcagacat |  |  |  |
| AC19S189F | 189,592 | taagtgatccccatgcctctgtct | 6-FAM | 407 | $*$ |
| AC19S189R |  | aacatggtgaaaccccgtctctac |  |  |  |
| CA19S872F | 872,286 | acagtgcaaatgcccatcaatcaa | 6-FAM | 411 | 67 |
| CA19S872R |  | gtggcgccgtctcacctgaa |  |  |  |
| GT19S949F | 949,752 | ctccttcgtccetcccagtc | 6-FAM | 271 | 68 |
| GT19S949R |  | ctcccgacggtgtctccccagat |  |  |  |
| ATA19S1001F | $1,001,575$ | cctggctaacatggtgaaacagtg | 6-FAM | 409 | 67 |
| ATA19S1001R |  | gacgtcgtggcccaggatgaag |  |  |  |
| AC19S1117F | $1,117,411$ | ccctccacatggattgaagacaca | 6-FAM | 414 | 67 |
| AC19S1117R |  | cagggtggtgcggctttctgt |  |  |  |
| CA19S2310F | $2,310,554$ | cttcccgggcaacatggtgagac | 6-FAM | 270 | 67 |
| CA19S2310R |  | agggcgtttctagggcactggtg |  |  |  |
| ATA19S2359F | $2,359,877$ | aaaaataaagtgccgggtgtggt | 6-FAM | 436 | $*$ |
| ATA19S2359R |  | ctcaggaggctaaggcaggagaat |  |  |  |
| GT19S2518F | $2,518,246$ | ttggccgtgatcattcctgtagtg | HEX | 290 | 68 |
| GT19S2518R |  | cctgggcaaaatggcgaaacc |  |  |  |

* represents markers which failed to amplify, therefore the annealing temperature remains unknown.


### 5.3.2 Calculation of Genetic positions

Using the formula described above, the genetic positions were calculated for the new microsatellite marker. Below is an example of how this formula was used to calculate the genetic distance of marker AC19S1117 located at 1,117,411bp on chromosome 19.


Genetic Map
Physical Map

Figure 5.4: Calculating the genetic position of marker AC19S1117
The black horizontal lines represent chromosome 19. A1, B1 and B2 represent the genetic positions of markers D19S886, AC19S1117 and D19S883 respectively. A2, B2 and B3 represent the physical positions of markers D19S886, AC19S1117 and D19S883 respectively. Marker B represents the microsatellite marker AC19S1117 with an unknown genetic location. Using the known locations of the other markers and the known physical location of marker AC19S1117, a formula is used to find the genetic position of AC19S1117.

Using the formula the genetic position of marker AC19S1117 is calculated.

$$
\frac{B 2-A 2}{C 2-A 2}=\frac{x}{C 1-A 1}, \text { where } x=B 1-A 1
$$

$\underline{1,117,411 \mathrm{bp}-949,644 \mathrm{bp}=\underline{\mathrm{X}}}$
$1,364,757 \mathrm{bp}-949,644 \mathrm{bp} \quad 5.54 \mathrm{cM}-0.00 \mathrm{cM}$
$\underline{167,767} \mathrm{bp}=\underline{\mathrm{X}}$
$415,113 \mathrm{bp} \quad 5.54 \mathrm{cM}$
$\mathrm{X}=2.23 \mathrm{cM}$

The relative genetic distance ' X ' is then added to the known genetic position of marker A1.
$0.00 \mathrm{cM}(\mathrm{A} 1)+2.23 \mathrm{cM}(\mathrm{X})=2.23 \mathrm{cM}$

Therefore marker AC19S1117 is located at 2.23 cM on chromosome 19. The remaining new markers were calculated for genetic position. Table 5.2 lists the known markers that were used to calculate the genetic position (cM) of the novel microsatellite markers. As this was a marker poor region, there were no microsatellite markers located at the region distal to markers CA19S322, AC19S189 and CA19S872. Therefore a superficial marker ' X ' was used which had a physical location at 0.00 bp and a genetic position at 0.00 cM . In table 5.2 , Marker B represents the novel microsatellite marker for which the genetic location is unknown and the physical location (B2) is known. Markers A and B represent known marker for which the genetic location (A1 and B1) and the physical location (A2 and B2) is known. Marker ' X ' represents the superficial marker used to calculate the genetic position of novel microsatellite markers CA19S322, AC19S189 and CA19S872.

Table 5.2: Known markers used for calculating the genetic position of novel microsatellite markers used for fine mapping the chromosome 19 locus.

| Marker (B) | Known <br> Marker <br> A | Known <br> Marker <br> C | A1 <br> $(\mathbf{c M})$ | A2 (bp) | B2 (bp) | C1 <br> $(\mathbf{c M})$ | C2 (bp) |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| CA19S322 | X | D19S886 | 0.00 | 0 | 322,195 | 0.00 | 949,644 |
| AC19S189 | X | D19S886 | 0.00 | 0 | 872,284 | 0.00 | 949,644 |
| CA19S872 | X | D19S886 | 0.00 | 0 | 872,390 | 0.00 | 949,644 |
| GT19S949 | D19S886 | D19S883 | 0.00 | 949,644 | 949,752 | 5.54 | $1,364,757$ |
| ATA19S1001 | D19S886 | D19S883 | 0.00 | 949,644 | $1,001,575$ | 5.54 | $1,364,757$ |
| AC19S1117 | D19S886 | D19S883 | 0.00 | 949,644 | $1,117,411$ | 5.54 | $1,364,757$ |
| CA19S2310 | D19S883 | D19S878 | 5.54 | $1,364,757$ | 2,310697 | 6.57 | $2,310,697$ |
| GT19S2359 | D19S878 | D19S565 | 6.57 | $2,310,697$ | $2,359,877$ | 6.57 | $2,518,075$ |
| GT19S2518 | D19S565 | D19S591 | 6.57 | $2,518,075$ | $2,518,246$ | 9.84 | $3,026,844$ |

The genetic positions of the novel microsatellite markers were calculated using the figures in Table 5.2. The results of these calculations, along with the physical and genetic positions of known microsatellite markers are detailed in Table 5.3. This table lists their physical position (bp) and Genetic position (cM).

Table 5.3: Genetic positions of known and novel microsatellite markers examined for fine-mapping chromosome 19 RLS locus.

| Marker | Physical position (bp) | Genetic position (cM) |
| :--- | :--- | :--- |
| CA19S322 | 322,195 | 0.00 |
| AC19S189 | 872,284 | 0.00 |
| CA19S872 | 872,390 | 0.00 |
| GT19S949 | 949,644 | 0.00 |
| ATA19S1001 | $1,001,575$ | 0.69 |
| AC19S1117 | $1,117,411$ | 2.23 |
| D19S883 | $1,413,918$ | 5.54 |
| CA19S2310 | $2,310,554$ | 6.57 |
| GT19S2359 | $2,359,877$ | 6.57 |
| GT19S2518 | $2,518,246$ | 6.57 |
| D19S565 | $2,567,233$ | 6.57 |
| D19S247 | $3,139,982$ | 9.84 |
| D19S120 | $3,189,343$ | 10.97 |
| D19S424 | $3,226,373$ | 10.97 |

### 5.3.3 Genotyping results

Fourteen microsatellite markers were ordered for fine-mapping the chromosome 19 locus, nine of which were novel microsatellite markers. These microsatellites were newly designed. It was not known if these markers were polymorphic and therefore if they were going to be informative. Six of the nine novel microsatellite markers were amplified, all of which were polymorphic, and provided LOD scores. This was a fantastic result, as these were newly designed primers, and it was not known if the novel microsatellite markers would firstly amplify and secondly be polymorphic. Of the known microsatellite markers four out of five amplified and provided LOD scores. Four out of the fourteen known and novel markers ordered for fine-mapping the chromosome 19 locus were not informative: CA19S322, AC19S189, D19S883 and ATA19S2359. These microsatellite marker did not produce specific bands on agarose gel electrophoresis. Standard procedures were carried out to attempt to amplify these markers e.g. range of annealing temperatures, range of $\mathrm{Mg}^{2+}$ concentrations, and primers were re-suspended. Nonetheless, these markers failed to amplify.

As previously mentioned ten of the fourteen microsatellite markers were successfully amplified for the chromosome 19 RLS locus. Table 5.3 lists all the markers examined and outlines their genetic positions (cM), the LOD score obtained at $\theta=0.00$, the corresponding maximum excluded distances in cM .

Table 5.4: Markers used to fine-map the chromosome 19 RLS locus.

| Marker | Position $(\mathbf{c M})$ | Max LOD $(\boldsymbol{\theta = 0})$ | Excluded cM |
| :--- | :--- | :--- | :--- |
| CA19S872 | 0.00 | 2.47 | 0.00 |
| GT19S949 | 0.00 | 2.12 | 0.00 |
| ATA19S1001 | 0.69 | 2.16 | 0.00 |
| AC19S1117 | 2.23 | 2.46 | 0.00 |
| CA19S2310 | 6.57 | 3.46 | 0.00 |
| D19S565 | 6.57 | -0.78 | 0.00 |
| GT19S2518 | 6.57 | -0.89 | 0.00 |
| D19S247 | 9.84 | 0.92 | 0.00 |
| D19S120 | 10.97 | -1.66 | 0.00 |
| D19S424 | 10.97 | -1.72 | 0.00 |

Of the ten microsatellite markers which worked, one of them obtained a statistically significant LOD score of $>3$, thereby confirming linkage. None of the microsatellite markers showed a negative LOD score of <-2, therefore there was no area of exclusion.

### 5.3.4 Haplotype Analysis

From these results it is not possible to determine the refined candidate region. In order to determine this region a haplotype for the chromosome 19 locus was reconstructed with the new microsatellite markers added. All 11 affected individuals carried a common haplotype which was identified by markers CA19S872, D19S886, GT19S949, AC19S1117, CA19S2310, and D19S878. Although individual PATH208 is unaffected, he does share part of the affected haplotype (figure 5.6).

A recombination event between markers D19S878 and D19S565 in individual PATH053 allowed for the defining of the RLS region. Both marker D19S878 and D19S565 are located at 6.57 cM , however D19S878 is located at 2.3 Mb and D19S565 is located at 2.5 Mb on chromosome 19. Therefore a recombination event took place somewhere within this 0.2 Mb region. Haplotype reconstruction allowed determination of the overall candidate region which is flanked by the telomere and microsatellite marker D19S565. This candidate region has a genetic distance of 6.57 cM and corresponds to a physical distance of 2.5 Mb on chromosome 19p13.3. Figure 5.5 represents the newly reconstructed haplotype for the chromosome 19 RLS locus.


Figure 5.5: Haplotype analysis of RLS3002 pedigree for chromosome 19 region A list of the examined markers is present on the left hand side. The alleles for each individual obtained from genotyping using these markers are listed below each individual. Each haplotype formed from the series of alleles is assigned a coloured bar so it can be recognised easily. The red bar (haplotype) is in all affected individuals (filled squares and circles), and it is assumed that this red bar is inherited from the affected grandmother in generation I. The unaffected (squares and circles containing the letter N ) individuals do not inherit a red bar, and it is assumed they inherit their haplotype from the unaffected grandfather (generation I). Hence there is a shared haplotype between all the affected individuals that is absent in normal individuals. A recombination event was observed in individual PATH053 and the point of recombination is marked with an asterix (*). This recombination event defined the critical RLS gene location as distal to marker D19S565.

### 5.3.5 Multipoint Linkage analysis

Once linkage has been detected, multipoint linkage analysis can help localize the position of the gene more precisely (Elston and Olson 2002). Multipoint linkage analysis is the analysis of linkage data involving three or more linked loci. Multipoint linkage analysis was performed for this region for a number of different marker combinations. However the combination of markers which gave the highest LOD score was: D19S886 ( 0.00 cM ), D19S878 ( 6.57 cM ) and D19S591 ( 9.84 cM ). A maximum multipoint LOD score of 3.77 was obtained at a position of 6.64 cM on chromosome 19, the nearest marker to this being D19S878 which is positioned at 6.57 cM . This maximum multipoint LOD score is illustrated in figure 5.6 which also shows the surrounding markers. A number of markers are located at the same genetic position on chromosome 19 , these markers are represented with a + sign.


Figure 5.6: Graph of Multipoint parametric LOD scores for family RLS3002 on chromosome 19p
Genetic distance ( cM ) are plotted on the x axis and LOD score $[\mathrm{Z}(\theta)$ ] is plotted on the y axis. Multipoint Linkage analysis was performed with markers D19S886, D19S878 and D19S591, which are shown in red. Names of a number of microsatellite markers are not shown on the graph as a number of the markers share the same genetic position. The markers which generated the highest LOD scores are shown on this map.

Multipoint Linkage analysis can help localize the position of the gene more precisely. However, it can also support the findings of the genome-wide scan. The results of the multipoint linkage analysis in figure 5.6 support the findings that family RLS3002 is linked to chromosome 19 for RLS. A maximum multipoint LOD score of 3.77 was obtained at a position of 6.64 cM on chromosome 19 .

From a multipoint LOD score graph it is usually possible to determine a support interval for the likely placement of the disease locus. Current Protocols of Human Genetics suggest that in order to do this, a horizontal line is to be drawn across the graph at a value equal to the highest LOD score minus 3 LOD units. All points on
the map that are above this horizontal line are included within the 3-LOD-unit support interval and cannot be entirely excluded. For two-point linkage analysis graph only a 1-LOD unit support is required, however the 3-LOD-unit support is needed for multipoint analysis to ensure a higher degree of confidence in locus placement for multipoint situation (Speer 2006). Unfortunately it is not possible to use this approach for our study. The maximum LOD score is equal to 3.77 , which would indicate that a horizontal line be drawn at $\mathrm{Z}(\theta=0.77)$. This line would not cross the LOD score curve at any point, therefore no support interval can be inferred.

Nevertheless, the graph can be used to interpret the location of the disease gene. The highest LOD score was obtained just proximal to microsatellite marker D19S878. The value of the maximum LOD score is equal to 3.77 . Therefore the most likely location of the causative gene is in close proximity to microsatellite marker D19S878. This information should be considered when selecting candidate genes for screening.

### 5.4 Discussion

Following confirmation of linkage to chromosome 19 it was necessary to refine the region. Refining the region will help to the smallest possible area will reduce the candidate region and will help locate the gene. Initial detection of a genetic locus generally does not yield much precision on its location, thus leaving too vast a region for choosing candidate genes. Fine-mapping refines the genetic region, thereby reducing the number of genes within the candidate region. Fine mapping is therefore an essential step before beginning candidate gene selection in order to reduce the number of potential candidate genes.

To fine map the chromosome 19 candidate region it was necessary to locate other microsatellite markers in the region. Only four other microsatellite markers were available in this region, (www.ncbi.nih.nlm, and www.ensembl.org). These markers were: D19S565, D19S247, D19S120 and D19S424. This region is a microsatellite poor region; therefore it was necessary to find additional microsatellite repeats in the region and to design primers to amplify these regions. An additional 10 microsatellite markers were ordered. Linkage analysis results of these markers obtained a maximum LOD score of 3.46 for marker CA19S2310 which is located at 6.57 cM .

Haplotype reconstruction revealed that all 11 affected individuals carried a common haplotype. A recombination event between markers D19S878 and D19S565 in individual PATH053 allowed for the defining of the 19p13.3 RLS region flanked by the telomere and microsatellite marker D19S565. Therefore an overall candidate region of 6.57 cM corresponding to 2.5 Mega bases $(\mathrm{Mb})$ on chromosome 19p13.3 has been identified.

Individual PATH208, is unaffected, however shares part of the affected haplotype. Two theories can explain this individual's phenotype. In the first scenario, individual PATH208 is a carrier of only a portion of the overall candidate region, spanning 1.1 Mb on the physical map. The gene responsible for RLS in this family may be positioned elsewhere on the chromosome 19p13.3 locus. The second theory is that PATH208 is a non-symptomatic member. He may carry the affected haplotype but symptoms and genetic traits are not being expressed (incomplete penetrance). It has been previously noted that incomplete penetrance is seen in one third of cases with autosomal dominant RLS (Hamet and Tremblay 2006).

A maximum multipoint LOD score of 3.77 was obtained at a position of 6.64 cM on chromosome 19, the nearest marker being D19S878 which is positioned at 6.57 cM . This confirmed linkage to the chromosome 19p13.3 region and also localises the position of the gene more precisely. It is probably not a coincidence that the highest two-point LOD score was also observed at this microsatellite marker, D19S878 (LOD $=3.59$ at $\theta=0.00$ ). Therefore the multipoint analysis shows that the most likely map location for the RLS gene is close to D19S878. Multipoint LOD scores are potentially more informative than two point LOD scores as they have the ability to pinpoint a disease gene location in the fine-mapping of a Mendelian disorder. Nonetheless, as seen in this study multipoint LOD scores will not produce a higher absolute value at a specific marker if the marker itself is already fully informative for linkage. In this study a maximum LOD score of $3.77(\theta=0.00)$ was observed, which was only slightly higher than that seen for the two-point LOD score (3.59 at $\theta=0.00$ ).

In conclusion a novel locus for RLS has been identified on chromosome 19p13.3. The next step is to search the region for candidate genes. It can be anticipated that
the detection of the underlying gene defect at 19 p13.3 will add to our current understanding of the underlying pathophysiology of RLS.

## Chapter 6

## Sequencing

### 6.1 Introduction

The novel RLS locus which was identified spanned a region of 6.57 cM corresponding to 2.5 Mb on chromosome 19p13.3. The next obvious step was to search the region for candidate genes in the hope of finding the underlying gene defect.

The first stage of this part of the study was to select candidate genes from within thes novel locus. Due to the fact that no causative gene had yet been identified in any of the previously described loci, it was not possible to choose candidate genes based on known genes. The candidate genes therefore were selected on the rationale that the pathophysiology of RLS may be closely linked to the dopaminergic system and/or iron metabolism. As previously mentioned (section 1.6), the pharmacological data that strongly lend support to a dopaminergic abnormality and iron depletion is regarded as the most relevant additional factor for the clinical manifestations of the syndrome. The chromosome 19 p 13.3 locus is flanked by the telomere and microsatellite marker D19S565. The region is 6.57 cM (genetic length) corresponding to 2.5 Mb (physical length). There are approximately 100 genes in this region and were identified using the human genome public database websites http://genome.ucsc.edu/ and www.ensembl.org. Many of the genes had known functions, for example MADCAM1, which is a cell adhesion leukocyte receptor expressed by mucosal venules; and PPAP2C, which is a member of the phosphatidic acid phosphatise family and is found mainly in the brain and placenta. Other genes were expressed in tissues unlikely to be related to RLS, for example C19orf22 which is expressed in whole blood, B cells and T cells. The genes within the chromosome

19p13.3 locus were narrowed down to 8 possible candidate genes, and from this two of the more promising genes within the candidate interval were chosen for screening.

### 6.1.1. KLF16

The first gene selected for screening was KLF16 (Krueppel-like factor 16) otherwise known as Transcription factor BTEB4 (Basic transcription element-binding protein 4) and $\operatorname{DRRF}$ (dopamine receptor regulating factor). Transcriptional regulation is governed by the coordinated action of regulatory factors that bind to specific DNA elements. KLF16 (DRRF) binds to GC and GT boxes in the D1 and D2 dopamine receptor promoters and effectively displaces Sp 1 and Sp 3 from these sequences. Subsequently, $D R R F$ can modulate the activity of these dopamine receptor promoters. In a study published in 2001, it was reported that mice treated with drugs that increase extracellular striatal dopamine levels (cocaine), block dopamine receptors (haloperidol), or destroy dopamine terminals (1-methyl-4-phenyl-1,2,3,6tetrahydropyridine) show significant alterations in DRRF mRNA (Hwang, D'Souza et al. 2001). KLF16, therefore, is an excellent candidate gene for RLS as it is important for modulating dopaminergic transmission in the brain.

### 6.1.2 GAMT

The second gene selected for screening was GAMT (guanidinoacetate N methyltransferase. It is also known as PIG2 and TP53I2. GAMT supplies instructions for making the enzyme guanidinoacetate methyltransferase. It is active in the pancreas, liver and kidneys. This enzyme participates in the two-step synthesis of the compound creatine from the amino acids glycine, arginine and methionine. GAMT gene mutations impair the ability of the guanidinoacetate methyltransferase enzyme to participate in creatine synthesis (http://ghr.nlm.nih.gov/). The effects of guanidinoacetate methyltransferase deficiency are most severe in organs and tissues that require large amounts of energy, such as the brain and muscles. Increased serum concentration of muscle creatine phosphokinase is observed in the majority of affected individuals with choreoacanthocytosis (ChAc) (Hiersemenzel, Johannes et al. 1996). Choreoacanthocytosis is characterised by a progressive movement disorder. Although the movement disorder is mostly limb chorea, some individuals present with a parkinsonian syndrome (http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene\&part=chac).

### 6.1.3 DNA sequencing

The principles of DNA sequencing is based on the dideoxy sequencing method developed by Fred Sanger. The DNA is present in a single stranded form and acts as a template for making a new complementary DNA strand in vitro using a suitable DNA polymerase. Cycle sequencing is similar to PCR as it includes denaturation, annealing and DNA synthesis and it uses a heat stable DNA polymerase. In addition, however, it uses dideoxynucleotide triphosphates (ddNTPs), and only a single oligonucleotide primer is required. The ddNTPs are closely related to the normal dNTPs; they differ only in that they lack a hydroxyl group at the 3 ' carbon position as well as the $2^{\prime}$ carbon. Due to the fact that ddNTPs lack a 3 ' hydroxyl group, any ddNTP that is incorporated into a growing DNA chain cannot participate in phosphodiester bonding at it 3' carbon atom. This thereby causes abrupt termination of chain synthesis. By setting the concentration of the ddNTP to be much lower than that of the dNTPs, there will be competition between a specific ddNTP molecule and an surplus of analogous dNTP molecules for incorporation into the growing DNA chain. Therefore if a dNTP is incorporated, chain extension will continue; however if a ddNTP is (occasionally) included it causes chain termination. Thus, each reaction is only a partial reaction, as chain termination will take place randomly at one of the possible choices for a specific type of base in any one DNA strand. The sequence cycle will therefore result in the synthesis of differently sized labelled DNA fragments. Figure 6.1 demonstrates the dideoxy DNA sequencing with the random incorporation of ddNTP causing a chain termination (Strachan and Read 2003).


Figure 6.1: A schematic diagram explaining Sanger's dideoxy DNA sequencing
The sequencing primer binds to a region $3^{\prime}$ of the desired DNA sequence and primes synthesis of a complementary DNA strand in the indicated direction. Four parallel base-specific reactions are carried out, each with all four dNTPs and with one ddNTP. Competition for inclusion into the growing DNA chain between a ddNTP and its normal dNTP analog results in a population of fragments of different lengths. The fragments will have a common $5^{\prime}$ end (defined by the sequencing primer) but variable $3^{\prime}$ ends, depending on where a dideoxynucleotide (shown with a filled circle above) has been inserted. For example, in the A-specific reaction chain, extension occurs until a ddA nucleotide (shown as A with a filled black circle above) is incorporated. This will lead to a population of DNA fragments of lengths $n+2, n+$ $5, n+13, n+16$ nucleotides, etc.

### 6.2 Materials and Methods

### 6.2.1 Primer design

KLF16

According to the UCSC Genome Browser (Feb. 2009), two versions (A and B) of GAMT exist (figure 6.2). Introns are depicted as lines with arrows indicating the direction of transcription. The arrows are pointing to the left, therefore transcription occurs on the negative strand. Version A of KLF16 has two exons in total. The second exon, which is the one to the far right, is quite large, spanning approximately 2 kb . Version B of KLF16 has three exons in total. Exon one is identical to that seen in version A. Exons two and three are located in the same location as the second exon in version A , the only difference being there is approximately 1.0 kb of an intronic region dividing the two exons. To ensure comprehensive screening of KLF16, both versions of the gene required screening. Exon 1 was identical in both versions and was designated x 1 . By screening the second exon of version A , exons two and three of version B was also covered.

## UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly mpe <br>  200min <br> $\qquad$ zoom oul $1.5 \times 3 x 10 x$

 position search chri9:1,850,319-1,865,840 gene jump clear size 15,522 bp. configure


Figure 6.2: Chromosome 19 and KLF16 gene
This figure shows the chromosome 19 ideogram, with a red vertical bar indicating the position of KLF16 on the chromosome. A represents the UCSC version of the KLF16 gene. The two exons are represented by the blue filled rectangles. The introns are represented by the arrowed lines in between the exons, with the arrows indicating the direction of transcription. The arrows are pointing to the left, therefore transcription occurs on the negative strand. B is a representation of the KLF16 gene based on another database. This version only has three exons. UCSC Genome Browser, February2009 Assembly (http://genome.ucsc.edu).

## GAMT (Primer Design)

According to the UCSC Genome Browser (Feb. 2009), two versions of GAMT exist. These two versions can be observed in figure 6.3 below. Introns are depicted as lines with arrows indicating the direction of transcription. In this case intron orientation is negative, therefore arrows point to the left. Version A of GAMT has six exons (black rectangles). Version B of GAMT has only five exons, however. Exons 1 to 4 (represented by the blue rectangles) are identical to exons 1-4 in version $\mathbf{A}$. Nonetheless, exon 5 is somewhat larger than that of version $\mathbf{A}$, and exon 6 does not exist. Hence, to ensure comprehensive screening of GAMT, both versions of the gene required screening. Exons 1 to 4 were identical and were therefore designated $\mathrm{x} 1, \mathrm{x} 2$, x3, x4. By screening the fifth exon of version $\mathbf{B}$, exon 5 of version $\mathbf{A}$ was also covered. The fifth exon of version $\mathbf{B}$ was designated x5. Exon 6 of version $\mathbf{A}$ was designated x 6 . According to ensembl (www.ensembl.org) only one version of GAMT exists, and this is identical to version A (http://genome.ucsc.edu).

## UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly

 move $\lll \lll \ggg>$ zoom in 1.5 x 3x 10 x base zoom out 1.5 x 3x 10 x

Figure 6.3: Chromosome 19 and GAMT gene
This figure shows the chromosome 19 ideogram, with a red vertical bar indicating the position of GAMT on the chromosome. A represents the UCSC version of the $G A M T$ gene. The six exons are represented by the black filled rectangles. The introns are represented by the arrowed lines in between the exons, with the arrows indicating the direction of transcription. The arrows are pointing to the left, therefore transcription occurs on the negative strand. B is a representation of the GAMT gene based on another database. This version only has five exons. UCSC Genome Browser, February2009 Assembly (http://genome.ucsc.edu).

### 6.2.2 Pedigree RLS3002

When carrying out the genome-wide scan it was necessary to genotype all members of the RLS3002 family. However, when sequencing a candidate gene, it is not necessary to screen every member of the family. For the purpose of screening the two candidate genes KLF16 and GAMT, DNA from one unaffected and two affected members of the pedigree were screened. The two affected members screened were first cousins, PATH048 and PATH054. One unaffected member of the family was screened, PATH047. This individual is the father of individual PATH048, which would mean that any variant observed in the daughter (PATH048) and not in the father would have been inherited from the affected mother or less likely, it would be a novel mutation. PATH047 is married into pedigree RLS3002, which also means that any variation he has is unlikely to be disease causing. The three members chosen for screening the candidate gene can be observed in figure 6.4.


Figure 6.4: Members of RLS3002 chosen for candidate gene screening
Only three members of RLS3002 were used for screening the candidate genes. Two affected members, PATH048 and PATH054, who are first cousins, were screened. PATH047, father of PATH048 is an unaffected member and was also screened. The three individuals used for this part of the study are indicated by the red boxes.

### 6.2.3 PCR

PCR was carried out in $50 \mu \mathrm{l}$ reactions. A total of $5 \mu \mathrm{l}$ of genomic DNA was used as a template for amplification reactions. Table 6.1 provides details of the reagents used. The table contains the name of the reagent, the stock concentration, the concentration used, and the amount of each reagent in a $50 \mu \mathrm{l}$ reaction volume.

Table 6.1 Reagents used for PCR in $50 \mu \mathrm{l}$ reaction volumes.

| Reagent | $[$ Stock $]$ | $[$ Final $]$ | 1 sample $(\boldsymbol{\mu l})$ |
| :--- | :--- | :--- | :--- |
| 10 x buffer | 10 x | 1 x | 5.00 |
| $\mathrm{MgCl}_{2}$ | 25 mM | 1.5 mM | 3.00 |
| dNTP | 10 mM | 0.2 mM | 1.00 |
| Forward Primer | $100 \mathrm{ng} / \mu \mathrm{l}$ | 100 ng | 1.00 |
| Reverse Primer | $100 \mathrm{ng} / \mu \mathrm{l}$ | 100 ng | 1.00 |
| Taq polymerase | $5 \mathrm{U} / \mu \mathrm{l}$ | 0.05 U | 0.15 |
| $\mathrm{H}_{2} \mathrm{O}$ |  |  | 33.85 |
| DNA |  | $50-100 \mathrm{ng}$ | 5.00 |
| Total Volume |  |  | 50.0 |

Once the samples were prepared, each $50 \mu 1$ reaction was placed in a 0.2 ml capped PCR tube and placed on the GStorm Thermal Cycler PCR machine. A temperature gradient was initially used to discover the optimal annealing temperature for each individual marker (as outlined earlier in section 3.2). The success of PCR was verified by agarose gel electrophoresis (as outlined in section 2.4).

### 6.2.4 DNA sequencing

Automated DNA sequencing requires 1) fluorescence labelling of DNA and 2) a detection system. Dideoxynucleotides to which fluorophores (chemical groups capable of fluorescing) are attached are used. This results in each nucleotide base being attached to a specific fluorophore. Since a different label is used for each nucleotide, the cycle sequencing will produce differently sized DNA fragments of the desired sequence with each base in the sequence tagged with a different fluorescent label. During electrophoresis, using the ABI3130 Genetic Analyser, the fluorescence signal is detected and recorded as the DNA passes through a fixed point in the capillary. The output is in the form of intensity profiles for each of the differently coloured fluorophores and the information is simultaneously stored electronically (Strachan and Read 2003).

Following amplification of the desired region by PCR (section 6.2.2 above), the sequencing protocol used for this study comprises of the following steps:
a. PCR purification;
b. Sequencing reaction;
c. Post-sequencing purification;
d. Detection of sequences using the ABI3130 genetic analyser.

## a. PCR purification

To achieve good quality sequence data using the Sanger dideoxy sequencing method and fluorescently labelled nucleotides, it is necessary to remove the excess primer and dNTPs from the initial PCR reaction performed (section 6.2.2). JETQUICK Spin Columns were used according to the manufacturer's guidelines as described in section 2.8.

## b. Sequencing reaction

The purified PCR products were then sequenced. As previously mentioned this technique is based on Fred Sanger's dideoxy sequencing method (section 6.1.3). This will result in differently sized DNA fragments of the desired sequence with each base in the sequence tagged with a different fluorescent label. The reaction was carried out according to the protocol detailed in section 2.9.

## c. Post-Sequencing Purification

Purification of sequencing products was carried out using the AmpliPurify ${ }^{\text {TM }}$ ExTerminator Kit. This optimised purification procedure removes unincorporated dyes, dNTPs and excess salt. It also removes the sequencing enzyme. This was performed according to the protocol outlined in section 2.10.
d. Detection of sequences using the ABI3130 genetic analyser The instrument is used to perform the electrophoresis step and to monitor the different fluorophores (colours) as they pass by and are detected by the laser. The samples are run on the ABI 3130 according to guidelines outlined in section 2.11.1.

### 6.2.5 Sequence Analysis

The sequences acquired from the ABI3130 were analysed using SeqMan programme from DNAstar software. SEQMAN provides a number of useful tools for simplifying the process of assembling data from a sequencing project. It allows the user to perform alignments and discover variants, along with many other analyses on de novo or template assemblies. SeqMan allows the user to assemble anything from two sequences to tens of thousands of sequences into contigs (figure 6.5).


Figure 6.5: DNA sequence data from the Seqman programme
A series of peaks are seen each of which corresponds to a specific base. Blue $=\mathrm{C}$ (Cytosine), black $=\mathrm{G}$ (Guanine), green $=\mathrm{A}$ (Adenine) and red $=\mathrm{T}$ (Thymine). X points to the position of the sequence (bp), Y points to the consensus sequence and Z points to the sequence of the sample. The example illustrated here shows a section of sequence from GAMT exon 6a, sample PATH054.

Sequences were compared with the expected amplicon and with other samples. Any variation present was recorded and both affected and unaffected individuals were compared for the presence or absence of variations. For a variation to be significant within this family, it must be present in samples from both of the affected individuals (PATH048 and PATH054) and not present in the unaffected individual (PATH047). If a variation was detected in both affected individuals, various websites (http://genome.ucsc.edu, http://www.ensembl.org and http://www.ncbi.nlm.nih.gov/) should be searched to determine whether these variations are known single nucleotide polymorphisms (SNPs).

### 6.3. Results

### 6.3.1 Primer Design

KLF16

As described in section 6.2.1, two versions of KLF16 exist according to UCSC Genome Browser, February2009 Assembly (http://genome.ucsc.edu). However, by screening version A of KLF16, version B is included within this. Therefore, two exons in total required screening.

The first exon (KLF16x1) was 524 bp long. Initially, only one set of primers was designed to amplify this exon. However, after numerous attempts to amplify this fragment, another set of primers were designed. One of the main limitation within a sequencing reaction is sequence length, therefore this exon was divided into smaller overlapping fragments. Exon 1 was divided into four overlapping fragments, x1a, $\mathrm{x} 1 \mathrm{~b}, \mathrm{x} 1 \mathrm{c}$, and x 1 d . Only a forward primer was ordered for x 1 c , as the reverse of x 1 d was to be used to amplify this inner region of exon 1 .

The second exon, $K L F 16 x 2$, was a large exon, encompassing $2,362 \mathrm{bp}$. Similar to exon 1 this exon was also divided into smaller overlapping fragments. Exon 2 was divided into five overlapping fragments, which were designated: x2a, x2b, x2c, x2d and $x 2 e$ (illustrated in figure 6.6).

As work progressed, a further three sets of primers were ordered. This was because gaps remained between sequences after analysis of the sequences using the original primers and so a complete sequence of exon 2 was not obtained. One gap was located between x2b and x2c. In total, two more pairs of primers (KLF16x2gap1 and KLF16x2bgap) were required to obtain sequences to close this gap.

A second gap of sequence was present between $x 2 d$ and $x 2 e$. It was necessary to design and order an additional set of primers (KLF16x2gap2) to cover this gap.

The primer sequences, annealing temperatures, and expected sizes of all the KLF16 fragments are shown in table 6.2. A number of the fragments failed to amplify, therefore an asterix (*) represents unknown annealing temperature in Table 6.2. Figure 6.6 shows KLF16 and its the positions of the fragments used to sequence the gene.

Table 6.2: Features of primer fragments used to screen KLF16.

| Fragment | Forward Primer Sequence | Reverse Primer Sequence | Annealing <br> Temp ${ }^{\circ} \mathbf{C} \mathbf{C}$ | Size <br> (bp) |
| :--- | :--- | :--- | :--- | :--- |
| KLF16x1 | CCCCACGCGCCACGCA <br> GTTT | GGGCGGGGCGGGCTGT <br> G | $*$ | 761 |
| KLF16x1a | AGAGGCCCAGCAGGAG <br> CAGGAAC | AGCACGTCGGCGGCGA <br> AGTAATC | $*$ | 186 |
| KLF16x1b | TCGCCGCCGACGTGCT <br> CAT | TCAATCCCGCCCGCAG <br> ACCTC | $*$ | 691 |
| KLF16x1c | GCGCGGCTCGGAGAGT <br> GGTG |  | $*$ | $*$ |
| KLF16x1d | CCGTGGCGTGCGTGGA <br> TTACTT | CTGGCGGGGGAGGGGT <br> CTC | $*$ | 509 |
| KGGGGCGGGTAAGGGT <br> TGTATTCT | GGGGGCAGGGGTGTCT <br> TCAGG | 67 | 600 |  |
| KLF16x2c | GCCCTCGTGCAGCCCA <br> CCCTGAC | CGCTGCCCCACTCCTG <br> GCTCTACC | 70 | 630 |
| GLF16x2d | CCGCCGGGGAAGCCATGG <br> CTCTT | CACAGCCAGCCCTCTG <br> CTTCCAGT | 70 | 657 |
| KLF16x2e | GGGGGCTTTGGATGGC <br> ACTG | TCCCCCACCCACCCCC <br> ACAGACTA | 69 | 766 |
| GG |  |  |  |  |



Figure 6.6: KLF16 gene, its exons and amplicons (primer fragments)
The horizontal line with arrows at the base of the picture represents the KLF16 gene to be sequenced. The two blue rectangles at either end represent exon 1 and exon 2. The fragments to be sequenced using the primers which were previously described (Table 6.2 ) are represented by the black rectangles. The name of each fragment is indicated. Both exons are covered by the fragments and from the diagram it is clear that many of the exons overlap. This is to ensure that all nucleotides are examined.

GAMT
As previously mentioned (section 6.2.1), two versions of the GAMT gene exist. To ensure comprehensive screening of GAMT, it was necessary to screen both versions of the gene. GAMT had six exons which required screening and they were designated $x 1, x 2, x 3, x 4, x 5$ and $x 6$. Initially five sets of primers were designed using the PrimerSelect program, and were ordered from Biomers (http://biomers.net). Exons 1 and 6 had one set of primers each (GAMTx1 and GAMTx6). Three sets of primers were designed to cover exons 2 to 3 (x2-3) and exons 4 to 5 (x4-5a and x4-5b). As work progressed, a further two sets of primers were required to obtain sequences not detected by the original primer products. The first additional set which was designed included primers for fragments to cover x1 (GAMTx1a and GAMTx1b), and primers for fragments to cover x6 (GAMTx6a and GAMTx6b). It was later necessary to design and order an additional set of primers (GAMTx1.2) to cover a large gap which remained in x 1 . The primer sequences, annealing temperatures, and expected sizes of the GAMT fragments are shown in Table 6.3.

Table 6.3: Features of primer fragments used to screen GAMT. (*) represents unknown annealing temperatures.

| Fragment | Forward Primer Sequence | Forward Primer <br> Sequence | Annealing <br> Temp ( $\left.{ }^{\circ} \mathbf{C}\right)$ | Size <br> $(\mathbf{b p})$ |
| :--- | :--- | :--- | :--- | :--- |
| GAMTx1 | CCGACCCCACTCCCGCCACC <br> TCTC | GCCCCCACCTGCAGCCCC <br> ACTTC | $*$ | 509 |
| GAMTx1a | GTAAGCGGCAAGGCGGGGA <br> TTC | CAGCGCGTGCATATAGG <br> GGGTCTC | $*$ | 465 |
| GAMTx1b | CCCAGCGCGACCCCCATCTT | GGACCCGGTTCCACCCAG <br> TTTCA | 63 | 477 |
| GAMTx1.2 | AGCGCCTGGAAGATCCTCAG <br> AT | CAGCCCCGGCCTCAGTTT | 53 | 631 |
| GAMTx2-3 | CGCCCCCTCCTCCAGGCAGT <br> CT | CGCCCCCTCCTCCAGGCA <br> GTCT | 63 | 701 |
| GAMTx4- <br> 5 a | GGGTGTGGGCCTGGCTGTGG | CACCCCCATCCAAGGTCA <br> CTTCCT | 70 | 555 |
| GAMTx4- <br> $5 b$ | GGAGGCCCTCGGGGAAGCG | TACCCAGGCTGGAGTGC <br> AGTGGTT | 70 | 702 |
| GAMTx6 | CCCGCACTGGGGAGCTGGA <br> GATTC | CTCCAGGTTTCTGAGCGG <br> CCCCAG | $*$ | 615 |
| GAMTx6a | CACTGGGGAGCTGGAGATTC <br> C | AGCGCCGGCGTTTACTTC <br> AC | 69 | 574 |
| GAMTx6b | CCGCTACTACGCCTTCCC | GGGGCCGCTCAGAAACC | 69 | 422 |

Figure 6.7 illustrates the positions of the fragments used to sequence the GAMT gene.


Figure 6.7. GAMT gene, its exons and amplicons (primer fragments)
The two horizontal lines with arrows at the base of the picture represents the two versions of GAMT to be sequenced. The black and blue rectangles located at intervals along these lines represent exons 1 to 6 . From the figure, it can be seen that two versions of exon 5 exist. The larger exons was screened. The fragments to be sequenced using the primers which were previously described (Table 6.3) are represented by the black rectangles. The name of each fragment is indicated to the left hand side. All six exons are covered by the fragments and from the diagram it is clear that many of the exons overlap. This is to ensure that all nucleotides are examined.

### 6.3.2 PCR

As mentioned for the purpose of screening KLF16 and GAMT for mutations, three individuals from RLS3002 were selected, two affected individuals (PATH048 and PATH054) and one unaffected individual (PATH047). DNA was amplified for all fragment following PCR optimisation.


Figure 6.8: Agarose gel electrophoresis of PCR products of GAMT x2-3
Samples used are PATH047, PATH048, PATH054 and $\mathrm{H}_{2} \mathrm{O}$ (negative control). The 100 bp ladder is to the far left of the gel and the $500 \mathrm{bp}, 700 \mathrm{bp}$ and $1,000 \mathrm{bp}$ bands are indicated. The ladder allows assessment of size of the products and also the quality of gel staining. The three samples (PATH047, PATH048 and PATH054) have one single band each, which is located at approximately 700 bp . This is consistent with the expected PCR product size of 701 bp .

KLF16

All fragments for KLF16x2 (KLF16x2a, KLF16x2b, KLF16x2c, KLF16x2d, KLF16x2e) were amplified successfully. An additional three sets of primers (KLF16x2gap1, KLF16x2gap2, KLF16x2bgap) were ordered to cover gaps in the sequence. Fragment KLF16x1 failed to amplify. A number of approaches were applied to attempt to amplify the fragment, e.g. altering annealing temperature, increasing/decreasing $\mathrm{MgCl}_{2}$, however none of these approaches were successful. Upon examining the content of the region, the reason that the fragment was failing to amplify became clear. The region has a very high GC content (81\%), and amplification of these regions is usually very difficult. Problems usually observed with GC-rich DNA are constraint of template amplification by stable secondary structures that stall or reduce the DNA polymerase progress, and the presence of secondary annealing sites giving rise to nonspecific amplified bands. Four additional primer sets were designed and ordered for exon 1 (KLf16x1a, KLf16x1b, KLf16x1c, KLf16x1d). When designing these primers, it was ensured that the primers themselves did not have a high GC content. Unfortunately, these four primer pairs failed to amplify also. Therefore, exon 1 of KLF16 was not sequenced.

GAMT

Following determination of PCR conditions, GAMTx1b, GAMTx2-3, GAMTx4-5a and GAMTx4-5 were amplified successfully. GAMTx1a and GAMTx6 did not amplify successfully. Upon examining the content of GAMTx 1 , it was discoved that this region has a high GC content (79\%) and was possibly the reason GAMTx 1a failed to amplify. After several attempts to amplify this region e.g increasing annealing temperature, adjusting $\mathrm{MgCl}_{2}$ concentration, and using betaine, the fragment failed to amplify. An additional set of primers was designed and ordered for this region (GAMTx1.2). These primers were successful in amplifying the remaining region on exon 1. GAMTx6 also failed to amplify after several attempts. Two new sets of primers were ordered for this region (GAMTx6a and GAMTx6b). These primers allowed successful amplification of exon 6 .

### 6.3.3 DNA sequencing and Sequencing analysis

The next step was DNA sequencing and sequence analysis of all three individuals to check for variation in the exons of KLF16 and GAMT. Sequences were compared with the expected amplicon and with one another. Any variation present was recorded and examined for presence/absence on the affected and unaffected individuals. For a variation to be significant with respect to RLS within this family, it must be present in the affected individuals (PATH048 and PATH054), and not in the unaffected one (PATH047).

## KLF16

Three variants were detected in KLF16x2. Table 6.4 lists these variations, the chromosome position, the variant type, and the SNP title.

Table 6.4: Variants found in gene KLF16.

| Exon | Chromosome 19 <br> Position (bp) <br> [UCSC Genome <br> Browser Feb. <br> 2009] | Variant type | Comment | SNP title |
| :--- | :--- | :--- | :--- | :--- |
| KLF16x2b | $1,805,159$ | $\mathrm{C} \rightarrow$ G | Present in an unaffected <br> individual (PATH107) and <br> affected family members. | rs12972720 |
| KLF16x2b | $1,805,260$ | GG Insertion | Present in unaffected <br> (PATH047) and affected <br> family members (PATH048 <br> and PATH054) | New <br> variant |
| KLF16x2d | $1,853,667$ | G $\rightarrow$ C (Het) | Present only in unaffected <br> (PATH047) | rs3746043 |

The first SNP detected was rs12972720 in exon 2b of KLF16. The SNP is a known C to G change in the coding sequence if KLF16 exon 2 . The unaffected member PATH047 was homozygous C/C; his affected son PATH048 was heterozygous (C/G) at that position; and the affected individual PATH054 was homozygous G/G. Therefore both affected individuals carry at least one G, while the unaffected sample has no G. An additional unaffected control PATH107 (which has been used for other RLS studies in the Dept. of Pathology) was sequenced; this was to ensure that the SNP was not significant with respect to RLS within this family. PATH107 was also homozygous G/G.

Although the two affected individuals (PATH048 and PATH054) do not have the same genotype at this position, both do carry at least one G while the unaffected sample has no G (PATH047). Furthermore, the G in PATH048 must have been inherited from her affected parent as the unaffected parent is CC at this position. Another unaffected sample (PATH107) was screened and was determined to be GG at this position. This individual does not have RLS; therefore, the genotype is not the causative variant. Furthermore, the frequency data on the SNP website www.ncbi.nlm.gov/projects/SNP/ published data for four populations for this SNP rs12972720. The European data (pilot.1.CEU) found that $54.2 \%$ of the population have a C at this position, while $45.8 \%$ of the population have a G .

One can conclude that this SNP is not a disease causing variant for two reasons. Due to the fact that the SNP was also seen in PATH107, an unaffected control, and that it is a known variant common in the European population, one can conclude that this is not disease causing variant.


Figure 6.9: A segment of DNA sequence data demonstrating $\mathbf{C} \rightarrow \mathbf{G}$ heterozygous change
Exon 2 b of GAMT. The four individuals screened for the variant are identified in the figure. The unaffected individual has a single blue peak at this position, and is therefore homozygous (C/C). Instead for PATH048 two overlapping peaks (black and blue) are present representing a $\mathrm{C} \rightarrow \mathrm{G}$ heterozygous change. Furthermore, a $\mathrm{C} \rightarrow \mathrm{G}$ homozygous change is present in PATH054 and PATH107, represented by a single black peak. This change is present in both an affected individual and an unaffected control. Blue $=\mathrm{C}($ Cytosine $)$, black $=\mathrm{G}($ Guanine $)$, green $=\mathrm{A}$ (Adenine) and red $=\mathrm{T}$ (Thymine).

The second variant detected in KLF16x2 was a single nucleotide insertion seen in both the affected and unaffected family members. PATH054 is homozygous for a single insertion of a G. The insertion was also observed in PATH048, however this sample is heterozygous, carrying one copy of the fragment with the insertion and one copy of the fragment without the insertion. This results in non-identical sequences downstream of the insertion point and this is observed in sequence data when there are two peaks at each nucleotide position. The position is 507 bp from the beginning of exon 2 in an untranslated region (UTR). The consensus sequence has 5 Guanines (ggggg); however, PATH054 has 6 Guanines (gggggg) in both copies of sequence.

PATH048 has one copy of each. A number of other affected and unaffected family members were sequenced for this region to determine if they also had this insertion (Table 6.5). Both affected and unaffected family members have a heterozygous insertion and a homozygous G insertion. Hence, it is non-disease segregating.


Figure 6.10: A segment of DNA sequence data demonstrating an insertion The arrows point to positions on PATH047, PATH052 and PATH054 where according to the database sequence there should be a single blue peak representing the base C. However, PATH048 has a G (heterozygous) insertion at this position, represented by the blue and black peak. PATH052 and PATH054 both have a G (homozygous) insertion at this position represented by the single black peak. This inserion is present in both affected and unaffected members of Pedigree RLS3002. Blue $=\mathrm{C}$ (Cytosine), black $=\mathrm{G}$ (Guanine), green $=\mathrm{A}$ (Adenine) and red $=\mathrm{T}$ (Thymine).

Table 6.5: Results of family members tested for the G/G insertion in KLF16 x2.

| Individual | Status | Insertion |
| :--- | :--- | :--- |
| PATH046 | Affected | G/G (Hom) |
| PATH048 | Affected | G (Het) |
| PATH053 | Affected | G/G (Hom) |
| PATH054 | Affected | G/G (Hom) |
| PATH047 | Unaffected | Not present |
| PATH052 | Unaffected | G/G (Hom) |
| PATH199 | Unaffected | G (Het) |
| PATH200 | Unaffected | G (Het) |

The third variant detected was a G (Guanine) to C (Cytosine) heterozygous shift, in the coding sequence of KLF16 exon 2. This variant was detected in the control/unaffected family member, PATH047 (figure 6.9). The variant was not present in either of the two affected family members. A number of other family members were sequenced for this SNP, two affected members (PATH053, PATH055) and two unaffected members (PATH052, PATH186). This variant was not detected in any of the other family members. Therefore this is not the disease causing mutation. It was only detected in one 'married in' family member.


Figure 6.11: A segment of DNA sequence data demonstrating $\mathbf{G} \rightarrow \mathbf{C}$ heterozygous change.
Exon 2bc of GAMT. The arrow points to a position (PATH047) where according to the amplicon should have a single black peak representing the base G, but instead two overlapping peaks (black and blue) are present representing a $\mathrm{G} \rightarrow \mathrm{C}$ heterozygous change. This is only present in PATH047 (unaffected parent) and not present in the affected individuals. Blue $=\mathrm{C}$ (Cytosine), black $=\mathrm{G}$ (Guanine), green $=\mathrm{A}$ (Adenine) and red $=\mathrm{T}$ (Thymine) .

KLF16x1 failed to amplify therefore it could not be sequenced.

GAMT

GAMT was successfully sequenced and analysed. No variants were detected within any of the amplicons. The UCSC genome browser database (www.genome.ucsc.edu) was used to investigate if there were any known SNPs within the regions amplified for GAMT. Figure 6.10 illustrates the SNPs located within the amplified regions of GAMT.


Figure 6.12: SNPs within the GAMT amplicons
The amplicons are represented by the black rectangles. Each amplicon has its own unique name e.g. GAMTx6a, which indicates the exon the fragment is incorporating. The SNPs are shown below the two versions of GAMT. There are 25 SNPs within these amplified regions. The SNPs are represented by colours. Black represents Unknown, Untranslated, Locus and Intron SNPs. Green represents Coding/Synonymous SNPs. Red represents Splice sites and Coding/Nonsynonymous SNPs. UCSC Genome Browser, February2009 Assembly (http://genome.ucsc.edu).

None of the above known SNPs or no new variant was detected within the three investigated members of pedigree RLS3002 for the gene GAMT.

### 6.4 Discussion

This part of the study examined two candidate genes within the novel RLS locus on chromosome 19p13.3. The first candidate gene which was studied was KLF16. Although this candidate region was not very near to the area which gave the highest multipoint LOD score, it was an excellent candidate gene. KLF16 is important for modulating dopaminergic transmission in the brain. The pathophysiology of RLS is thought to be closely linked to the dopaminergic system and iron metabolism.

Exon 2 of KLF16 was large and therefore had to be divided into five overlapping fragments of sizes suitable for sequencing. Amplification and sequencing of this exon was successful and three variants were detected. The first SNP detected was rs12972720. This is a synonymous SNP present in the untranslated region of exon 2. The $\mathrm{C} \rightarrow \mathrm{G}$ change was observed in both unaffected individuals PATH048 and PATH054. However a second unaffected control for RLS was sequenced and was found to also have this variation. This SNP was a known variant, and according to the population data both the C and G are common in the European population. Therefore this disease most likely has no role in the pathogenesis of RLS.

The second variant was a $G$ insertion in the untranslated region of exon 2. A number of additional family members were examined for this variation and it was found that both affected and unaffected family members carry either a heterozygous or homozygous G insertion. It did not have an effect on amino acid as it is in an untranslated region. This was an unknown SNP; therefore no frequency data was available. Due to the fact the variation was seen in both affected and unaffected family members, this variation was not disease segregating and most likely has no role in the pathogenesis of RLS.

The third variation that was observed in exon 2 of KLF16 was a G to C change in the unaffected individual, PATH047. This is a known SNP (rs3746043), and is a synonymous SNP, therefore it does not have any effect on the resulting amino acid. A number of additional affected and unaffected family members were examined for this variation, and it was not detected in these samples. The frequency data on the SNP website www.ncbi.nlm.gov/projects/SNP/ published data for five studies for this SNP rs3746043. The European data (pilot.1.CEU) found that $26.4 \%$ of the population have a C at this position, while $73.6 \%$ of the population have a G . Subsequently, the variant rs3746043 detected in exon 2 of KLF16 is common within the European population and is unlikely to have a role in the pathogenesis of RLS in this family.

Exon 1 of KLF16 is not a very large exon, howver it has a high GC content (81\%). This exon proved very difficult to amplify, and after several attemps including trying to amplify the fragment with betaine and reordering new primers it failed to amplify. Therefore, KLF16 cannot be fully excluded as to having a role in the aetiology of RLS in this pedigree.

The second candidate gene which was investigated was GAMT. GAMT is required for producing the enzyme guanidinoacetate methyltransferase. A deficiency in this enzyme mainly affects organs and tissues that require large amounts of energy, such as the brain and muscles. RLS is a neurological disorder which is characterised by cramps in the lower limbs and often accompanied with muscle spasms.

Exon 1 of GAMT was difficult to amplify as it has a high GC content. Additional primers were ordered for this region, and were designed avoiding the GC rich areas.

All of the GAMT fragments were amplified successfully for the three family members PATH047, PATH048 and PATH 054.

No SNP or new variant was detected within any of the amplicons analysed for the members of pedigree RLS3002. Firstly there were not many SNPs within the amplicons. Figure 6.12 confirms that there were a total of 25 SNPs within these amplified regions. One of these SNPs, rs73525059, was deleted from the database on May 13, 2010. Therefore there are a total of 24 SNPs within the amplicons of GAMT. Furthermore, when the database of these SNPs were investigated further, it was became clear that many of these SNPs are not common in people of European descent. For example, SNP rs266811 had frequency data for two populations. The first was for pilot.1.YRI (Nigerian population) which found an allele frequency of 0.24 for C and an allele frequency of 0.76 for G . The CEPH data (European population), also for SNP rs266811, found an allele frequency of 1.00 for G. Another SNP, rs 1050914 , found an allele frequency of 1.00 for G. In addition to this, a number of SNPs had only been detected in non-European populations. For example, rs76461299 and rs73515058, had only been detected in Chinese/Japanese and Nigerian population respectively. Therefore it is not unusual that any of the known variants were not detected in the members of pedigree RLS3002. The SNPs within the amplified region were seen mostly within non-European populations, and are not common within the European population.

All six exons of GAMT were analysed including splice sites. No mutation was found within the exons of GAMT when comparing the two affected members (PATH047 and PATH054) to the unaffected member (PATH047). Sequencing of the candidate
gene GAMT did not identify mutations, however variants in either the promoter region or the intronic regulatory elements cannot be excluded.

## Chapter 7

## Discussion

### 7.1 Introduction

Restless Legs Syndrome is one of the most common disorders seen in sleep clinics. It is a sensorimotor disorder, characterised by the desire to move the lower limbs due to unpleasant sensations that are worse at rest and in the evenings and are relieved, at least temporarily, by movement. It can be either primary (genetic/idiopathic) or secondary (caused by other conditions). RLS causes tremendous distress and affects the quality of life of those affected. Generally speaking, RLS is not well understood in terms of pathophysiology and symptomatology. Ironically, RLS can be described as one of the most common conditions one has never heard of.

In order to find the causative gene for primary RLS, several genome wide studies have been completed and 10 loci have been identified on chromosomes $5 \mathrm{q}, 12 \mathrm{q}, 14 \mathrm{q}$, $9 p, 2 q, 20 p, 4 q, 17 p, 16 p$ and 19p. Several association studies have also been performed and to date five genomic regions associated with RLS have been identified on chromosomes $6 \mathrm{p}, 2 \mathrm{p}, 15 \mathrm{q}, 9 \mathrm{p}$ and 12 q .

The UCC Pathology lab has identified and collected Irish families with RLS. In one large autosomal dominant (AD) family, a novel RLS locus has been determined. The research lab also identified and recruited a second Irish AD RLS family (RLS3002). Previous research on this family had partially excluded four of the known loci (Abdulrahim 2008). The aim of this study was to examine all known loci and associated regions for linkage to this family, and if linkage was not found to perform a genome-wide search to map and identify the causative gene in this RLS family

### 7.2 Discussion

RLS is a difficult to define condition that is possibly more prevalent than previously thought. Public and professional awareness of RLS has certainly increased in the last number of years, but questions, even skepticism about the very existence of the condition, persist (Varga, Ako-Agugua et al. 2009). According to Professor Richard P. Allen, Secretary of the World Association of Sleep Medicine and Committee Member of World Sleep Day 2009 he has " conducted medical research in the field of RLS for over 30 years, but still encounter many misconceptions about the condition."

In 2005 Requip (ropinirole) became the first drug approved by the FDA for treatment of RLS. This was followed, in 2006, by Mirapex (pramipexole). In 2006 an article published in the Public Library of Science Medicine Journal, discussed the issue of "disease mongering" in relation to RLS. This is the effort by pharmaceutical companies (or others with similar financial interests) to enlarge the market for a treatment by convincing people that they are sick and need medical intervention. They state that "the restless legs campaign has developed into a multimillion dollar international effort to push restless legs syndrome into the consciousness of doctors and consumers alike" (Woloshin and Schwartz 2006).

Although there are a number of negative claims regarding RLS, scientific evidence proves this is a "real" syndrome. The oldest reference of 'Restless Legs syndrome' syndrome on PubMed (www.ncbi.nlm.nih.gov/pubmed), dates back to 1960 (Ekbom 1960). Ekbom also published and presented eight cases in a paper published in 1945, titled 'Restless Legs.' It was claimed that Mirapex and Requip were in fact failed sleep medications, however, both of these medications were also approved by the

FDA as treatments for Parkinson 's disease. The drugs are dopamine agonists, which means they act in the brain mimicking the neurotransmitter dopamine, which is lacking in PD patients. The pathophysiology of RLS is thought to be closely linked to dopamine dysfunction; L-dopa ( a precursor to dopamine) relieves the symptoms of RLS. Furthermore akathisia, which is present in PD patients, is similar in character to RLS. It was therefore understandable why dopamine agonists, such as Mirapex and Requip, were considered for the treatment of RLS. It was not surprising that such drugs helped relieve symptoms of RLS.

RLS is a known disorder known for over four decades, it fits well within our knowledge of movement disorders and the role of dopamine agonists in treating them.

The UCC Pathology lab recruited pedigree RLS3002 in 2004/2005. There was a strong family history seen in this family, the grandmother (generation I), all of her four children (generation II) and seven of her grandchildren (generation III) being affected with RLS. Suggestive family history is therefore consistent with an autosomal mode of inheritance. All 18 family members answered a questionnaire and all affected individuals undertook a neurological examination. Age of onset in most affected individuals in this family ranged from 3 years of age to the teens. Although most RLS patients have lower extremity symptoms, arm restlessness occurs in up to $49 \%$ of patients with RLS (Freedom and Merchut 2003). In pedigree RLS3002, most of the individuals experienced symptoms confined to the lower limbs bar one who reported upper limb and trunk symptoms as well. Of the 11 affected individuals, five were affected mildly and the remaining six individuals
were moderately affected according to the IRLSSG rating scale for RLS severity (section 1.3.2).

Iron deficiency and chronic renal failure can play a part in the pathophysiology of RLS and basic biochemical tests such as serum iron and renal function can be carried out on affected individuals to rule out secondary RLS. Such tests were not performed on affected individuals of pedigree RLS3002 as the familial pattern, the early age of onset and the absence of any clinical history of other disease indicates primary/familial RLS in this family.

Mode of inheritance refers to the way in which genes are passed down from generation to generation (e.g. autosomal dominant, autosomal recessive, X-linked, multifactorial and mitochondrial inheritance). By analysing mode of inheritance, researchers can estimate the relative risk for and penetrance of a disease. Determining the mode of inheritance was crucial for this study. In order to perform linkage analysis and to compute a LOD score it is necessary to specify the mode of genetic transmission of the family being investigated. RLS has been identified as a Mendelian autosomal dominant disease with variable expressivity and high penetrance ((Winkelmann, Muller-Myhsok et al. 2002; Bonati, Ferini-Strambi et al. 2003; Levchenko, Montplaisir et al. 2004; Pichler, Marroni et al. 2006). An autosomal dominant mode of inheritance was displayed in pedigree RLS3002 as was reported in the other loci. This was evident by the fact that all the affected individuals have one parent who is affected. The chromosome 12 q locus mapped by Desautels et al in a French-Canadian (FC) family was the only exception to an autosomal dominant mode of inheritance in an RLS pedigree. This family displayed autosomal recessive inheritance (Desautels, Turecki et al. 2001). However, Desautels
and associates explained the autosomal recessive mode of inheritance in their family as pseudodominant mode of inheritance. This means that the real transmission of the gene is autosomal recessive but given a high frequency of the disease allele(s) there is a higher proportion of affected individuals, thus giving the appearance of a dominant pattern of inheritance. Desautels et al supported their pseudodominant mode of transmission, by stating that 1) the occurrence of RLS in the FC population was one of the highest in the world; 2) only FC families were linked to that locus at that time; and 3) that a founder effect has been well described in the FC population. The paper also maintained that RLS may be similar to Parkinson disease, where the presence of intrafamilial allelic heterogeneity is highly possible. After the original report of recessive juvenile Parkinsonism, a number of other studies later identified further mutations in the same gene and have also identified a mixed mode of inheritance of different mutations within and between families (Desautels, Turecki et al. 2005).

Linkage analysis of pedigree data is a powerful tool for mapping genomic regions that are likely to contain genes influencing human diseases. It is assumed that the phenotype arises from a single major gene effect with almost full (95\%) penetrance i.e. it is expressed regardless of other genetic or environmental factors. It has been successful in mapping hundreds of Mendelian diseases including Huntington's disease and cystic fibrosis. However for many common diseases with high prevalence, their penetrance are often incomplete. Also, phenocopies may exist. It is therefore difficult to specify the genetic model parameters in these circumstances. Misspecification of the model parameters as a result of incomplete penetrances and/or phenocopies may lead to a reduction in power to detect linkage. The reason there may be a reduction in linkage power is, incomplete penetrances have the ability
to reduce evidence for linkage. Furthermore, phenocopies can give false information about recombination patterns. For linkage calculations in this study, RLS was modelled as an autosomal dominant trait with a $95 \%$ penetrance as used in other RLS linkage studies (Chen et al, 2004). The reported value varies from $86 \%$ to full penetrance in most families; Trenkwalder et al., 1996 $\ddagger$; Lazzarini et al., 1999 $\downarrow$ ).

For many years, genetic mapping was limited by the lack of available markers. In 1980, it was suggested that restriction-fragment length polymorphisms (RFLPs) be used as simple Mendelian co-dominant genetic markers. Although RFLPs generally did not lie within a gene of interest, they had the advantage of requiring no prior knowledge about the sequence of DNA in which they lie, or of its function. Since the 1980's the field of molecular biology has grown rapidly, and there are currently a number of dense genetic maps available. Microsatellite markers are now more commonly used, and were therefore employed for this study. While this was efficient and powerful for the gene mapping part of this study, once linkage was estabished fine-mapping of our RLS locus was hampered by the lack of available markers in the area. Novel microsatellite markers were identified and primers were designed for these areas. It was not known if these novel microsatellites would be polymorphic, and subsequently, informative. Despite the great advances in genomic technology, the availability of microsatellite markers for fine-mapping the chromosome 19p13.3 region was limited.

Apart from a number of microsatellite markers failing to amplify i.e. those used for fine-mapping (section 5.3.3), no problems with genotyping was encountered throughout the study. I found it was best to attain the alleles without reference to the pedigree, and then to consider the results in the context of the family tree. This
avoided the subconscious bias of results. If there was doubt over a particular individual or a number of individuals in the family, these samples were re-analysed together with parent samples if available. This made it much easier to determine whether or not they all share the same allele. When genotyping, it is important to bear in mind that an inconsistency can arise through mutation of the microsatellite from one generation to the next. This is the process of how microsatellite polymorphisms occur over time. This, however, was not observed during this study.

When this study was initiated only four loci were identified. It was postulated that the newly recruited Irish family might share the same disease locus as the other Irish family in which the chromosome 5 locus was identified rather than being linked to the other loci identified in families of other origins. Hence, the remaining gap on chromosome 5 was examined first. A total area of 38.31 cM was excluded extending from 44.92 cM (flanked by the marker D5S418) and 83.23 cM (flanked by the marker D5S2003). The results exclude a large area which included the locus. All other nine loci identified by linkage analysis studies were examined for linkage to RLS3002. Linkage to all nine loci was successfully examined and excluded in this RLS family.

In 2007 the first RLS association studies were published. To date four RLS regions have been identified by association studies. This is a high-throughput approach which scans the entire genome or a specific region in an unbiased manner, using statistical methods to determine associations between chromosomal loci and a given phenotype. It requires a large number of cases and a large number of controls which is often difficult to collect, therefore would not have been a suitable method to use for our family. However, it was important to investigate if pedigree RLS3002 was
linked to any of these novel RLS regions. Following linkage analysis, our analysis shows that RLS3002 is not linked to any of these known risk-associated genes.

The RLS3002 pedigree must therefore carry a mutation in a novel genetic locus. Two microsatellite marker sets were used during the genome wide scan of family RLS3002. The first marker set used was the Research Genetics Inc. mapping set. When this panel of markers was completed and no linkage had been found, there were many questions raised as to whether another microsatellite marker set should be used or if another route should be taken. Two possible options to locate the RLS locus were considered. The first option was another microsatellite marker set with a higher density of markers. The second options considered was using microarraybased single nucleotide polymorphism (SNP) data. The second option was becoming more widely used as markers for linkage analysis in the identification of loci for disease-associated genes. Both options were considered. Although microarray based SNP analyses have markedly reduced genotyping time and cost compared with microsatellite-based analyses, applying these enormous data to linkage analysis programs is a time-consuming step. The Microsatellite marker set which was considered was the Applied Biosystems Linkage mapping set version 2.5, which has a resolution of 10 cM . The first mapping, Research Genetics Inc. has a resolution of approximately 20 cM .

Furthermore, since the beginning of the initial genome-wide scan an additional seven members of the RLS3002 family had been examined and had blood samples taken which significantly increase the power of the study. The maximum possible positive LOD score with initial eleven individuals in the study was calculated to be 1.86 . In order to detect linkage reliably, the family size must be large enough to give a
statistically significant answer. This means that in practice there must be at least ten potentially informative meiosis (those in which potential cross-overs could be recognised) within a family in order to give a LOD score $\geq 3.00$. With the addition of the seven other members, power analysis with the SLINK software was performed again and it was found that the maximum possible LOD score for this family was now 3.41. This was sufficient to prove linkage as a LOD score of $>3.00$ is required to prove linkage. It was therefore decided that the Applied Biosystems Linkage mapping set version 2.5 would be used to continue the investigation of RLS in pedigree RLS3002.

Several epidemiological investigations have shown a female preponderance of RLS(Berger, Luedemann et al. 2004). This could indeed be a result of reporting bias. As pedigree RLS3002 is quite small it is difficult to say if there is a female prevalence. There are six affected females (seven if the grandmother is included), and there are five affected males.

There are considerable geographical variations in the prevalence of RLS. The cause of these variations are not well understood. The majority of these prevalence studies have also been performed on Caucasian populations. Prevalence estimates in East Asians include $0.1 \%$ in a Singapore survey (Tan, Seah et al. 2001) and $12 \%$ in a Korean survey (Kim, Choi et al. 2005). This study on an Irish Caucasian family is consistent with these findings.

The racial differences in RLS have yet to be understood. The prevalence of RLS in some ethnic groups may have been underestimated due to their under-representation in RLS speciality clinics. In a study by Lee et al. (2006) the prevalence of RLS in African-Americans and Caucasians were compared in a biracial community sample.

The prevalence of RLS in the overall population was $4.1 \%$. The rates were similar for African-Americans (4.7\%) and Caucasians (3.8\%). After adjustment for age, gender, medical comorbidities, and socioeconomic status, no difference in the prevalence of RLS was found between African-Americans and Caucasians. It is quite possible that under-representation difficulties are present in prevalence studies in countries such as India where access to speciality clinics may also be a problem. Barriers affecting access to care settings for RLS patients worldwide should be investigated in the future. Further investigations into the geographical and racial differences in RLS need to be carried out also.

Two candidate genes within the novel RLS locus 19p13.3 were screened. Exon 1 of KLF16 failed to sequence; however the results show no disease causing mutations in the exons and exon-intron junction regions of KLF16 exon 2 and GAMT. There is a possibility that the mutation is one which is not detectable using current sequencing techniques, for example, large deletions.

In order to exclude mutations in the remaining introns of KLF16 and GAMT further work is needed. There may be possible mutations within the introns of these genes that cause a cryptic splice site. These mutations would not have been detected by the approaches used in this study.

Further research is needed to screen other candidate genes within the disease gene interval. Genes which play a role in iron and dopamine pathways will be identified and prioritised for mutation screening. Failing to find a disease causing mutation in these genes, all other genes within the locus need to be screened as many do not have a known function as yet. Furthermore new mechanisms which have not yet been described may be involved in the pathophysiology. Reducing the size of the RLS
locus would reduce the number of genes to be examined. A number of approaches could be used to potentially decrease the size of the locus. Since all family members of RLS3002 have been recruited this approach is not an option. One approach to narrow the linkage region is to add more markers to the region to find additional recombinants. Although all known microsatellite markers in the region were examined, there is a possibility new markers may be identified in the future. Additional clinically defined RLS families could also be studied to determine whether any might be linked to the RLS 19p13.3 locus. This might allow for reduction of the locus

### 7.3 Conclusion and Future work

The results obtained in the Irish pedigree RLS3002 with autosomal dominant RLS showed evidence of a new RLS locus located on chromosome 19p13.3. To date linkage studies in RLS families have revealed 11 loci but no causally related sequence variant has yet been identified using this approach. Replication of RLS linkage studies has met with limited success. The first RLS locus, reported on chromosome 12 q in a study of a single French-Canadian family, was confirmed in an additional 5 of 18 families (Desautels, Turecki et al. 2005) , and extended to a study of 100 families in Iceland (Hicks AA 2005). RLS2, the second susceptibility locus identified on chromosome 14 q was initially seen in two large families from South Tyrol (Bonati, Ferini-Strambi et al. 2003) and subsequently identified in a single French-Canadian family (Levchenko, Montplaisir et al. 2004). This suggests that RLS is a genetically highly-heterogeneous disorder thus presenting a challenge to researchers hoping to unravel its pathophysiology. Further confirmation of existing RLS loci in different ethnic groups would be important as these might help prioritise and promote efforts to identify potential causative genes.

Symptoms of RLS are quite common. The prevalence of RLS severe enough to cause impairment in quality of life (QOL) and warrant medical treatment is probably around 2-3\%. This makes clinically significant RLS 2 to 6 times as common as epilepsy in developed countries (estimated prevalence of epilepsy is $0.5-1.0 \%$ ) (Natarajan). RLS can cause significant impairment of sleep and about $94 \%$ of patients report sleep disturbance due to RLS. As a result patients have daytime fatigue, reduced motivation, emotional stress, anxiety and difficulty accomplishing their jobs and participating in social activities. Studies have shown that RLS patients
are more prone to headaches than healthy subjects. Headaches are similar to migraine in character, though with an element of tension-type headache (d'Onofrio, Bussone et al. 2008). In addition, RLS may be linked to cardiovascular disease (Winkelman, Shahar et al. 2008) Currently, RLS is diagnosed solely on the clinical history, there are four essential criteria and all of them must be established to make a definite diagnosis. In addition there are three supportive criteria that are not necessary for a diagnosis of RLS but are admittedly helpful in the differential diagnosis. No other method has yet been discovered to diagnose RLS as effectively as the clinical criteria. The treatment of RLS is mainly symptomatic. Many therapeutic measures have been considered, some less effective than others. At present dopaminergic receptor agonists are considered the first choice in treating moderate to severe cases of RLS. However they can often be problematic, causing side effects such as augmentation; where in some patients the symptoms begin in late afternoon or in early evening as a result of treatment. Patients should also realize that pharmacological treatment is life-long in most cases. Ultimately if the causative gene(s) for RLS is identified, it would be a stepping stone to unravelling the etiology, and the development of therapeutic drugs to treat, prevent, and hopefully to cure this chronic distressing condition.(Garcia-Borreguero, Allen et al. 2007).

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## Appendices

## Appendix I: Restless Legs Syndrome Rating Scale

The patient rated his/her symptoms for the ten questions listed below. Ratings range from: none (0); mild (1); moderate (2); severe (3); and very severe (4). Ratings for question 3 include: none ( 0 ); mild ( 1 day a week or less) (1); moderate ( 2 to 3 days per week) (2); severe ( 4 to 5 days per week) (3); and very severe ( 6 to 7 days per week) (4). Ratings for question 4 include: none ( 0 ); mild (less than 1 hour per 24 hour day) (1); moderate ( 1 to 3 hours per 24 hour day) (2); severe ( 3 to 8 hours per 24 day) (3); and very severe ( 8 hours per 24 hour day or more) (4).



|  | 菏 | 8 <br> 7 <br> 7 <br> 7 <br> 8 | 8 B B 首 | 范 | 菏 |  |  | 菏 | $\xrightarrow{B}$ |  | $\overrightarrow{2}$ $\underset{N}{N}$ N |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 7．How often do you get RLS symptoms？ | 1 | 2 | 1 | 1 | 1 | 4 | 1 | 2 | $\begin{gathered} 1-2 \\ (1.5) \end{gathered}$ | 1 | 1 |
| 8．When you have RLS symptoms，how severe are they on an average day？ | 0 | 1 | 1 | 2 | 1 | 4 | 1 | 1 | 1 | 1 | 1 |
| 9．Overall，how severe is the impact of your RLS symptoms on your ability to carry out your daily affairs，for example carrying out a satisfactory family，home，social，school， or work life？ | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| 10．How severe is your mood disturbance from your RLS symptoms－for example angry，depressed，sad，anxious or irritable？ | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 0 |
| Total score： | 4 | 14 | 13 | 10 | 15 | 17 | 11 | 10 | 16 | 8 | 10 |

Scoring criteria are：None $=0$ points；mild＝1－10 points；moderate $=11-20$ points；severe $=21-30$ points；and Very severe $=31-40$ points．

Appendix II: Research Genetics Inc. mapping set

| Chromosome | Microsatellite Marker | Fluorescent Label | Position (cM) | Repeat Type | Heterozygosity | Size Range (bp) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | D1S1622 | FAM | 56.74 | ATA | 0.73 | 249-273 |
| 1 | D1S3728 | HEX | 89.49 | GATA | 0.63 | 244-268 |
| 1 | D1S1728 | HEX | 109.04 | GATA | 0.58 | 158-174 |
| 1 | D1S3723 | HEX | 140.39 | GATA | 0.67 | 142-206 |
| 1 | D1S1589 | TET | 192.05 | ATA | 0.69 | 201-214 |
| 1 | GATA124F08 | FAM | 226.16 | GATA | 0.79 | 229-245 |
| 1 | D1S3462 | TET | 247.23 | ATA | 0.75 | 256-271 |
| 1 | D1S547 | TET | 267.51 | GATA | 0.87 | 282-308 |
| 2 | D2S2952 | TET | 17.88 | GATA | 0.77 | 177-209 |
| 2 | D2S1400 | FAM | 27.60 | GGAA | 0.50 | 111-140 |
| 2 | D2S405 | TET | 47.97 | GATA | 0.68 | 240-256 |
| 2 | D2S1356 | TET | 64.29 | ATA | 0.95 | 218-262 |
| 2 | D2S2739 | FAM | 73.61 | GATA | 0.94 | 281-329 |
| 2 | D2S1777 | FAM | 99.41 | GATA | 0.64 | 196-208 |
| 2 | D2S1334 | HEX | 145.08 | GATA | 0.71 | 266-310 |
| 2 | D2S1776 | TET | 173.00 | GATA | 0.88 | 288-312 |
| 2 | D2S434 | FAM | 215.78 | GATA | 0.82 | 262-286 |
| 2 | D2S2968 | HEX | 251.94 | GATA | 0.59 | 171-195 |
| 2 | D2S125 | HEX | 260.63 | AC | 0.86 | 96-100 |
| 3 | D3S1304 | HEX | 22.33 | AC | 0.86 | 253-269 |
| 3 | D3S3038 | HEX | 44.81 | GATA | 0.81 | 187-219 |
| 3 | D3S4542 | FAM | 89.91 | GATA | 0.68 | 236-260 |
| 3 | D3S2406 | HEX | 102.64 | GGAT | 0.92 | 306-350 |
| 3 | D3S3045 | FAM | 124.16 | GATA | 0.99 | 170-211 |
| 3 | D3S1303 | FAM | 136.32 | AC | 0.93 | 196-220 |
| 3 | D3S3053 | TET | 181.87 | GATA | 0.68 | 226-238 |


| 3 | D3S1262 | FAM | 201.14 | AC | 0.78 | 112-126 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 3 | D3S3023 | TET | 126.98 | ATA | 0.82 | 228-249 |
| 4 | D4S403 | TET | 25.90 | AC | 0.61 | 217-231 |
| 4 | D4S2632 | FAM | 50.53 | GATA | 0.82 | 124-164 |
| 4 | D4S3248 | FAM | 72.52 | GATA | 0.65 | 233-257 |
| 4 | D4S3243 | TET | 88.35 | GATA | 0.61 | 162-174 |
| 4 | D4S1647 | TET | 104.94 | GATA | 0.79 | 132-156 |
| 4 | D4S1629 | HEX | 157.99 | GATA | 0.68 | 137-157 |
| 4 | D4S2417 | TET | 181.93 | GATA | 0.67 | 251-271 |
| 4 | D4S408 | HEX | 195.06 | AC | 0.85 | 229-243 |
| 5 | D5S817 | TET | 22.88 | GATA | 0.62 | 260-272 |
| 5 | D5S1470 | TET | 45.34 | GATA | 0.85 | 163-207 |
| 5 | D5S2500 | FAM | 69.23 | GATA | 0.75 | 149-181 |
| 5 | D5S1725 | FAM | 97.82 | GATA | 0.86 | 188-212 |
| 5 | D5S1505 | FAM | 129.83 | GATA | 0.74 | 243-275 |
| 5 | D5S1480 | TET | 147.49 | ATA | 0.86 | 208-249 |
| 5 | D5S211 | HEX | 182.89 | AC | 0.79 | 186-204 |
| 6 | D6S2434 | TET | 25.08 | ATA | 0.57 | 224-236 |
| 6 | D6S2439 | FAM | 42.27 | GATA | 0.76 | 218-258 |
| 6 | D6S2410 | HEX | 73.13 | GATA | 0.56 | 238-254 |
| 6 | D6S1040 | TET | 128.93 | GATA | 0.74 | 257-285 |
| 6 | D6S2436 | TET | 154.64 | GATA | 0.63 | 121-149 |
| 7 | D7S513 | FAM | 17.74 | AC | 0.84 | 173-201 |
| 7 | D7S3051 | HEX | 29.28 | GATA | 0.68 | 146-182 |
| 7 | D7S2846 | TET | 57.79 | GATA | 0.85 | 172-196 |
| 7 | D7S3046 | FAM | 78.65 | GATA | 0.69 | 318-346 |
| 7 | D7S821 | FAM | 109.12 | GATA | 0.82 | 238-270 |
| 7 | D7S3061 | FAM | 128.41 | GGAA | 0.82 | 114-146 |
| 7 | D7S1804 | TET | 136.95 | GATA | 0.86 | 250-290 |
| 7 | D7S1823 | HEX | 173.71 | GATA | 0.76 | 207-235 |
| 8 | D8S1130 | HEX | 22.41 | GATA | 0.78 | 132-156 |


| 8 | D8S136 | TET | 43.96 | AC | 0.79 | 71-80 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 8 | D8S1477 | FAM | 60.34 | GGAA | 0.89 | 139-179 |
| 8 | D8S532 | FAM | 64.75 | AC | 0.86 | 239-255 |
| 8 | D8S285 | FAM | 71.00 | AC | 0.81 | 108-124 |
| 8 | D8S260 | HEX | 79.36 | AC | 0.81 | 187-213 |
| 8 | D8S2324 | HEX | 94.08 | GATA | 0.74 | 196-216 |
| 8 | GAAT1A4 | TET | 110.20 | GAAT | 0.67 | 140-160 |
| 8 | D8S1179 | TET | 135.08 | GATA | 0.91 | 162-194 |
| 8 | D8S256 | FAM | 148.12 | AC | 0.81 | 210-232 |
| 9 | D9S921 | HEX | 21.88 | GATA | 0.87 | 175-232 |
| 9 | D9S1121 | FAM | 44.28 | GATA | 0.71 | 184-212 |
| 9 | D9S1118 | TET | 58.26 | GATA | 0.85 | 142-178 |
| 9 | D9S1122 | TET | 75.88 | GATA | 0.79 | 186-217 |
| 9 | D9S257 | HEX | 91.87 | AC | 0.93 | 259-285 |
| 9 | D9S282 | FAM | 136.47 | AC | 0.74 | 233-242 |
| 9 | D9S2157 | TET | 146.83 | ATA | 0.82 | 132-173 |
| 10 | D10S189 | HEX | 19.00 | AC | 0.85 | 175-195 |
| 10 | D10S1423 | FAM | 46.23 | GATA | 0.83 | 218-238 |
| 10 | D10S1208 | FAM | 63.30 | ATA | 0.83 | 179-200 |
| 10 | ATA5A04 | FAM | 88.41 | GATA | 0.63 | 184-200 |
| 10 | D10S2327 | HEX | 100.92 | GGAT | 0.58 | 204-228 |
| 10 | D10S1239 | TET | 125.41 | GATA | 0.78 | 160-184 |
| 10 | D10S1230 | TET | 142.78 | ATA | 0.71 | 113-140 |
| 10 | D10S212 | TET | 170.94 | AC | 0.65 | 189-201 |
| 11 | D11S1981 | FAM | 21.47 | GATA | 0.84 | 134-178 |
| 11 | D11S2371 | FAM | 76.13 | GATA | 0.64 | 188-220 |
| 11 | D11S2000 | HEX | 100.62 | GATA | 0.92 | 199-235 |
| 11 | D11S4090 | FAM | 105.74 | CA | 0.80 | 167-191 |
| 11 | D11S1998 | HEX | 113.13 | GATA | 0.69 | 129-169 |
| 11 | D11S912 | TET | 131.26 | AC | 0.93 | 101-123 |
| 12 | D12S398 | FAM | 68.16 | GGAT | 0.71 | 120-144 |


| 12 | D12S1300 | TET | 104.12 | GATA | 0.68 | 112-136 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 12 | D12S2070 | TET | 125.30 | ATA | 0.89 | 89-107 |
| 12 | D12S2078 | TET | 149.60 | GATA | 0.89 | 250-282 |
| 13 | D13S1493 | FAM | 25.80 | GGAA | 0.92 | 215-260 |
| 13 | D13S800 | HEX | 55.31 | GATA | 0.74 | 295-319 |
| 13 | D13S793 | TET | 76.26 | GATA | 0.74 | 253-273 |
| 13 | D13S779 | TET | 82.93 | ATA | 0.50 | 175-210 |
| 14 | D14S608 | HEX | 28.01 | GATA | 0.78 | 188-224 |
| 14 | D14S599 | FAM | 40.68 | ATA | 0.85 | 98-133 |
| 14 | D14S978 | HEX | 53.19 | CA | 0.89 | 228-258 |
| 14 | D14S989 | FAM | 55.29 | CA | 0.57 | 161-185 |
| 14 | D14S587 | HEX | 55.82 | GGAA | 0.81 | 254-274 |
| 14 | D14S592 | TET | 66.80 | ATA | 0.78 | 220-250 |
| 14 | D14S588 | TET | 75.61 | GGAA | 0.68 | 110-145 |
| 14 | D14S53 | FAM | 86.29 | CA | 0.66 | 144-161 |
| 14 | D14S610 | FAM | 95.89 | GATA | 0.73 | 360-376 |
| 14 | D14S1279 | TET | 95.89 | GATA | 0.68 | 107-143 |
| 14 | D14S617 | FAM | 105.53 | GGAA | 0.76 | 141-173 |
| 15 | D15S1507 | TET | 60.17 | GATA | 0.65 | 205-221 |
| 15 | D15S211 | HEX | 75.85 | AC | 0.95 | 207-261 |
| 15 | D15S816 | FAM | 100.59 | GATA | 0.56 | 130-150 |
| 16 | D16S403 | HEX | 43.89 | AC | 0.86 | 120-160 |
| 16 | D16S3253 | TET | 71.77 | GATA | 0.78 | 167-199 |
| 17 | D17S974 | FAM | 22.24 | GATA | 0.82 | 201-217 |
| 17 | D17S947 | FAM | 31.96 | AC | 0.82 | 250-280 |
| 17 | D17S2196 | FAM | 44.62 | GATA | 0.87 | 139-163 |
| 17 | D17S2180 | HEX | 66.85 | ATC | 0.64 | 116-128 |
| 17 | D17S2193 | TET | 89.32 | ATA | 0.71 | 88-123 |
| 17 | D17S784 | TET | 116.86 | AC | 0.78 | 226-238 |
| 18 | D18S976 | HEX | 12.81 | GATA | 0.92 | 171-198 |
| 18 | D18S542 | FAM | 41.24 | GATA | 0.75 | 178-205 |


| 18 | D18S877 | TET | 54.40 | GATA | 0.75 | $110-150$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 18 | D18S1357 | HEX | 88.62 | ATA | 0.82 | $126-147$ |
| 18 | ATA82B02 | TET | 106.81 | ATA | 0.86 | $172-196$ |
| 19 | D19S1034 | FAM | 20.75 | GATA | 0.71 | $216-232$ |
| 19 | D19S586 | HEX | 32.94 | GATA | 0.79 | $222-250$ |
| 19 | D19S178 | FAM | 68.08 | AC | 0.77 | $155-189$ |
| 19 | D19S589 | HEX | 87.66 | GATA | 0.64 | $161-181$ |
| 20 | D20S482 | TET | 12.12 | GATA | 0.75 | $151-167$ |
| 20 | D20S851 | HEX | 24.70 | AV | 0.73 | $128-150$ |
| 21 | D21S2055 | HEX | 40.49 | GATA | 0.85 | $119-199$ |
| 22 | GCT10C10 | FAM | 18.10 | GCT | 0.68 | $192-210$ |

Appendix III: Applied Biosystems Linkage mapping set version 2.5

| Panel | Chromosome | Microsatellite Marker | Fluorescent Label | Position (cM) | Het | Size Range (bp) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | 1 | D1S2797 | FAM | 75.66 | 0.74 | 97-135 |
| 1 | 1 | D1S249 | FAM | 220.65 | 0.87 | 160-190 |
| 1 | 1 | D1S2800 | FAM | 252.12 | 0.77 | 205-221 |
| 1 | 1 | D1S234 | FAM | 55.1 | 0.81 | 262-284 |
| 1 | 1 | D1S450 | FAM | 20.61 | 0.81 | 315-341 |
| 1 | 1 | D1S255 | VIC | 65.47 | 0.75 | 85-107 |
| 1 | 1 | D1S2667 | VIC | 24.68 | 0.82 | 122-152 |
| 1 | 1 | D1S2785 | VIC | 266.27 | 0.76 | 171-185 |
| 1 | 1 | D1S2890 | VIC | 85.68 | 0.81 | 211-235 |
| 1 | 1 | D1S484 | VIC | 169.68 | 0.64 | 272-286 |
| 1 | 1 | D1S196 | VIC | 181.49 | 0.74 | 321-337 |
| 1 | 1 | D1S213 | NED | 242.34 | 0.86 | 103-129 |
| 1 | 1 | D1S2878 | NED | 177.86 | 0.84 | 148-176 |
| 1 | 1 | D1S206 | NED | 134.2 | 0.82 | 205-223 |
| 1 | 1 | D1S2836 | NED | 285.75 | 0.79 | 242-256 |
| 1 | 1 | D1S2726 | NED | 144.38 | 0.75 | 280-294 |
| 1 | 1 | D1S2842 | NED | 273.46 | 0.76 | 336-358 |
| 2 | 1 | D1S199 | FAM | 45.33 | 0.83 | 94-120 |
| 2 | 1 | D1S207 | FAM | 113.69 | 0.84 | 146-176 |
| 2 | 1 | D1S2868 | FAM | 126.16 | 0.76 | 206-220 |
| 2 | 1 | D1S413 | FAM | 212.44 | 0.76 | 249-265 |
| 2 | 1 | D1S238 | FAM | 202.73 | 0.86 | 292-326 |
| 2 | 1 | D1S252 | VIC | 150.27 | 0.81 | 86-112 |
| 2 | 1 | D1S230 | VIC | 95.31 | 0.78 | 150-164 |
| 2 | 1 | D1S468 | VIC | 4.22 | 0.76 | 191-211 |
| 2 | 1 | D1S2841 | VIC | 106.45 | 0.78 | 230-250 |
| 2 | 1 | D1S2697 | VIC | 37.05 | 0.70 | 286-302 |
| 2 | 1 | D1S214 | NED | 14.04 | 0.78 | 117-147 |


| 2 | 1 | D1S498 | NED | 155.89 | 0.82 | 187-209 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 2 | 1 | D1S218 | NED | 191.52 | 0.83 | 265-291 |
| 2 | 1 | D1S425 | NED | 231.11 | 0.81 | 332-358 |
| 3 | 2 | D2S286 | FAM | 94.05 | 0.66 | 80-102 |
| 3 | 2 | D2S165 | FAM | 47.43 | 0.85 | 141-175 |
| 3 | 2 | D2S160 | FAM | 122.96 | 0.78 | 206-224 |
| 3 | 2 | D2S2211 | FAM | 15.61 | 0.74 | 236-258 |
| 3 | 2 | D2S367 | FAM | 54.96 | 0.86 | 306-340 |
| 3 | 2 | D2S125 | VIC | 260.63 | 0.82 | 87-109 |
| 3 | 2 | D2S206 | VIC | 240.79 | 0.8 | 125-163 |
| 3 | 2 | D2S117 | VIC | 194.45 | 0.82 | 190-220 |
| 3 | 2 | D2S142 | VIC | 161.26 | 0.76 | 235-255 |
| 3 | 2 | D2S2333 | NED | 103.16 | 0.82 | 79-101 |
| 3 | 2 | D2S126 | NED | 221.13 | 0.82 | 113-145 |
| 3 | 2 | D2S325 | NED | 204.53 | 0.82 | 154-184 |
| 3 | 2 | D2S364 | NED | 186.21 | 0.80 | 230-256 |
| 3 | 2 | D2S337 | NED | 80.69 | 0.88 | 291-315 |
| 4 | 2 | D2S112 | FAM | 141.62 | 0.71 | 73-89 |
| 4 | 2 | D2S162 | FAM | 20.03 | 0.75 | 119-148 |
| 4 | 2 | D2S2330 | FAM | 169.41 | 0.81 | 166-186 |
| 4 | 2 | D2S2216 | FAM | 111.21 | 0.76 | 208-224 |
| 4 | 2 | D2S347 | FAM | 131.51 | 0.80 | 267-299 |
| 4 | 2 | D2S2259 | FAM | 64.29 | 0.79 | 321-341 |
| 4 | 2 | D2S319 | VIC | 7.6 | 0.73 | 128-140 |
| 4 | 2 | D2S168 | VIC | 27.06 | 0.82 | 156-180 |
| 4 | 2 | D2S151 | VIC | 152.04 | 0.82 | 224-252 |
| 4 | 2 | D2S2382 | VIC | 213.49 | 0.81 | 296-336 |
| 4 | 2 | D2S2368 | NED | 85.48 | 0.83 | 93-117 |
| 4 | 2 | D2S391 | NED | 70.31 | 0.79 | 143-159 |
| 4 | 2 | D2S335 | NED | 175.91 | 0.79 | 183-205 |
| 4 | 2 | D2S396 | NED | 232.9 | 0.83 | 232-250 |
| 4 | 2 | D2S338 | NED | 250.54 | 0.81 | 264-288 |
| 4 | 2 | D2S305 | NED | 38.87 | 0.72 | 314-336 |
| 5 | 4 | D4S392 | FAM | 78.97 | 0.82 | 79-109 |


| 5 | 3 | D3S1311 | FAM | 224.88 | 0.83 | 133-159 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 5 | 3 | D3S1565 | FAM | 186.04 | 0.64 | 178-194 |
| 5 | 4 | D4S406 | FAM | 117.06 | 0.87 | 242-268 |
| 5 | 4 | D4S1575 | FAM | 132.05 | 0.65 | 287-305 |
| 5 | 3 | D3S1271 | VIC | 117.76 | 0.73 | 84-104 |
| 5 | 3 | D3S3681 | VIC | 109.22 | 0.83 | 121-161 |
| 5 | 4 | D4S414 | VIC | 100.75 | 0.89 | 231-249 |
| 5 | 4 | D4S405 | VIC | 56.95 | 0.86 | 281-309 |
| 5 | 3 | D3S1614 | NED | 177.75 | 0.83 | 101-125 |
| 5 | 3 | D4S1534 | NED | 95.09 | 0.77 | 147-169 |
| 5 | 3 | D3S1263 | NED | 36.1 | 0.86 | 191-211 |
| 5 | 3 | D3S1285 | NED | 91.18 | 0.73 | 233-251 |
| 5 | 3 | D4S1597 | NED | 169.42 | 0.76 | 274-300 |
| 6 | 3 | D3S1262 | FAM | 201.14 | 0.80 | 110-132 |
| 6 | 3 | D3S1569 | FAM | 158.38 | 0.80 | 150-174 |
| 6 | 4 | D4S1572 | FAM | 107.95 | 0.84 | 195-213 |
| 6 | 3 | D3S1300 | FAM | 80.32 | 0.82 | 230-262 |
| 6 | 4 | D4S413 | FAM | 157.99 | 0.85 | 282-334 |
| 6 | 4 | D4S2935 | VIC | 13.96 | 0.62 | 85-105 |
| 6 | 4 | D4S1592 | VIC | 69.53 | 0.72 | 113-141 |
| 6 | 4 | D4S391 | VIC | 43.59 | 0.85 | 150-170 |
| 6 | 3 | D3S1304 | VIC | 22.33 | 0.80 | 254-276 |
| 6 | 3 | D3S1601 | VIC | 214.45 | 0.85 | 298-330 |
| 6 | 3 | D3S1297 | VIC | 8.31 | 0.82 | 351-369 |
| 6 | 3 | D3S1292 | NED | 146.6 | 0.85 | 111-145 |
| 6 | 4 | D4S426 | NED | 206.98 | 0.76 | 160-180 |
| 6 | 4 | D4S419 | NED | 33.42 | 0.77 | 225-245 |
| 6 | 4 | D4S415 | NED | 181.36 | 0.80 | 264-300 |
| 7 | 4 | D4S402 | FAM | 124.45 | 0.91 | 106-146 |
| 7 | 4 | D4S403 | FAM | 25.9 | 0.77 | 170-186 |
| 7 | 4 | D3S1580 | FAM | 207.73 | 0.84 | 215-235 |
| 7 | 4 | D3S1279 | FAM | 169.6 | 0.85 | 268-286 |
| 7 | 4 | D4S1539 | FAM | 176.19 | 0.68 | 316-326 |
| 7 | 4 | D3S2338 | VIC | 42.1 | 0.86 | 89-109 |


| 7 | 4 | D4S2964 | VIC | 88.35 | 0.76 | 119-143 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 7 | 4 | D4S412 | VIC | 4.74 | 0.77 | 158-176 |
| 7 | 4 | D4S424 | VIC | 144.56 | 0.83 | 194-212 |
| 7 | 4 | D3S1278 | VIC | 129.73 | 0.87 | 232-260 |
| 7 | 4 | D3S1266 | VIC | 52.6 | 0.73 | 289-305 |
| 7 | 4 | D3S1267 | NED | 139.12 | 0.88 | 93-131 |
| 7 | 4 | D3S1566 | NED | 97.75 | 0.84 | 155-177 |
| 7 | 4 | D3S1289 | NED | 71.41 | 0.81 | 202-224 |
| 7 | 4 | D4S1535 | NED | 195.06 | 0.77 | 248-262 |
| 7 | 4 | D3S1277 | NED | 61.52 | 0.82 | 289-311 |
| 8 | 5 | D5S407 | FAM | 64.67 | 0.86 | 82-110 |
| 8 | 6 | D6S289 | FAM | 29.93 | 0.79 | 160-182 |
| 8 | 6 | D6S1610 | FAM | 53.81 | 0.84 | 200-214 |
| 8 | 6 | D6S1581 | FAM | 164.78 | 0.72 | 257-271 |
| 8 | 6 | D6S422 | FAM | 35.66 | 0.77 | 298-320 |
| 8 | 5 | D5S644 | VIC | 104.76 | 0.85 | 82-112 |
| 8 | 6 | D6S281 | VIC | 190.14 | 0.68 | 131-151 |
| 8 | 6 | D6S262 | VIC | 130 | 0.82 | 169-189 |
| 8 | 5 | D5S424 | VIC | 81.95 | 0.76 | 212-234 |
| 8 | 5 | D5S419 | VIC | 39.99 | 0.81 | 255-287 |
| 8 | 5 | D5S433 | NED | 111.97 | 0.86 | 63-93 |
| 8 | 5 | D5S422 | NED | 164.19 | 0.84 | 113-135 |
| 8 | 5 | D5S406 | NED | 11.85 | 0.79 | 164-192 |
| 8 | 5 | D5S400 | NED | 174.8 | 0.82 | 217-239 |
| 8 | 6 | D6S309 | NED | 14.07 | 0.83 | 304-330 |
| 9 | 6 | D6S264 | FAM | 179.07 | 0.70 | 108-130 |
| 9 | 6 | D6S1574 | FAM | 9.18 | 0.84 | 146-172 |
| 9 | 6 | D6S276 | FAM | 44.41 | 0.83 | 201-233 |
| 9 | 5 | D5S408 | FAM | 195.49 | 0.73 | 249-285 |
| 9 | 6 | D6S308 | FAM | 144.46 | 0.75 | 326-354 |
| 9 | 6 | D6S287 | VIC | 121.97 | 0.85 | 105-139 |
| 9 | 6 | D6S292 | VIC | 136.97 | 0.83 | 155-177 |
| 9 | 6 | D6S434 | VIC | 109.19 | 0.86 | 202-246 |
| 9 | 5 | D5S426 | VIC | 51.99 | 0.80 | 275-299 |


| 9 | 5 | D5S1981 | NED | 1.72 | 0.73 | 115-125 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 9 | 6 | D6S257 | NED | 79.92 | 0.87 | 167-195 |
| 9 | 6 | D6S446 | NED | 189 | 0.62 | 217-229 |
| 9 | 5 | D5S641 | NED | 92.38 | 0.77 | 299-339 |
| 10 | 5 | D5S2027 | FAM | 119.5 | 0.78 | 180-202 |
| 10 | 5 | D5S436 | FAM | 147.49 | 0.83 | 238-258 |
| 10 | 6 | D6S460 | FAM | 89.83 | 0.81 | 279-303 |
| 10 | 5 | D5S410 | FAM | 156.47 | 0.79 | 329-351 |
| 10 | 6 | D6S462 | VIC | 99.01 | 0.68 | 104-121 |
| 10 | 5 | D5S2115 | VIC | 138.64 | 0.76 | 142-170 |
| 10 | 5 | D5S418 | VIC | 58.55 | 0.80 | 208-228 |
| 10 | 5 | D5S428 | VIC | 95.4 | 0.76 | 241-259 |
| 10 | 5 | D5S630 | VIC | 19.67 | 0.89 | 283-393 |
| 10 | 6 | D6S470 | NED | 18.22 | 0.80 | 120-140 |
| 10 | 6 | D6S441 | NED | 154.1 | 0.86 | 162-196 |
| 10 | 5 | D5S471 | NED | 129.83 | 0.76 | 235-255 |
| 10 | 5 | D5S416 | NED | 28.76 | 0.77 | 285-297 |
| 10 | 5 | D5S647 | NED | 74.07 | 0.82 | 326-365 |
| 11 | 7 | D7S484 | FAM | 53.5 | 0.74 | 97-115 |
| 11 | 8 | D8S264 | FAM | 0.73 | 0.83 | 136-160 |
| 11 | 8 | D8S260 | FAM | 79.36 | 0.81 | 191-217 |
| 11 | 7 | D7S517 | FAM | 7.44 | 0.83 | 243-261 |
| 11 | 8 | D8S1784 | FAM | 118.15 | 0.67 | 276-292 |
| 11 | 7 | D7S2465 | FAM | 180.24 | 0.83 | 319-343 |
| 11 | 8 | D8S549 | VIC | 31.73 | 0.63 | 73-83 |
| 11 | 7 | D7S530 | VIC | 134.55 | 0.78 | 105-123 |
| 11 | 8 | D8S258 | VIC | 41.55 | 0.70 | 142-156 |
| 11 | 7 | D7S669 | VIC | 90.42 | 0.80 | 172-194 |
| 11 | 8 | D8S272 | VIC | 154.02 | 0.81 | 211-261 |
| 11 | 7 | D7S502 | VIC | 78.65 | 0.84 | 289-309 |
| 11 | 7 | D7S630 | VIC | 98.44 | 0.73 | 327-355 |
| 11 | 7 | D7S510 | NED | 59.93 | 0.77 | 79-95 |
| 11 | 7 | D7S640 | NED | 137.83 | 0.85 | 110-150 |
| 11 | 7 | D7S513 | NED | 17.74 | 0.83 | 168-198 |


| 11 | 8 | D8S514 | NED | 130 | 0.77 | 212-232 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 11 | 7 | D7S657 | NED | 104.86 | 0.81 | 244-270 |
| 11 | 7 | D7S516 | NED | 41.69 | 0.76 | 306-326 |
| 11 | 8 | D8S1771 | NED | 50.05 | 0.75 | 343-367 |
| 12 | 7 | D7S507 | FAM | 28.74 | 0.89 | 81-109 |
| 12 | 7 | D7S515 | FAM | 112.32 | 0.82 | 139-201 |
| 12 | 7 | D7S486 | FAM | 124.08 | 0.81 | 221-235 |
| 12 | 7 | D7S519 | FAM | 69.03 | 0.81 | 257-284 |
| 12 | 7 | D7S661 | FAM | 155.1 | 0.75 | 305-337 |
| 12 | 7 | D7S798 | VIC | 168.98 | 0.84 | 71.26-93 |
| 12 | 8 | D8S505 | VIC | 60.87 | 0.79 | 110-124 |
| 12 | 8 | D8S277 | VIC | 8.34 | 0.73 | 151-185 |
| 12 | 7 | D7S493 | VIC | 34.69 | 0.88 | 203-235 |
| 12 | 8 | D8S284 | VIC | 143.82 | 0.83 | 272-306 |
| 12 | 7 | D7S684 | VIC | 147.22 | 0.81 | 341-363 |
| 12 | 8 | D8S270 | NED | 103.69 | 0.79 | 101-117 |
| 12 | 7 | D7S636 | NED | 162.33 | 0.90 | 136-172 |
| 12 | 8 | D8S550 | NED | 21.33 | 0.87 | 187-217 |
| 12 | 7 | D7S531 | NED | 5.28 | 0.77 | 276-294 |
| 12 | 8 | D8S285 | NED | 71 | 0.78 | 314-330 |
| 13 | 11 | D11S987 | FAM | 67.48 | 0.82 | 94-134 |
| 13 | 11 | D11S937 | FAM | 79.98 | 0.88 | 144-180 |
| 13 | 11 | D11S935 | FAM | 45.94 | 0.73 | 196-218 |
| 13 | 9 | D9S1677 | FAM | 117.37 | 0.81 | 229-255 |
| 13 | 11 | D11S1314 | VIC | 73.64 | 0.78 | 93-121 |
| 13 | 11 | D11S902 | VIC | 21.47 | 0.80 | 148-170 |
| 13 | 11 | D11S904 | VIC | 33.57 | 0.83 | 183-213 |
| 13 | 11 | D10S547 | VIC | 29.15 | 0.74 | 236-257 |
| 13 | 11 | D11S905 | VIC | 51.95 | 0.75 | 269-297 |
| 13 | 9 | D9S285 | NED | 29.52 | 0.78 | 80-110 |
| 13 | 10 | D10S249 | NED | 2.13 | 0.74 | 117-139 |
| 13 | 9 | D9S171 | NED | 42.73 | 0.79 | 160-186 |
| 13 | 9 | D9S273 | NED | 65.79 | 0.74 | 202-222 |
| 13 | 10 | D10S192 | NED | 124.27 | 0.77 | 238-264 |


| 13 | 11 | D11S4175 | NED | 91.47 | 0.89 | 288-340 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 14 | 9 | D9S161 | FAM | 51.81 | 0.78 | 122-139 |
| 14 | 10 | D10S197 | FAM | 52.1 | 0.75 | 166-180 |
| 14 | 10 | D10S185 | FAM | 116.34 | 0.77 | 201-219 |
| 14 | 9 | D9S175 | FAM | 70.33 | 0.85 | 255-289 |
| 14 | 11 | D11S901 | FAM | 85.48 | 0.82 | 311-327 |
| 14 | 10 | D10S1653 | VIC | 40.36 | 0.77 | 118-132 |
| 14 | 10 | D10S212 | VIC | 170.94 | 0.71 | 189-207 |
| 14 | 10 | D10S1686 | VIC | 105.04 | 0.86 | 243-281 |
| 14 | 9 | D9S287 | VIC | 103.42 | 0.67 | 295-315 |
| 14 | 11 | D11S4046 | NED | 2.79 | 0.86 | 101-125 |
| 14 | 9 | D9S288 | NED | 9.83 | 0.84 | 132-154 |
| 14 | 10 | D10S208 | NED | 60.64 | 0.79 | 173-193 |
| 14 | 9 | D9S157 | NED | 32.24 | 0.84 | 225-249 |
| 14 | 9 | D9S167 | NED | 83.41 | 0.87 | 304-338 |
| 15 | 9 | D9S164 | FAM | 147.91 | 0.80 | 84-102 |
| 15 | 9 | D9S286 | FAM | 18.06 | 0.88 | 136-170 |
| 15 | 9 | D9S1690 | FAM | 106.63 | 0.78 | 226-240 |
| 15 | 11 | D11S1320 | FAM | 141.91 | 0.68 | 259-277 |
| 15 | 11 | D11S4151 | FAM | 127.33 | 0.79 | 331-345 |
| 15 | 11 | D11S4191 | VIC | 60.09 | 0.87 | 89-119 |
| 15 | 11 | D11S968 | VIC | 147.77 | 0.81 | 140-162 |
| 15 | 9 | D9S1776 | VIC | 123.33 | 0.84 | 172-210 |
| 15 | 11 | D11S1338 | VIC | 12.92 | 0.74 | 253-271 |
| 15 | 10 | D10S591 | VIC | 13.49 | 0.71 | 309-339 |
| 15 | 10 | D10S587 | NED | 147.57 | 0.80 | 92-114 |
| 15 | 10 | D10S537 | NED | 91.13 | 0.83 | 139-165 |
| 15 | 10 | D10S189 | NED | 19 | 0.72 | 179-197 |
| 15 | 11 | D11S925 | NED | 118.47 | 0.84 | 260-290 |
| 16 | 10 | D10S217 | FAM | 157.89 | 0.81 | 96-120 |
| 16 | 11 | D11S898 | FAM | 98.98 | 0.85 | 141-165 |
| 16 | 10 | D10S548 | FAM | 45.7 | 0.70 | 182-198 |
| 16 | 9 | D9S1826 | FAM | 159.61 | 0.69 | 215-231 |
| 16 | 9 | D9S290 | FAM | 140.86 | 0.83 | 240-262 |


| 16 | 9 | D91817 | FAM | 59.34 | 0.88 | 279-315 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 16 | 9 | D9S158 | FAM | 161.71 | 0.69 | 330-356 |
| 16 | 10 | D10S196 | VIC | 70.23 | 0.77 | 103-115 |
| 16 | 9 | D9S1682 | VIC | 132.09 | 0.68 | 147-159 |
| 16 | 11 | D11S908 | VIC | 108.59 | 0.76 | 172-190 |
| 16 | 10 | D10S1693 | VIC | 137.39 | 0.80 | 213-227 |
| 16 | 10 | D10S597 | VIC | 128.73 | 0.64 | 273-297 |
| 16 | 9 | D9S283 | NED | 94.85 | 0.80 | 89-115 |
| 16 | 10 | D10S1651 | NED | 168.77 | 0.80 | 206-230 |
| 16 | 10 | D10S1652 | NED | 80.77 | 0.78 | 269-295 |
| 17 | 12 | D12S83 | FAM | 75.17 | 0.81 | 102-122 |
| 17 | 13 | D13S218 | FAM | 32.9 | 0.66 | 141-153 |
| 17 | 12 | D12S78 | FAM | 111.87 | 0.91 | 174-212 |
| 17 | 13 | D13S217 | FAM | 17.21 | 0.68 | 242-262 |
| 17 | 12 | D12S1659 | FAM | 155.94 | 0.78 | 290-316 |
| 17 | 13 | D13S285 | VIC | 110.55 | 0.81 | 89-115 |
| 17 | 13 | D13S170 | VIC | 63.9 | 0.90 | 143-173 |
| 17 | 12 | D12S1723 | VIC | 164.63 | 0.67 | 198-216 |
| 17 | 13 | D13S175 | NED | 6.03 | 0.76 | 101-119 |
| 17 | 13 | D13S263 | NED | 38.32 | 0.84 | 146-174 |
| 17 | 12 | D12S346 | NED | 104.65 | 0.84 | 188-214 |
| 17 | 12 | D12S1617 | NED | 44.03 | 0.80 | 245-265 |
| 18 | 12 | D12S85 | FAM | 61.34 | 0.67 | 99-131 |
| 18 | 12 | D12S351 | FAM | 95.56 | 0.75 | 147-169 |
| 18 | 12 | D12S368 | FAM | 66.03 | 0.81 | 202-222 |
| 18 | 13 | D13S1265 | FAM | 98.82 | 0.80 | 275-305 |
| 18 | 12 | D12S86 | VIC | 134.54 | 0.89 | 129-169 |
| 18 | 13 | D13S156 | VIC | 55.85 | 0.80 | 277-297 |
| 18 | 12 | D12S336 | NED | 19.68 | 0.82 | 111-129 |
| 18 | 12 | D12S79 | NED | 125.31 | 0.87 | 160-186 |
| 18 | 12 | D12S345 | NED | 53.09 | 0.87 | 211-247 |
| 18 | 12 | D12S99 | NED | 12.6 | 0.83 | 264-296 |
| 19 | 13 | D13S158 | FAM | 84.87 | 0.82 | 116-133 |
| 19 | 13 | D13S159 | FAM | 79.49 | 0.90 | 154-196 |


| 19 | 13 | D13S173 | FAM | 93.52 | 0.82 | 232-252 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 19 | 12 | D12S364 | FAM | 30.6 | 0.87 | 298-326 |
| 19 | 13 | D13S265 | VIC | 98.82 | 0.70 | 89-127 |
| 19 | 12 | D12S352 | VIC | 0 | 0.73 | 154-174 |
| 19 | 12 | D12S326 | VIC | 86.4 | 0.80 | 207-233 |
| 19 | 12 | D12S310 | VIC | 36.06 | 0.69 | 244-252 |
| 19 | 13 | D13S153 | NED | 45.55 | 0.81 | 89-121 |
| 19 | 13 | D13S171 | NED | 25.08 | 0.73 | 177-205 |
| 19 | 12 | D12S324 | NED | 147.17 | 0.69 | 233-255 |
| 20 | 14 | D14S292 | FAM | 134.3 | 0.73 | 83-100 |
| 20 | 14 | D14S275 | FAM | 28.01 | 0.70 | 145-159 |
| 20 | 14 | D14S258 | FAM | 76.28 | 0.79 | 192-213 |
| 20 | 14 | D14S280 | FAM | 105 | 0.68 | 237-258 |
| 20 | 14 | D14S70 | VIC | 40.11 | 0.75 | 98-114 |
| 20 | 14 | D14S283 | VIC | 13.89 | 0.81 | 127-158 |
| 20 | 14 | D14S63 | VIC | 69.18 | 0.76 | 177-195 |
| 20 | 14 | D14S985 | VIC | 126.61 | 0.76 | 240-255 |
| 20 | 14 | D14S74 | VIC | 87.36 | 0.79 | 297-321 |
| 20 | 14 | D14S65 | NED | 117.3 | 0.79 | 124-156 |
| 20 | 14 | D14S288 | NED | 47.51 | 0.83 | 192-215 |
| 20 | 14 | D14S276 | NED | 56.36 | 0.76 | 236-249 |
| 20 | 14 | D14S261 | NED | 6.46 | 0.75 | 273-305 |
| 20 | 14 | D14S68 | NED | 95.89 | 0.91 | 318-346 |
| 21 | 16 | D16S3075 | FAM | 23.28 | 0.79 | 76-94 |
| 21 | 16 | D16S3136 | FAM | 62.11 | 0.69 | 173-185 |
| 21 | 16 | D16S3068 | FAM | 48.53 | 0.77 | 219-235 |
| 21 | 15 | D15S130 | FAM | 100.59 | 0.66 | 285-299 |
| 21 | 16 | D16S515 | FAM | 92.1 | 0.80 | 325-357 |
| 21 | 15 | D15S1002 | VIC | 14.58 | 0.78 | 104-134 |
| 21 | 16 | D16S520 | VIC | 125.82 | 0.84 | 149-165 |
| 21 | 15 | D15S165 | VIC | 20.24 | 0.79 | 182-213 |
| 21 | 15 | D15S131 | VIC | 71.28 | 0.83 | 240-281 |
| 21 | 16 | D16S503 | VIC | 83.55 | 0.81 | 299-319 |
| 21 | 15 | D15S127 | NED | 86.81 | 0.86 | 118-154 |


| 21 | 16 | D16S3091 | NED | 111.12 | 0.73 | 166-182 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 21 | 15 | D15S153 | NED | 62.4 | 0.87 | 240-274 |
| 21 | 15 | D15S117 | NED | 51.21 | 0.78 | 321-339 |
| 22 | 16 | D16S3046 | FAM | 40.65 | 0.74 | 83-109 |
| 22 | 15 | D15S205 | FAM | 78.92 | 0.88 | 126-166 |
| 22 | 16 | D16S415 | FAM | 67.62 | 0.72 | 213-241 |
| 22 | 15 | D15S1012 | VIC | 35.95 | 0.72 | 94-112 |
| 22 | 16 | D16S423 | VIC | 10.36 | 0.73 | 137-161 |
| 22 | 15 | D15S978 | VIC | 45.62 | 0.83 | 184-212 |
| 22 | 16 | D16S404 | VIC | 18.07 | 0.80 | 261-281 |
| 22 | 16 | D16S3103 | VIC | 32.07 | 0.81 | 315-343 |
| 22 | 15 | D15S1007 | NED | 25.86 | 0.86 | 84-108 |
| 22 | 15 | D15S120 | NED | 112.58 | 0.73 | 155-183 |
| 22 | 15 | D15S128 | NED | 6.11 | 0.78 | 197-215 |
| 22 | 16 | D16S516 | NED | 100.39 | 0.73 | 245-267 |
| 22 | 15 | D15S994 | NED | 40.25 | 0.73 | 303-315 |
| 23 | 18 | D18S70 | FAM | 126 | 0.83 | 111-131 |
| 23 | 17 | D17S949 | FAM | 93.27 | 0.80 | 210-228 |
| 23 | 18 | D18S478 | FAM | 52.86 | 0.64 | 242-256 |
| 23 | 17 | D17S1852 | FAM | 22.24 | 0.87 | 279-310 |
| 23 | 17 | D17S831 | VIC | 6.6 | 0.82 | 107-129 |
| 23 | 17 | D17S1857 | VIC | 43.01 | 0.64 | 164-174 |
| 23 | 17 | D17S799 | VIC | 31.96 | 0.68 | 186-208 |
| 23 | 17 | D17S1868 | VIC | 64.16 | 0.73 | 254-268 |
| 23 | 17 | D17S798 | VIC | 53.41 | 0.80 | 298-322 |
| 23 | 18 | D18S1102 | NED | 62.84 | 0.79 | 90-102 |
| 23 | 17 | D17S787 | NED | 74.99 | 0.81 | 138-174 |
| 23 | 18 | D18S61 | NED | 105.03 | 0.87 | 209-239 |
| 23 | 17 | D17S849 | NED | 0.63 | 0.67 | 253-267 |
| 23 | 18 | D18S462 | NED | 120.05 | 0.70 | 296-318 |
| 24 | 18 | D18S474 | FAM | 71.32 | 0.82 | 121-143 |
| 24 | 18 | D18S53 | FAM | 41.24 | 0.79 | 152-182 |
| 24 | 17 | D17S938 | FAM | 14.69 | 0.76 | 238-258 |
| 24 | 18 | D18S464 | FAM | 31.17 | 0.65 | 298-314 |


| 24 | 18 | D18S63 | VIC | 8.3 | 0.79 | 74-100 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 24 | 18 | D18S59 | VIC | 0 | 0.81 | 152-174 |
| 24 | 17 | D17S921 | VIC | 36.14 | 0.72 | 193-211 |
| 24 | 17 | D17S784 | VIC | 116.86 | 0.77 | 230-244 |
| 24 | 18 | D18S64 | VIC | 84.8 | 0.74 | 319-345 |
| 24 | 17 | D17S928 | NED | 126.46 | 0.76 | 68-102 |
| 24 | 18 | D18S452 | NED | 18.7 | 0.83 | 126-144 |
| 24 | 17 | D17S785 | NED | 103.53 | 0.83 | 165-193 |
| 24 | 18 | D18S1161 | NED | 114.26 | 0.82 | 211-237 |
| 24 | 18 | D18S68 | NED | 96.48 | 0.68 | 269-295 |
| 24 | 17 | D17S944 | NED | 82.56 | 0.75 | 318-334 |
| 25 | 20 | D20S889 | FAM | 11.2 | 0.83 | 87-123 |
| 25 | 20 | D20S117 | FAM | 2.83 | 0.84 | 151-187 |
| 25 | 20 | D20S112 | FAM | 39.25 | 0.81 | 213-237 |
| 25 | 19 | D19S220 | FAM | 62.03 | 0.84 | 267-291 |
| 25 | 19 | D19S221 | VIC | 36.22 | 0.86 | 87-110 |
| 25 | 20 | D20S171 | VIC | 95.7 | 0.78 | 127-155 |
| 25 | 19 | D19S210 | VIC | 100.01 | 0.74 | 172-192 |
| 25 | 20 | D20S100 | VIC | 84.78 | 0.76 | 209-235 |
| 25 | 19 | D19S420 | NED | 66.3 | 0.79 | 95-117 |
| 25 | 19 | D19S414 | NED | 54.01 | 0.78 | 164-194 |
| 25 | 20 | D20S115 | NED | 21.15 | 0.66 | 234-246 |
| 25 | 20 | D20S196 | NED | 75.01 | 0.81 | 259-295 |
| 26 | 20 | D20S119 | FAM | 61.77 | 0.82 | 103-123 |
| 26 | 21 | D21S266 | FAM | 45.87 | 0.59 | 156-178 |
| 26 | 20 | D20S107 | FAM | 55.74 | 0.80 | 197-221 |
| 26 | 19 | D19S902 | FAM | 72.72 | 0.79 | 237-273 |
| 26 | 20 | D20S186 | VIC | 32.3 | 0.86 | 113-139 |
| 26 | 22 | D22S420 | VIC | 4.06 | 0.77 | 153-169 |
| 26 | 22 | D22S280 | VIC | 31.3 | 0.82 | 213-225 |
| 26 | 19 | D19S216 | VIC | 20.01 | 0.76 | 256-274 |
| 26 | 22 | D22S423 | VIC | 46.42 | 0.82 | 287-309 |
| 26 | 19 | D19S884 | NED | 26.37 | 0.86 | 93-113 |
| 26 | 21 | D21S1252 | NED | 35.45 | 0.80 | 144-176 |


| 26 | 22 | D22S539 | NED | 14.44 | 0.58 | $199-217$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 26 | 22 | D22S274 | NED | 51.54 | 0.77 | $276-298$ |
| 27 | 20 | D20S195 | FAM | 50.81 | 0.81 | $128-154$ |
| 27 | 22 | D22S315 | FAM | 21.47 | 0.78 | $180-210$ |
| 27 | 19 | D19S209 | FAM | 10.97 | 0.77 | $238-254$ |
| 27 | 19 | D19S418 | VIC | 92.56 | 0.66 | $87-107$ |
| 27 | 20 | D20S173 | VIC | 98.09 | 0.67 | $128-182$ |
| 27 | 21 | D21S263 | VIC | 27.4 | 0.75 | $194-229$ |
| 27 | 21 | D21S1914 | VIC | 19.39 | 0.86 | $258-280$ |
| 27 | 21 | D21S1256 | NED | 9.72 | 0.65 | $96-116$ |
| 27 | 22 | D22S283 | NED | 38.62 | 0.89 | $127-155$ |
| 27 | 20 | D20S178 | NED | 66.16 | 0.83 | $179-195$ |
| 27 | 19 | D19S226 | NED | 42.28 | 0.85 | $238-270$ |
| 27 | 19 | D19S571 | NED | 84.08 | 0.81 | $287-319$ |

## Appendix IV：Corrected CEPH sizes for microsatellite markers and corresponding members of RLS3002

| Marker |  | $\frac{\pi}{d}$ | $\stackrel{B}{A}$ |  | 菏 |  | 㵄 |  | 式 | $\begin{aligned} & \text { B } \\ & \text { H } \\ & \text { H } \\ & \text { 首 } \end{aligned}$ | 淢 | 島 | $\begin{aligned} & \text { J } \\ & \text { H } \\ & \text { 首 } \end{aligned}$ | 菏 | 范 | 荡 | 式 | B | B |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D1S468 | 4.22 | $\begin{aligned} & 183, \\ & 189 \end{aligned}$ |  | $\begin{aligned} & 189, \\ & 189 \end{aligned}$ | $\begin{aligned} & 189, \\ & 183 \end{aligned}$ | $\begin{aligned} & 183, \\ & 183 \end{aligned}$ | $\begin{gathered} 189, \\ 189 \end{gathered}$ | $\begin{aligned} & 189, \\ & 183 \end{aligned}$ | $\begin{aligned} & 183, \\ & 189 \end{aligned}$ | $\begin{aligned} & 183, \\ & 189 \end{aligned}$ | $\begin{aligned} & 189, \\ & 189 \end{aligned}$ | $\begin{aligned} & 189, \\ & 183 \end{aligned}$ | $\begin{gathered} 189, \\ 189 \end{gathered}$ | $\begin{aligned} & 189, \\ & 189 \end{aligned}$ | $\begin{aligned} & 189, \\ & 189 \end{aligned}$ | $\begin{aligned} & 191, \\ & 191 \end{aligned}$ | $\begin{aligned} & 189, \\ & 191 \end{aligned}$ | $\begin{aligned} & 189, \\ & 191 \end{aligned}$ | $\begin{aligned} & 189, \\ & 191 \end{aligned}$ |
| D1S214 | $\begin{gathered} 14.0 \\ 4 \end{gathered}$ | $\begin{aligned} & 136, \\ & 136 \end{aligned}$ | $\begin{aligned} & 136 \\ & 141 \end{aligned}$ | $\begin{aligned} & 136, \\ & 136 \end{aligned}$ | $\begin{aligned} & 136, \\ & 14, \end{aligned}$ | $\begin{gathered} 136, \\ 141 \end{gathered}$ | $\begin{gathered} 136, \\ 136 \end{gathered}$ | $\begin{aligned} & 136, \\ & 141 \end{aligned}$ | $\begin{aligned} & 136, \\ & 136 \end{aligned}$ | $\begin{aligned} & 12, \\ & 14, \end{aligned}$ | $\begin{gathered} 136 \\ 136 \end{gathered}$ | $\begin{gathered} 120, \\ 136 \end{gathered}$ | $\begin{aligned} & 136, \\ & 141 \end{aligned}$ | $\begin{aligned} & 141, \\ & 136 \end{aligned}$ | $\begin{aligned} & 136, \\ & 136 \end{aligned}$ | $\begin{gathered} 131, \\ 136 \end{gathered}$ | $\begin{aligned} & 131, \\ & 136 \end{aligned}$ | $\begin{aligned} & 136, \\ & 136 \end{aligned}$ | $\begin{aligned} & 136, \\ & 136 \end{aligned}$ |
| D1S450 | $\begin{gathered} 20.6 \\ 1 \end{gathered}$ | $\begin{aligned} & 251, \\ & 225 \end{aligned}$ | $\begin{aligned} & 251, \\ & 279 \end{aligned}$ | $\begin{aligned} & 251, \\ & 259 \end{aligned}$ | $\begin{aligned} & 255, \\ & 259 \end{aligned}$ | $\begin{aligned} & 255, \\ & 259 \end{aligned}$ | $\begin{aligned} & 251, \\ & 259 \end{aligned}$ | $\begin{aligned} & 251, \\ & 259 \end{aligned}$ | $\begin{aligned} & 251, \\ & 255 \end{aligned}$ | $\begin{aligned} & 251, \\ & 259 \end{aligned}$ | $\begin{aligned} & 251, \\ & 251 \end{aligned}$ | $\begin{aligned} & 251, \\ & 251 \end{aligned}$ | $\begin{aligned} & 251, \\ & 259 \end{aligned}$ | $\begin{aligned} & 255, \\ & 259 \end{aligned}$ | $\begin{aligned} & 251, \\ & 255 \end{aligned}$ | $\begin{aligned} & 259, \\ & 261 \end{aligned}$ | $\begin{aligned} & 251, \\ & 259 \end{aligned}$ |  |  |
| D1S2667 | $\begin{gathered} 24.6 \\ 8 \end{gathered}$ | $\begin{aligned} & 144, \\ & 148 \end{aligned}$ | $\begin{aligned} & 133, \\ & 144 \end{aligned}$ | $\begin{aligned} & 144, \\ & 148 \end{aligned}$ | $\begin{aligned} & \hline 134, \\ & 148 \end{aligned}$ | $\begin{aligned} & \hline 134, \\ & 148 \end{aligned}$ | $\begin{aligned} & \hline 144, \\ & 148 \end{aligned}$ | $\begin{aligned} & 134, \\ & 148 \end{aligned}$ | $\begin{aligned} & \hline 144, \\ & 148 \end{aligned}$ | $\begin{aligned} & \hline 142, \\ & 148 \end{aligned}$ | $\begin{aligned} & 134, \\ & 134 \end{aligned}$ | $\begin{aligned} & 134, \\ & 148 \end{aligned}$ | $\begin{aligned} & 134, \\ & 142 \end{aligned}$ | $\begin{aligned} & \hline 134, \\ & 148 \end{aligned}$ | $\begin{aligned} & 148, \\ & 148 \end{aligned}$ | $\begin{aligned} & \hline 140, \\ & 146 \end{aligned}$ |  |  |  |
| D1S2697 | $\begin{gathered} 37.0 \\ 5 \end{gathered}$ | $\begin{gathered} 273, \\ 181 \end{gathered}$ | $\begin{gathered} 277, \\ 181 \end{gathered}$ | $\begin{aligned} & 281, \\ & 281 \end{aligned}$ | $\begin{array}{\|l\|} \hline 272, \\ 277 \\ \hline \end{array}$ | $\begin{aligned} & 277, \\ & 281 \end{aligned}$ | $\begin{aligned} & 273, \\ & 281 \end{aligned}$ | $\begin{aligned} & 277, \\ & 281 \end{aligned}$ | $\begin{aligned} & \hline 273, \\ & 281 \end{aligned}$ | $\begin{aligned} & 281, \\ & 281 \end{aligned}$ | $\begin{aligned} & 273, \\ & 281 \end{aligned}$ | $\begin{aligned} & 273, \\ & 281 \end{aligned}$ | $\begin{gathered} 281, \\ 281 \end{gathered}$ | $\begin{aligned} & 273, \\ & 273 \end{aligned}$ | $\begin{aligned} & 273, \\ & 273 \end{aligned}$ | $\begin{aligned} & 273, \\ & 281 \end{aligned}$ | $\begin{aligned} & 283, \\ & 281 \end{aligned}$ |  |  |
| D1S199 | $\begin{gathered} 45.3 \\ 3 \end{gathered}$ | $\begin{aligned} & 100, \\ & 108 \end{aligned}$ | $\begin{aligned} & 110, \\ & 114 \end{aligned}$ | $\begin{aligned} & 108, \\ & 110 \end{aligned}$ | $\begin{aligned} & 100, \\ & 10 \end{aligned}$ | $\begin{aligned} & 108, \\ & 114 \end{aligned}$ | $\begin{aligned} & 100, \\ & 110 \end{aligned}$ | $\begin{aligned} & 108, \\ & 114 \end{aligned}$ | $\begin{aligned} & 100, \\ & 110 \end{aligned}$ | $\begin{gathered} 96,1 \\ 00 \end{gathered}$ | $\begin{gathered} 98,1 \\ 12 \end{gathered}$ | $\begin{gathered} 96,1 \\ 12 \end{gathered}$ | $\begin{gathered} 96,1 \\ 12 \end{gathered}$ | $\begin{gathered} 96,1 \\ 00 \end{gathered}$ | $\begin{gathered} 96,1 \\ 00 \end{gathered}$ | $\begin{gathered} 98,1 \\ 00 \end{gathered}$ | $\begin{gathered} 96,1 \\ 00 \end{gathered}$ | $\begin{gathered} 96,0 \\ 8 \end{gathered}$ | $\begin{gathered} 96,0 \\ 8 \end{gathered}$ |
| D1S1622 | $\begin{gathered} 56.7 \\ 4 \end{gathered}$ | $\begin{aligned} & 258, \\ & 258 \end{aligned}$ | $\begin{aligned} & 258, \\ & 279 \end{aligned}$ | $\begin{aligned} & 258, \\ & 258 \end{aligned}$ | $\begin{aligned} & 258, \\ & 279 \\ & \hline \end{aligned}$ | $\begin{aligned} & 258, \\ & 279 \end{aligned}$ | $\begin{aligned} & 258, \\ & 258 \end{aligned}$ |  |  | $\begin{gathered} 261, \\ 270 \end{gathered}$ | $\begin{aligned} & 258, \\ & 270 \end{aligned}$ | $\begin{aligned} & 258, \\ & 261 \end{aligned}$ | $\begin{aligned} & 258, \\ & 270 \end{aligned}$ | $\begin{aligned} & 258, \\ & 270 \end{aligned}$ | $\begin{aligned} & 258, \\ & 258 \end{aligned}$ | $\begin{aligned} & 258, \\ & 258 \end{aligned}$ | $\begin{aligned} & 258, \\ & 258 \end{aligned}$ |  |  |
| D1S255 | $65.4$ | $\begin{gathered} 72,8 \\ 0 \end{gathered}$ | $\begin{gathered} 74,7 \\ 4 \end{gathered}$ | $\begin{gathered} 74,8 \\ 0 \end{gathered}$ | $\begin{gathered} \hline 74,8 \\ 0 \end{gathered}$ | $\begin{gathered} 74,7 \\ 4 \end{gathered}$ | $\begin{gathered} 74,7 \\ 4 \end{gathered}$ | $\begin{gathered} \hline 74,8 \\ 0 \end{gathered}$ | $\begin{gathered} \hline 74,8 \\ 0 \end{gathered}$ | $\begin{gathered} 74,8 \\ 0 \end{gathered}$ | $\begin{gathered} 74,7 \\ 4 \end{gathered}$ | $\begin{gathered} 74,7 \\ 4 \end{gathered}$ | $\begin{gathered} 74,7 \\ 4 \end{gathered}$ | $\begin{gathered} 74,7 \\ 4 \end{gathered}$ | $\begin{gathered} 74,8 \\ 0 \end{gathered}$ | $\begin{gathered} 80,8 \\ 4 \end{gathered}$ | $\begin{gathered} 80,8 \\ 0 \end{gathered}$ |  |  |
| D1S2797 | $\begin{gathered} 75.6 \\ 6 \end{gathered}$ | $\begin{aligned} & 168, \\ & 168 \end{aligned}$ | $\begin{aligned} & \hline 168, \\ & 168 \end{aligned}$ | $\begin{aligned} & \hline 168, \\ & 168 \end{aligned}$ | $\begin{aligned} & 168, \\ & 168 \end{aligned}$ | $\begin{aligned} & \hline 168, \\ & 168 \end{aligned}$ | $\begin{aligned} & \hline 168, \\ & 168 \end{aligned}$ | $\begin{aligned} & 168, \\ & 168 \end{aligned}$ | $\begin{aligned} & \hline 168, \\ & 168 \end{aligned}$ | $\begin{aligned} & \hline 168, \\ & 168 \end{aligned}$ | $\begin{aligned} & \hline 168, \\ & 170 \end{aligned}$ | $\begin{aligned} & \hline 168, \\ & 170 \end{aligned}$ | $\begin{aligned} & 168, \\ & 168 \end{aligned}$ | $\begin{aligned} & \hline 168, \\ & 168 \end{aligned}$ | $\begin{aligned} & \hline 166, \\ & 168 \end{aligned}$ | $\begin{aligned} & \hline 164, \\ & 168 \end{aligned}$ | $\begin{aligned} & \hline 166, \\ & 168 \end{aligned}$ |  |  |
| D1S2890 | $\begin{gathered} 85.6 \\ 8 \end{gathered}$ | $\begin{aligned} & 175, \\ & 179 \end{aligned}$ | $\begin{aligned} & 177, \\ & 179 \end{aligned}$ | $\begin{aligned} & 175, \\ & 177 \end{aligned}$ | $\begin{array}{\|l\|} \hline 175, \\ 177 \end{array}$ | $\begin{aligned} & 179, \\ & 179 \end{aligned}$ | $\begin{aligned} & 177, \\ & 179 \end{aligned}$ | $\begin{aligned} & 179, \\ & 179 \end{aligned}$ | $\begin{aligned} & 175, \\ & 179 \end{aligned}$ | $\begin{aligned} & \hline 179, \\ & 183 \end{aligned}$ | $\begin{aligned} & 175, \\ & 185 \end{aligned}$ | $\begin{aligned} & 179, \\ & 185 \end{aligned}$ | $\begin{aligned} & 179, \\ & 185 \end{aligned}$ | $\begin{aligned} & 175, \\ & 187 \end{aligned}$ | $\begin{aligned} & 175, \\ & 187 \end{aligned}$ | $\begin{aligned} & 179, \\ & 179 \end{aligned}$ | $\begin{aligned} & 179, \\ & 187 \end{aligned}$ |  |  |
| D1S3728 | $\begin{gathered} 89.4 \\ 9 \end{gathered}$ | $\begin{aligned} & 248, \\ & 260 \end{aligned}$ | $\begin{aligned} & 252, \\ & 268 \end{aligned}$ | $\begin{aligned} & 248, \\ & 252 \end{aligned}$ | $\begin{aligned} & 248, \\ & 252 \\ & \hline \end{aligned}$ | $\begin{aligned} & 260, \\ & 268 \end{aligned}$ | $\begin{aligned} & 252, \\ & 260 \end{aligned}$ |  |  | $\begin{aligned} & 248, \\ & 260 \end{aligned}$ | $\begin{aligned} & 248, \\ & 260 \end{aligned}$ | $\begin{aligned} & 260, \\ & 260 \end{aligned}$ | $\begin{aligned} & 256, \\ & 260 \end{aligned}$ | $\begin{aligned} & 248, \\ & 252 \end{aligned}$ | $\begin{aligned} & 248, \\ & 252 \end{aligned}$ | $\begin{aligned} & 252, \\ & 264 \end{aligned}$ | $\begin{aligned} & 252, \\ & 252 \end{aligned}$ |  |  |


| D1S230 | $\begin{gathered} 95.3 \\ 1 \end{gathered}$ | $\begin{gathered} 177, \\ 179 \end{gathered}$ | $\begin{aligned} & 179, \\ & 185 \end{aligned}$ | $\begin{aligned} & 179, \\ & 185 \end{aligned}$ | $\begin{aligned} & 179 \\ & 185 \end{aligned}$ | $\begin{gathered} 177, \\ 179 \end{gathered}$ | $\begin{aligned} & 179 \\ & 185 \end{aligned}$ | $\begin{aligned} & 177, \\ & 185 \end{aligned}$ |  | $\begin{gathered} 177, \\ 179 \end{gathered}$ | $\begin{aligned} & 179, \\ & 185 \end{aligned}$ | $\begin{gathered} 177, \\ 179 \end{gathered}$ | $\begin{aligned} & 177, \\ & 185 \end{aligned}$ | $\begin{aligned} & 179, \\ & 189 \end{aligned}$ | $\begin{aligned} & 179, \\ & 189 \end{aligned}$ | $\begin{gathered} 183, \\ 189 \end{gathered}$ | $\begin{gathered} 183, \\ 189 \end{gathered}$ | $\begin{aligned} & 189, \\ & 189 \end{aligned}$ | $\begin{aligned} & 179, \\ & 183 \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D1S2841 | $\begin{gathered} 106 . \\ 45 \end{gathered}$ | $\begin{aligned} & 277, \\ & 283 \end{aligned}$ | $\begin{aligned} & 283, \\ & 285 \end{aligned}$ | $\begin{aligned} & 277, \\ & 283 \end{aligned}$ | $\begin{aligned} & 277, \\ & 283 \end{aligned}$ | $\begin{aligned} & 277, \\ & 285 \end{aligned}$ | $\begin{aligned} & 277, \\ & 283 \end{aligned}$ | $\begin{aligned} & 277, \\ & 283 \end{aligned}$ | $\begin{aligned} & 277, \\ & 285 \end{aligned}$ | $\begin{aligned} & 277, \\ & 283 \end{aligned}$ | $\begin{aligned} & 283, \\ & 283 \end{aligned}$ | $\begin{aligned} & 283, \\ & 283 \end{aligned}$ |  | $\begin{aligned} & 275, \\ & 281 \end{aligned}$ | $\begin{aligned} & 277, \\ & 281 \end{aligned}$ | $\begin{aligned} & 271, \\ & 279 \end{aligned}$ | $\begin{aligned} & 271, \\ & 281 \end{aligned}$ | $\begin{aligned} & 277, \\ & 279 \end{aligned}$ |  |
| D1S1728 | $\begin{gathered} 109 . \\ 04 \end{gathered}$ | $\begin{aligned} & 162, \\ & 166 \end{aligned}$ | $\begin{aligned} & 158, \\ & 174 \end{aligned}$ | $\begin{aligned} & 158, \\ & 166 \end{aligned}$ | $\begin{aligned} & 158 \\ & 166 \end{aligned}$ | $\begin{gathered} 158, \\ 166 \end{gathered}$ | $\begin{aligned} & 166, \\ & 174 \end{aligned}$ |  |  | $\begin{aligned} & 162, \\ & 166 \end{aligned}$ | $\begin{aligned} & 162, \\ & 162 \end{aligned}$ | $\begin{aligned} & 162, \\ & 162 \end{aligned}$ | $\begin{aligned} & 162, \\ & 162 \end{aligned}$ | $\begin{aligned} & 166, \\ & 166 \end{aligned}$ | $\begin{aligned} & 166, \\ & 166 \end{aligned}$ | $\begin{aligned} & 162, \\ & 166 \end{aligned}$ | $\begin{aligned} & 162, \\ & 166 \end{aligned}$ |  |  |
| D1S2868 | $\begin{gathered} 126 . \\ 16 \end{gathered}$ | $\begin{aligned} & 146 \\ & 180 \end{aligned}$ | $\begin{aligned} & 146, \\ & 148 \end{aligned}$ | $\begin{aligned} & 146, \\ & 148 \end{aligned}$ | $\begin{gathered} \hline 146, \\ 148 \end{gathered}$ | $\begin{gathered} 146, \\ 148 \end{gathered}$ | $\begin{aligned} & 146 . \\ & 146 \end{aligned}$ | $\begin{aligned} & 146, \\ & 148 \end{aligned}$ | $\begin{aligned} & 146 \\ & 148 \end{aligned}$ | $\begin{aligned} & 146, \\ & 148 \end{aligned}$ |  | $\begin{aligned} & \hline 144, \\ & 148 \end{aligned}$ | $\begin{gathered} 146, \\ 148 \end{gathered}$ | $\begin{aligned} & 148 \\ & 148 \end{aligned}$ | $\begin{aligned} & 146, \\ & 148 \end{aligned}$ | $\begin{aligned} & \hline 146 \\ & 148 \end{aligned}$ | $\begin{aligned} & 148 \\ & 148 \end{aligned}$ |  | $\begin{aligned} & 146 \\ & 148 \end{aligned}$ |
| D1S206 | $\begin{gathered} 134 . \\ 20 \end{gathered}$ | $\begin{aligned} & 210, \\ & 212 \end{aligned}$ | $\begin{aligned} & \hline 210, \\ & 214 \end{aligned}$ | $\begin{aligned} & 210, \\ & 212 \end{aligned}$ | $\begin{aligned} & 210, \\ & 210 \end{aligned}$ | $\begin{aligned} & 210, \\ & 212 \end{aligned}$ | $\begin{aligned} & 212, \\ & 214 \end{aligned}$ | $\begin{aligned} & 210, \\ & 212 \end{aligned}$ | $\begin{aligned} & 212, \\ & 214 \end{aligned}$ | $\begin{aligned} & 210, \\ & 212 \end{aligned}$ | $\begin{aligned} & 210, \\ & 212 \end{aligned}$ | $\begin{aligned} & 212, \\ & 212 \end{aligned}$ | $\begin{aligned} & \hline 210, \\ & 210 \end{aligned}$ | $\begin{aligned} & 210, \\ & 212 \end{aligned}$ | $\begin{aligned} & 210, \\ & 212 \end{aligned}$ | $\begin{aligned} & 206, \\ & 206 \end{aligned}$ | $\begin{aligned} & 206, \\ & 210 \end{aligned}$ |  |  |
| D1S3723 | $\begin{gathered} 140 . \\ 39 \end{gathered}$ | $\begin{aligned} & 154, \\ & 198 \end{aligned}$ | $\begin{aligned} & 146, \\ & 198 \end{aligned}$ | $\begin{aligned} & 146, \\ & 154 \end{aligned}$ | $\begin{aligned} & 146, \\ & 154 \end{aligned}$ | $\begin{aligned} & 146, \\ & 154 \end{aligned}$ | $\begin{gathered} 198, \\ 198 \end{gathered}$ |  |  | $\begin{gathered} 154, \\ 198 \end{gathered}$ | $\begin{aligned} & 194, \\ & 150 \end{aligned}$ | $\begin{aligned} & 154, \\ & 194 \end{aligned}$ | $\begin{gathered} 150, \\ 198 \end{gathered}$ | $\begin{aligned} & 198, \\ & 182 \end{aligned}$ | $\begin{aligned} & 154, \\ & 194 \end{aligned}$ | $\begin{gathered} 190, \\ 194 \end{gathered}$ | $\begin{gathered} 190, \\ 194 \end{gathered}$ |  |  |
| D1S2726 | $\begin{gathered} 144 . \\ 28 \end{gathered}$ | $\begin{aligned} & 276, \\ & 280 \end{aligned}$ | $\begin{aligned} & 276, \\ & 282 \end{aligned}$ | $\begin{aligned} & 276, \\ & 280 \end{aligned}$ | $\begin{aligned} & 276, \\ & 280 \end{aligned}$ | $\begin{aligned} & 276, \\ & 280 \end{aligned}$ | $\begin{aligned} & 276 \\ & 276 \end{aligned}$ | $\begin{aligned} & 276, \\ & 282 \end{aligned}$ | $\begin{aligned} & 276, \\ & 280 \end{aligned}$ | $\begin{aligned} & 276, \\ & 280 \end{aligned}$ | $\begin{aligned} & 278 \\ & 280 \end{aligned}$ | $\begin{aligned} & 280, \\ & 280 \end{aligned}$ | $\begin{aligned} & 276 \\ & 278 \end{aligned}$ | $\begin{aligned} & 276, \\ & 280 \end{aligned}$ | $\begin{aligned} & 278, \\ & 280 \end{aligned}$ | $\begin{aligned} & 278, \\ & 288 \end{aligned}$ | $\begin{aligned} & 278, \\ & 280 \end{aligned}$ |  |  |
| $\begin{gathered} \hline \text { D1S498/ } \\ \text { D1S252 } \end{gathered}$ | $\begin{gathered} 150 . \\ 27 \end{gathered}$ | $\begin{aligned} & 189, \\ & 193 \end{aligned}$ | $\begin{aligned} & 191, \\ & 203 \end{aligned}$ | $\begin{aligned} & 189, \\ & 203 \end{aligned}$ | $\begin{aligned} & 193, \\ & 203 \end{aligned}$ | $\begin{aligned} & 193, \\ & 203 \end{aligned}$ | $\begin{aligned} & 189, \\ & 191 \end{aligned}$ | $\begin{aligned} & 189, \\ & 203 \end{aligned}$ | $\begin{gathered} 191, \\ 193 \end{gathered}$ | $\begin{gathered} 191, \\ 195 \end{gathered}$ | $\begin{aligned} & 191, \\ & 195 \end{aligned}$ | $\begin{gathered} 191, \\ 195 \end{gathered}$ | $\begin{gathered} 191, \\ 195 \end{gathered}$ | $\begin{aligned} & 189, \\ & 193 \end{aligned}$ | $\begin{aligned} & 189, \\ & 193 \end{aligned}$ | $\begin{gathered} 197, \\ 197 \end{gathered}$ |  |  | $\begin{aligned} & 193, \\ & 197 \end{aligned}$ |
| D1S484 | $\begin{gathered} 169 . \\ 69 \end{gathered}$ | $\begin{aligned} & 136, \\ & 138 \end{aligned}$ | $\begin{gathered} 136, \\ 138 \end{gathered}$ | $\begin{aligned} & 136, \\ & 136 \end{aligned}$ | $\begin{gathered} 136, \\ 138 \end{gathered}$ | $\begin{gathered} 136, \\ 138 \end{gathered}$ | $\begin{aligned} & 136 \\ & 136 \end{aligned}$ | $\begin{aligned} & 136, \\ & 138 \end{aligned}$ | $\begin{aligned} & 138, \\ & 138 \end{aligned}$ | $\begin{aligned} & 136, \\ & 142 \end{aligned}$ | $\begin{aligned} & 136, \\ & 142 \end{aligned}$ | $\begin{aligned} & 136, \\ & 142 \end{aligned}$ | $\begin{aligned} & 136 \\ & 136 \end{aligned}$ | $\begin{aligned} & 136, \\ & 142 \end{aligned}$ | $\begin{aligned} & 136 \\ & 138 \end{aligned}$ | $\begin{gathered} 136, \\ 136 \end{gathered}$ | $\begin{aligned} & 136, \\ & 136 \end{aligned}$ |  |  |
| D1S2878 | $\begin{gathered} 177 . \\ 86 \end{gathered}$ | $\begin{aligned} & 169, \\ & 177 \end{aligned}$ | $\begin{aligned} & 177, \\ & 189 \end{aligned}$ | $\begin{aligned} & 177, \\ & 189 \end{aligned}$ | $\begin{gathered} 169, \\ 189 \end{gathered}$ | $\begin{gathered} 169, \\ 189 \end{gathered}$ | $\begin{gathered} 177, \\ 177 \end{gathered}$ | $\begin{aligned} & 177, \\ & 189 \end{aligned}$ | $\begin{gathered} 169, \\ 177 \end{gathered}$ | $\begin{gathered} 175 \\ 179 \end{gathered}$ | $\begin{aligned} & 177, \\ & 183 \end{aligned}$ | $\begin{aligned} & 179, \\ & 183 \end{aligned}$ | $\begin{aligned} & 175, \\ & 183 \end{aligned}$ | $\begin{aligned} & 169 \\ & 177 \end{aligned}$ | $\begin{aligned} & 169, \\ & 177 \end{aligned}$ | $\begin{gathered} 196, \\ 175 \end{gathered}$ | $\begin{aligned} & 169, \\ & 169 \end{aligned}$ |  |  |
| D1S196 | $\begin{gathered} 181 . \\ 49 \end{gathered}$ | $\begin{aligned} & 267, \\ & 277 \end{aligned}$ | $\begin{aligned} & 277, \\ & 277 \end{aligned}$ | $\begin{aligned} & 277, \\ & 277 \end{aligned}$ | $\begin{aligned} & 277, \\ & 277 \end{aligned}$ | $\begin{aligned} & 267, \\ & 277 \end{aligned}$ | $\begin{aligned} & 277, \\ & 277 \end{aligned}$ | $\begin{aligned} & 277, \\ & 277 \end{aligned}$ | $\begin{aligned} & 267, \\ & 277 \end{aligned}$ | $\begin{aligned} & 275 \\ & 283 \end{aligned}$ | $\begin{aligned} & 267, \\ & 275 \end{aligned}$ | $\begin{aligned} & 267, \\ & 275 \end{aligned}$ |  | $\begin{aligned} & 277, \\ & 267 \end{aligned}$ | $\begin{aligned} & 277, \\ & 267 \end{aligned}$ | $\begin{aligned} & 273 \\ & 275 \end{aligned}$ | $\begin{aligned} & 273 \\ & 277 \end{aligned}$ |  |  |
| D1S1589 |  | $\begin{aligned} & 205, \\ & 220 \end{aligned}$ | $\begin{aligned} & 205 \\ & 205 \end{aligned}$ | $\begin{aligned} & 205, \\ & 220 \end{aligned}$ | $\begin{aligned} & 205 \\ & 220 \end{aligned}$ | $\begin{aligned} & 205 \\ & 220 \end{aligned}$ | $\begin{aligned} & 205, \\ & 220 \end{aligned}$ |  |  | $\begin{aligned} & 205 \\ & 205 \end{aligned}$ | $\begin{aligned} & 208 \\ & 208 \end{aligned}$ | $\begin{aligned} & 205, \\ & 208 \end{aligned}$ | $\begin{aligned} & 205, \\ & 208 \end{aligned}$ | $\begin{aligned} & 205, \\ & 220 \end{aligned}$ | $\begin{aligned} & 205, \\ & 220 \end{aligned}$ | $\begin{aligned} & 205 \\ & 217 \end{aligned}$ | $\begin{aligned} & 205 \\ & 217 \end{aligned}$ |  |  |
| D1S413 | $\begin{gathered} 212 . \\ 42 \end{gathered}$ | $\begin{aligned} & \hline 246, \\ & 246 \end{aligned}$ | $\begin{aligned} & 254, \\ & 254 \end{aligned}$ | $\begin{aligned} & 246, \\ & 254 \end{aligned}$ | $\begin{aligned} & 246 \\ & 254 \end{aligned}$ | $\begin{aligned} & 246, \\ & 254 \end{aligned}$ | $\begin{aligned} & 246 \\ & 254 \end{aligned}$ | $\begin{aligned} & 246, \\ & 254 \end{aligned}$ | $\begin{aligned} & 246, \\ & 254 \end{aligned}$ | $\begin{aligned} & 250, \\ & 254 \end{aligned}$ | $\begin{aligned} & 254, \\ & 254 \end{aligned}$ | $\begin{aligned} & 250, \\ & 254 \end{aligned}$ | $\begin{aligned} & 250, \\ & 254 \end{aligned}$ |  | $\begin{aligned} & 246, \\ & 250 \end{aligned}$ | $\begin{aligned} & 246 \\ & 246 \end{aligned}$ | $\begin{aligned} & 246 \\ & 246 \end{aligned}$ |  | $\begin{aligned} & 246, \\ & 250 \end{aligned}$ |
| D1S249 | $\begin{gathered} 220 . \\ 65 \end{gathered}$ | $\begin{aligned} & 173, \\ & 175 \end{aligned}$ | $\begin{aligned} & 177, \\ & 181 \end{aligned}$ | $\begin{aligned} & 174, \\ & 177 \end{aligned}$ | $\begin{gathered} 173, \\ 181 \end{gathered}$ | $\begin{aligned} & 175 \\ & 181 \end{aligned}$ |  | $\begin{aligned} & 173, \\ & 181 \end{aligned}$ | $\begin{aligned} & 173, \\ & 181 \end{aligned}$ | $\begin{aligned} & 161, \\ & 173 \end{aligned}$ | $\begin{aligned} & 171, \\ & 177 \end{aligned}$ | $\begin{aligned} & 171, \\ & 173 \end{aligned}$ | $\begin{aligned} & 173, \\ & 177 \end{aligned}$ | $\begin{aligned} & 173 \\ & 175 \end{aligned}$ | $\begin{aligned} & 173, \\ & 175 \end{aligned}$ | $\begin{gathered} 167, \\ 175 \end{gathered}$ | $\begin{aligned} & 173, \\ & 175 \end{aligned}$ |  |  |
| $\begin{gathered} \text { GATA124 } \\ \text { F08 } \end{gathered}$ | $\begin{gathered} 226 . \\ 16 \end{gathered}$ | $\begin{aligned} & 233, \\ & 241 \end{aligned}$ | $\begin{aligned} & 233 \\ & 237 \end{aligned}$ | $\begin{aligned} & 233, \\ & 237 \end{aligned}$ | $\begin{aligned} & 233, \\ & 241 \end{aligned}$ | $\begin{aligned} & 233, \\ & 233 \end{aligned}$ | $\begin{aligned} & 233, \\ & 241 \end{aligned}$ |  |  | $\begin{aligned} & 233, \\ & 237 \end{aligned}$ | $\begin{aligned} & 229 \\ & 233 \end{aligned}$ | $\begin{aligned} & 229, \\ & 233 \end{aligned}$ | $\begin{aligned} & 233 \\ & 233 \end{aligned}$ | $\begin{aligned} & 233 \\ & 233 \end{aligned}$ | $\begin{aligned} & 233, \\ & 237 \end{aligned}$ | $\begin{aligned} & 233, \\ & 237 \end{aligned}$ | $\begin{aligned} & 237, \\ & 237 \end{aligned}$ |  |  |


| D1S425 | $\begin{gathered} 231 . \\ 11 \end{gathered}$ | $\begin{aligned} & \hline 100, \\ & 100 \end{aligned}$ | $\begin{array}{\|c\|} \hline 96,1 \\ 00 \end{array}$ | $\begin{aligned} & 100, \\ & 100 \end{aligned}$ | $\begin{gathered} \hline 96,1 \\ 00 \end{gathered}$ | $\begin{gathered} 96,1 \\ 00 \end{gathered}$ | $\begin{gathered} 96,1 \\ 00 \end{gathered}$ | $\begin{gathered} \hline 96,1 \\ 00 \end{gathered}$ | $\begin{gathered} \hline 96,1 \\ 00 \end{gathered}$ | $\begin{aligned} & \hline 100, \\ & 100 \end{aligned}$ | $\begin{gathered} 96,9 \\ 6 \end{gathered}$ | $\begin{gathered} 96,1 \\ 00 \end{gathered}$ |  | $\begin{gathered} \hline 92,1 \\ 00 \end{gathered}$ | $\begin{gathered} \hline 92,1 \\ 02 \end{gathered}$ | $\begin{gathered} \hline 96,1 \\ 02 \end{gathered}$ | $\begin{gathered} \hline 96,1 \\ 02 \end{gathered}$ |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D1S213 | $\begin{gathered} 242 . \\ 23 \end{gathered}$ | $\begin{aligned} & 114, \\ & 124 \end{aligned}$ | $\begin{aligned} & \hline 104, \\ & 118 \end{aligned}$ | $\begin{aligned} & \hline 104, \\ & 114 \end{aligned}$ | $\begin{array}{\|l\|} \hline 118, \\ 124 \\ \hline \end{array}$ | $\begin{gathered} 114, \\ 118 \end{gathered}$ | $\begin{array}{\|l\|} \hline 104, \\ 114 \\ \hline \end{array}$ | $\begin{aligned} & 114, \\ & 118 \end{aligned}$ | $\begin{aligned} & 118, \\ & 124 \end{aligned}$ | $\begin{aligned} & \hline 108, \\ & 124 \end{aligned}$ | $\begin{aligned} & 114, \\ & 118 \end{aligned}$ | $\begin{aligned} & \hline 118, \\ & 124 \\ & \hline \end{aligned}$ | $\begin{aligned} & \hline 114, \\ & 124 \end{aligned}$ | $\begin{aligned} & 108, \\ & 124 \end{aligned}$ | $\begin{aligned} & 104, \\ & 108 \end{aligned}$ | $\begin{aligned} & 108, \\ & 114 \end{aligned}$ | $\begin{aligned} & 104, \\ & 114 \end{aligned}$ |  |  |
| D1S3462 | $\begin{gathered} 247 . \\ 23 \end{gathered}$ | $\begin{aligned} & 257, \\ & 263 \end{aligned}$ | $\begin{array}{\|l\|} \hline 257, \\ 260 \\ \hline \end{array}$ | $\begin{aligned} & 257, \\ & 257 \end{aligned}$ | $\begin{aligned} & \hline 257, \\ & 257 \\ & \hline \end{aligned}$ | $\begin{gathered} 260, \\ 263 \end{gathered}$ | $\begin{aligned} & 257, \\ & 260 \end{aligned}$ | $\begin{aligned} & 257, \\ & 260 \end{aligned}$ | $\begin{aligned} & 260, \\ & 263 \end{aligned}$ | $\begin{aligned} & 257, \\ & 263 \end{aligned}$ | $\begin{aligned} & 257, \\ & 260 \end{aligned}$ | $\begin{aligned} & 257, \\ & 257 \end{aligned}$ | $\begin{aligned} & 257, \\ & 260 \end{aligned}$ | $\begin{aligned} & 257, \\ & 263 \end{aligned}$ | $\begin{aligned} & 257, \\ & 257 \end{aligned}$ | $\begin{gathered} 260, \\ 263 \end{gathered}$ | $\begin{aligned} & 260, \\ & 257 \end{aligned}$ |  |  |
| D1S2800 | $\begin{gathered} 252 . \\ 12 \end{gathered}$ | $\begin{aligned} & 182, \\ & 184 \end{aligned}$ | $\begin{aligned} & \hline 182, \\ & 184 \end{aligned}$ | $\begin{aligned} & \hline 182, \\ & 184 \end{aligned}$ | $\begin{aligned} & \hline 184, \\ & 184 \end{aligned}$ | $\begin{aligned} & 184, \\ & 184 \end{aligned}$ | $\begin{aligned} & 182, \\ & 184 \end{aligned}$ | $\begin{aligned} & 182, \\ & 184 \end{aligned}$ | $\begin{aligned} & 184, \\ & 184 \end{aligned}$ | $\begin{aligned} & 178, \\ & 184 \end{aligned}$ | $\begin{aligned} & \hline 178, \\ & 186 \end{aligned}$ | $\begin{aligned} & \hline 178, \\ & 186 \end{aligned}$ | $\begin{aligned} & \hline 178, \\ & 184 \end{aligned}$ | $\begin{aligned} & \hline 178, \\ & 184 \end{aligned}$ | $\begin{aligned} & 182, \\ & 190 \end{aligned}$ | $\begin{aligned} & 182, \\ & 182 \end{aligned}$ | $\begin{aligned} & 182, \\ & 182 \end{aligned}$ |  |  |
| D1S2785 | $\begin{gathered} 266 . \\ 27 \end{gathered}$ | $\begin{aligned} & \hline 168, \\ & 178 \end{aligned}$ | $\begin{array}{\|l\|} \hline 168, \\ 178 \\ \hline \end{array}$ | $\begin{aligned} & 168, \\ & 168 \end{aligned}$ | $\begin{array}{\|c\|} \hline 168, \\ 178 \\ \hline \end{array}$ | $\begin{aligned} & 168, \\ & 178 \end{aligned}$ | $\begin{aligned} & 178, \\ & 178 \end{aligned}$ | $\begin{aligned} & \hline 168, \\ & 178 \end{aligned}$ | $\begin{aligned} & \hline 168, \\ & 178 \end{aligned}$ | $\begin{aligned} & 174, \\ & 178 \end{aligned}$ | $\begin{aligned} & \hline 174, \\ & 180 \end{aligned}$ | $\begin{aligned} & 174, \\ & 178 \end{aligned}$ | $\begin{aligned} & \hline 174, \\ & 178 \end{aligned}$ | $\begin{aligned} & \hline 174, \\ & 178 \end{aligned}$ |  | $\begin{aligned} & \hline 168, \\ & 178 \end{aligned}$ | $\begin{aligned} & \hline 168, \\ & 174 \end{aligned}$ |  |  |
| D1S547 | $\begin{gathered} 267 . \\ 51 \end{gathered}$ | $\begin{aligned} & 290, \\ & 290 \end{aligned}$ | $\begin{array}{\|c\|} \hline 290, \\ 300 \\ \hline \end{array}$ | $\begin{aligned} & 290, \\ & 300 \end{aligned}$ | $\begin{array}{\|l\|} \hline 290, \\ 290 \\ \hline \end{array}$ | $\begin{aligned} & 290, \\ & 290 \end{aligned}$ | $\begin{aligned} & 290, \\ & 290 \end{aligned}$ |  |  | $\begin{aligned} & 286, \\ & 290 \end{aligned}$ | $\begin{aligned} & 286, \\ & 290 \end{aligned}$ | $\begin{aligned} & 286, \\ & 29 \end{aligned}$ | $\begin{aligned} & 290, \\ & 290 \end{aligned}$ | $\begin{aligned} & 290, \\ & 290 \end{aligned}$ | $\begin{aligned} & 290, \\ & 290 \end{aligned}$ | $\begin{aligned} & 290, \\ & 290 \end{aligned}$ | $\begin{aligned} & 290, \\ & 290 \end{aligned}$ |  |  |
| D1S2842 | $\begin{gathered} \hline 273 . \\ 96 \end{gathered}$ | $\begin{aligned} & 227, \\ & 227 \end{aligned}$ | $\begin{array}{\|l\|} \hline 217, \\ 217 \\ \hline \end{array}$ | $\begin{aligned} & 217, \\ & 227 \end{aligned}$ | $\begin{aligned} & 217, \\ & 227 \end{aligned}$ | $\begin{aligned} & \hline 217, \\ & 227 \end{aligned}$ | $\begin{aligned} & 217, \\ & 227 \end{aligned}$ | $\begin{aligned} & 217, \\ & 227 \end{aligned}$ | $\begin{aligned} & 217, \\ & 227 \end{aligned}$ | $\begin{aligned} & 221, \\ & 229 \end{aligned}$ | $\begin{aligned} & \hline 221, \\ & 221 \end{aligned}$ | $\begin{aligned} & \hline 221, \\ & 229 \\ & \hline \end{aligned}$ |  | $\begin{aligned} & \hline 221, \\ & 229 \end{aligned}$ | $\begin{aligned} & \hline 227, \\ & 229 \end{aligned}$ | $\begin{aligned} & \hline 221, \\ & 223 \end{aligned}$ | $\begin{aligned} & 221, \\ & 229 \end{aligned}$ |  |  |
| D1S2836 | $\begin{gathered} 285 . \\ 75 \end{gathered}$ | $\begin{aligned} & 273, \\ & 277 \end{aligned}$ | $\begin{aligned} & 270, \\ & 277 \end{aligned}$ | $\begin{aligned} & \hline 273, \\ & 277 \end{aligned}$ | $\begin{aligned} & \hline 270, \\ & 273 \\ & \hline \end{aligned}$ |  | $\begin{array}{\|l\|} \hline 277, \\ 277 \\ \hline \end{array}$ | $\begin{aligned} & 270, \\ & 273 \end{aligned}$ | $\begin{aligned} & 270, \\ & 273 \end{aligned}$ | $\begin{aligned} & 270, \\ & 273 \end{aligned}$ | $\begin{aligned} & 270, \\ & 270 \end{aligned}$ | $\begin{aligned} & 270, \\ & 273 \end{aligned}$ |  | $\begin{aligned} & 273, \\ & 277 \end{aligned}$ | $\begin{aligned} & 270, \\ & 277 \end{aligned}$ |  |  |  |  |
| D2S2211 | $\begin{gathered} 15.6 \\ 1 \end{gathered}$ | $\begin{aligned} & 254, \\ & 254 \end{aligned}$ | $\begin{array}{\|l\|} \hline 258, \\ 258 \\ \hline \end{array}$ | $\begin{aligned} & 254, \\ & 258 \end{aligned}$ | $\begin{array}{\|l\|} \hline 254, \\ 258 \\ \hline \end{array}$ | $\begin{aligned} & 254, \\ & 258 \end{aligned}$ | $\begin{aligned} & \hline 254, \\ & 258 \\ & \hline \end{aligned}$ | $\begin{aligned} & 254, \\ & 258 \end{aligned}$ | $\begin{aligned} & 254, \\ & 258 \end{aligned}$ | $\begin{aligned} & 254, \\ & 254 \end{aligned}$ | $\begin{aligned} & 254, \\ & 256 \end{aligned}$ | $\begin{aligned} & \hline 254, \\ & 256 \end{aligned}$ | $\begin{aligned} & \hline 254, \\ & 254 \end{aligned}$ | $\begin{aligned} & 254, \\ & 258 \end{aligned}$ | $\begin{aligned} & 254, \\ & 254 \end{aligned}$ | $\begin{aligned} & 254, \\ & 254 \end{aligned}$ | $\begin{aligned} & 254, \\ & 254 \end{aligned}$ | $\begin{aligned} & 254, \\ & 254 \end{aligned}$ | $\begin{aligned} & 254, \\ & 254 \end{aligned}$ |
| D2S2952 | $\begin{gathered} 17.8 \\ 8 \end{gathered}$ | $\begin{aligned} & 185 \\ & 189 \end{aligned}$ | $\begin{aligned} & \text { 181, } \\ & 201 \end{aligned}$ | $\begin{aligned} & 181, \\ & 185 \end{aligned}$ | $\begin{array}{\|c\|} \hline 181, \\ 185 \\ \hline \end{array}$ | $\begin{aligned} & 181, \\ & 189 \end{aligned}$ | $\begin{aligned} & 185, \\ & 201 \end{aligned}$ |  |  | $\begin{aligned} & 185 \\ & 189 \end{aligned}$ | $\begin{aligned} & 185, \\ & 185 \end{aligned}$ | $\begin{aligned} & 189, \\ & 185 \end{aligned}$ | $\begin{aligned} & 185, \\ & 185 \end{aligned}$ | $\begin{aligned} & 185, \\ & 185 \end{aligned}$ | $\begin{aligned} & 185, \\ & 189 \end{aligned}$ | $\begin{aligned} & 185, \\ & 185 \end{aligned}$ | $\begin{aligned} & 189 \\ & 185 \end{aligned}$ |  |  |
| D2S168 | $\begin{gathered} 27.0 \\ 6 \end{gathered}$ | $\begin{aligned} & 198 \\ & 200 \end{aligned}$ | $\begin{array}{\|l\|} \hline 210, \\ 210 \\ \hline \end{array}$ | $\begin{aligned} & 200, \\ & 210 \end{aligned}$ | $\begin{gathered} 200, \\ 210 \end{gathered}$ | $\begin{aligned} & 198, \\ & 210 \end{aligned}$ | $\begin{aligned} & 200, \\ & 210 \end{aligned}$ | $\begin{aligned} & 198, \\ & 210 \end{aligned}$ | $\begin{aligned} & 200, \\ & 210 \end{aligned}$ | $\begin{aligned} & 198, \\ & 200 \end{aligned}$ | $\begin{gathered} 200, \\ 210 \end{gathered}$ | $\begin{aligned} & 200, \\ & 200 \end{aligned}$ | $\begin{aligned} & 200, \\ & 200 \end{aligned}$ | $\begin{aligned} & 200, \\ & 210 \end{aligned}$ | $\begin{aligned} & 198, \\ & 200 \end{aligned}$ | $\begin{aligned} & 198, \\ & 210 \end{aligned}$ | $\begin{aligned} & 198, \\ & 198 \end{aligned}$ |  | $\begin{aligned} & 198, \\ & 198 \end{aligned}$ |
| D2S405 | $\begin{gathered} 47.9 \\ 7 \end{gathered}$ | $\begin{aligned} & 245 \\ & 245 \end{aligned}$ | $\begin{array}{\|l\|} \hline 245, \\ 253 \end{array}$ | $\begin{aligned} & 245, \\ & 253 \\ & \hline \end{aligned}$ | $\begin{aligned} & 245, \\ & 253 \end{aligned}$ | $\begin{aligned} & 245, \\ & 253 \end{aligned}$ | $\begin{array}{\|l\|} \hline 245, \\ 245 \end{array}$ |  |  | $\begin{gathered} 245, \\ 249 \end{gathered}$ | $\begin{aligned} & 245, \\ & 249 \end{aligned}$ | $\begin{aligned} & 245, \\ & 249 \end{aligned}$ | $\begin{aligned} & 249, \\ & 249 \end{aligned}$ | $\begin{aligned} & 245, \\ & 249 \end{aligned}$ | $\begin{aligned} & 245, \\ & 249 \end{aligned}$ | $\begin{aligned} & 245, \\ & 253 \end{aligned}$ | $\begin{aligned} & 249, \\ & 253 \end{aligned}$ |  |  |
| D2S367 | $\begin{gathered} 54.9 \\ 6 \end{gathered}$ | $\begin{aligned} & 161, \\ & 165 \end{aligned}$ | $\begin{aligned} & 137, \\ & 143 \end{aligned}$ | $\begin{aligned} & 143, \\ & 161 \end{aligned}$ | $\begin{array}{\|c\|} \hline 137, \\ 165 \\ \hline \end{array}$ | $\begin{gathered} 143, \\ 165 \end{gathered}$ | $\begin{aligned} & \hline 137, \\ & 165 \end{aligned}$ | $\begin{aligned} & 137, \\ & 165 \end{aligned}$ | $\begin{gathered} 137, \\ 161 \end{gathered}$ | $\begin{aligned} & \hline 163, \\ & 165 \end{aligned}$ | $\begin{gathered} 137, \\ 143 \end{gathered}$ | $\begin{aligned} & \hline 143, \\ & 165 \end{aligned}$ | $\begin{gathered} 137, \\ 165 \end{gathered}$ | $\begin{aligned} & 159, \\ & 161 \end{aligned}$ | $\begin{aligned} & 163, \\ & 165 \end{aligned}$ | $\begin{aligned} & \hline 161, \\ & 165 \end{aligned}$ | $\begin{aligned} & \hline 161, \\ & 163 \end{aligned}$ |  | $\begin{gathered} 161, \\ 165 \end{gathered}$ |
| D2S2259 | $\begin{gathered} 64.2 \\ 9 \end{gathered}$ | $\begin{aligned} & 229, \\ & 231 \end{aligned}$ | $\begin{array}{\|l\|} \hline 227, \\ 229 \\ \hline \end{array}$ | $\begin{aligned} & 229, \\ & 231 \end{aligned}$ | $\begin{aligned} & 229, \\ & 231 \end{aligned}$ |  | $\begin{array}{\|l} \hline 227, \\ 229 \\ \hline \end{array}$ | $\begin{aligned} & 227, \\ & 229 \end{aligned}$ | $\begin{aligned} & 227, \\ & 231 \end{aligned}$ | $\begin{aligned} & 229, \\ & 229 \end{aligned}$ | $\begin{aligned} & 229, \\ & 243 \end{aligned}$ | $\begin{aligned} & \hline 229, \\ & 229 \end{aligned}$ | $\begin{aligned} & \hline 229, \\ & 243 \end{aligned}$ | $\begin{aligned} & 229, \\ & 229 \end{aligned}$ | $\begin{aligned} & 227, \\ & 229 \end{aligned}$ | $\begin{aligned} & 227, \\ & 231 \end{aligned}$ | $\begin{aligned} & 227, \\ & 229 \end{aligned}$ | $\begin{aligned} & 229, \\ & 231 \end{aligned}$ | $\begin{aligned} & 227, \\ & 229 \end{aligned}$ |
| D2S1356 | $\begin{gathered} 64.2 \\ 9 \end{gathered}$ | $\begin{aligned} & 237, \\ & 240 \end{aligned}$ | $\begin{array}{\|l\|} \hline 240, \\ 252 \\ \hline \end{array}$ | $\begin{aligned} & 240, \\ & 240, \end{aligned}$ | $\begin{aligned} & 237, \\ & 252 \\ & \hline \end{aligned}$ | $\begin{aligned} & 240, \\ & 240 \end{aligned}$ | $\begin{aligned} & 237, \\ & 252 \end{aligned}$ |  |  | $\begin{aligned} & 237, \\ & 252 \end{aligned}$ | $\begin{aligned} & 252, \\ & 252 \end{aligned}$ | $\begin{aligned} & 237, \\ & 252 \end{aligned}$ | $\begin{aligned} & 252, \\ & 252 \end{aligned}$ | $\begin{aligned} & 237, \\ & 252 \end{aligned}$ | $\begin{aligned} & 237, \\ & 252 \end{aligned}$ | $\begin{aligned} & 237, \\ & 240 \end{aligned}$ | $\begin{aligned} & 237, \\ & 252 \end{aligned}$ |  |  |


| D2S391 | $\begin{gathered} 70.3 \\ 1 \end{gathered}$ | $\begin{aligned} & \hline 144, \\ & 150 \end{aligned}$ | $\begin{aligned} & 150, \\ & 152 \end{aligned}$ | $\begin{aligned} & \hline 150, \\ & 152 \end{aligned}$ | $\begin{aligned} & \hline 144, \\ & 150 \end{aligned}$ | $\begin{aligned} & \hline 144, \\ & 152 \end{aligned}$ | $\begin{aligned} & 144, \\ & 150 \end{aligned}$ | $\begin{aligned} & \hline 150, \\ & 150 \end{aligned}$ | $\begin{aligned} & \hline 150, \\ & 150 \end{aligned}$ | $\begin{aligned} & \hline 144, \\ & 152 \end{aligned}$ | $\begin{aligned} & 144, \\ & 144 \end{aligned}$ | $\begin{aligned} & \hline 144, \\ & 144 \end{aligned}$ | $\begin{aligned} & \hline 144, \\ & 144 \end{aligned}$ | $\begin{aligned} & 144, \\ & 144 \end{aligned}$ | $\begin{aligned} & \hline 144, \\ & 144 \end{aligned}$ | $\begin{aligned} & \hline 144, \\ & 150 \end{aligned}$ | $\begin{aligned} & 144, \\ & 144 \end{aligned}$ |  | $\begin{aligned} & 144, \\ & 144 \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D2S2739 | $\begin{gathered} 73.6 \\ 1 \end{gathered}$ | $\begin{aligned} & 319, \\ & 323 \end{aligned}$ | $\begin{aligned} & \hline 291, \\ & 315 \end{aligned}$ | $\begin{aligned} & \hline 315, \\ & 319 \\ & \hline \end{aligned}$ | $\begin{aligned} & 291, \\ & 323 \end{aligned}$ | $\begin{aligned} & 315, \\ & 323 \end{aligned}$ | $\begin{array}{\|l\|} \hline 391, \\ 323 \\ \hline \end{array}$ |  |  | $\begin{aligned} & 309, \\ & 323 \end{aligned}$ | $\begin{gathered} 321, \\ 327 \end{gathered}$ | $\begin{aligned} & 321, \\ & 323 \end{aligned}$ | $\begin{gathered} 309, \\ 327 \end{gathered}$ | $\begin{aligned} & 285, \\ & 309 \end{aligned}$ | $\begin{aligned} & 309, \\ & 323 \end{aligned}$ | $\begin{gathered} \hline 391, \\ 315 \end{gathered}$ | $\begin{gathered} 309, \\ 315 \end{gathered}$ |  |  |
| D2S337 | $\begin{gathered} 80.6 \\ 9 \end{gathered}$ | $\begin{aligned} & 235, \\ & 249 \end{aligned}$ | $\begin{aligned} & 233, \\ & 235 \end{aligned}$ | $\begin{aligned} & 233, \\ & 235 \\ & \hline \end{aligned}$ | $\begin{aligned} & 233, \\ & 249 \end{aligned}$ | $\begin{aligned} & 235, \\ & 249 \end{aligned}$ | $\begin{array}{\|c\|} \hline 233, \\ 235 \\ \hline \end{array}$ |  |  | $\begin{aligned} & 239, \\ & 249 \end{aligned}$ | $\begin{aligned} & 247, \\ & 253 \end{aligned}$ | $\begin{aligned} & 249, \\ & 253 \end{aligned}$ | $\begin{aligned} & 239, \\ & 247 \end{aligned}$ | $\begin{aligned} & 239, \\ & 247 \end{aligned}$ | $\begin{aligned} & 239, \\ & 249 \end{aligned}$ | $\begin{aligned} & 235, \\ & 237 \end{aligned}$ | $\begin{aligned} & 237, \\ & 239 \end{aligned}$ |  |  |
| D2S290 | $\begin{gathered} 84.4 \\ 2 \end{gathered}$ | $\begin{aligned} & 205, \\ & 215 \end{aligned}$ | $\begin{aligned} & 215, \\ & 219 \end{aligned}$ | $\begin{aligned} & 205, \\ & 215 \\ & \hline \end{aligned}$ | $\begin{aligned} & 215, \\ & 215 \end{aligned}$ | $\begin{gathered} 215, \\ 219 \end{gathered}$ | $\begin{array}{\|c\|} \hline 205, \\ 215 \\ \hline \end{array}$ |  |  | $\begin{aligned} & 205, \\ & 215 \end{aligned}$ | $\begin{gathered} 207, \\ 219 \end{gathered}$ | $\begin{aligned} & 205, \\ & 207 \end{aligned}$ | $\begin{gathered} 205, \\ 219 \end{gathered}$ | $\begin{aligned} & 205, \\ & 219 \end{aligned}$ | $\begin{aligned} & 205, \\ & 215 \end{aligned}$ | $\begin{gathered} 217, \\ 219 \end{gathered}$ | $\begin{aligned} & 205, \\ & 217 \end{aligned}$ |  |  |
| D2S166 | $\begin{gathered} 82.4 \\ 2 \end{gathered}$ | $\begin{aligned} & 240, \\ & 242 \end{aligned}$ | $\begin{aligned} & 240, \\ & 242 \end{aligned}$ | $\begin{aligned} & 240, \\ & 240 \\ & \hline \end{aligned}$ | $\begin{aligned} & \hline 240, \\ & 242 \end{aligned}$ | $\begin{aligned} & 242, \\ & 242 \end{aligned}$ | $\begin{array}{\|c\|} \hline 240, \\ 240 \\ \hline \end{array}$ | $\begin{aligned} & 240, \\ & 242 \end{aligned}$ | $\begin{aligned} & \hline 240, \\ & 240 \end{aligned}$ | $\begin{aligned} & \hline 240, \\ & 242 \end{aligned}$ | $\begin{aligned} & 242, \\ & 242 \end{aligned}$ | $\begin{aligned} & 240, \\ & 242 \end{aligned}$ | $\begin{aligned} & 240, \\ & 242 \end{aligned}$ | $\begin{aligned} & 240, \\ & 242 \end{aligned}$ | $\begin{aligned} & \hline 240, \\ & 242 \end{aligned}$ | $\begin{aligned} & \hline 240, \\ & 244 \end{aligned}$ | $\begin{aligned} & 240, \\ & 244 \end{aligned}$ |  |  |
| D2S285 | $\begin{gathered} 86.0 \\ 2 \end{gathered}$ | $\begin{aligned} & \hline 191, \\ & 193 \end{aligned}$ | $\begin{aligned} & \hline 191, \\ & 203 \end{aligned}$ | $\begin{array}{\|l\|} \hline 193, \\ 203 \\ \hline \end{array}$ | $\begin{aligned} & 191, \\ & 203 \end{aligned}$ | $\begin{aligned} & \hline 191, \\ & 191 \end{aligned}$ | $\begin{array}{\|l\|} \hline 193, \\ 203 \\ \hline \end{array}$ | $\begin{aligned} & \hline 193, \\ & 203 \end{aligned}$ | $\begin{aligned} & \hline 191, \\ & 203 \\ & \hline \end{aligned}$ | $\begin{array}{\|l\|} \hline 191, \\ 193 \\ \hline \end{array}$ | $\begin{aligned} & 189, \\ & 191 \end{aligned}$ | $\begin{aligned} & \hline 189, \\ & 193 \end{aligned}$ | $\begin{aligned} & \hline 191, \\ & 193 \end{aligned}$ | $\begin{aligned} & \hline 189, \\ & 913 \end{aligned}$ | $\begin{aligned} & \hline 181, \\ & 193 \\ & \hline \end{aligned}$ | $\begin{aligned} & \hline 189, \\ & 199 \\ & \hline \end{aligned}$ | $\begin{aligned} & \hline 189, \\ & 193 \end{aligned}$ |  |  |
| D2S327 | $\begin{gathered} 88.1 \\ 5 \end{gathered}$ | $\begin{aligned} & 123, \\ & 125 \end{aligned}$ | $\begin{aligned} & 125, \\ & 125 \end{aligned}$ | $\begin{aligned} & 123, \\ & 125 \end{aligned}$ | $\begin{aligned} & 125, \\ & 125 \end{aligned}$ | $\begin{aligned} & 125, \\ & 125 \end{aligned}$ | $\begin{aligned} & 123, \\ & 125 \end{aligned}$ | $\begin{aligned} & 123, \\ & 125 \end{aligned}$ | $\begin{aligned} & 125, \\ & 125 \end{aligned}$ | $\begin{aligned} & 123, \\ & 125 \end{aligned}$ | $\begin{aligned} & 127, \\ & 129 \end{aligned}$ | $\begin{aligned} & 123, \\ & 127 \end{aligned}$ | $\begin{aligned} & 125, \\ & 129 \end{aligned}$ | $\begin{aligned} & 123, \\ & 127 \end{aligned}$ | $\begin{aligned} & 123, \\ & 125 \end{aligned}$ | $\begin{aligned} & \hline 125, \\ & 129 \end{aligned}$ | $\begin{aligned} & 123, \\ & 125 \end{aligned}$ |  |  |
| D2S2113 | $\begin{gathered} 88.1 \\ 5 \end{gathered}$ | $\begin{aligned} & \hline 194, \\ & 194 \end{aligned}$ | $\begin{aligned} & \hline 194, \\ & 200 \end{aligned}$ | $\begin{aligned} & \hline 194, \\ & 200 \end{aligned}$ | $\begin{aligned} & \hline 194, \\ & 200 \end{aligned}$ | $\begin{aligned} & \hline 194, \\ & 194 \end{aligned}$ | $\begin{array}{\|l\|} \hline 194, \\ 200 \\ \hline \end{array}$ |  |  | $\begin{aligned} & \hline 194, \\ & 194 \end{aligned}$ | $\begin{aligned} & \hline 194, \\ & 194 \end{aligned}$ | $\begin{aligned} & \hline 194, \\ & 194 \end{aligned}$ | $\begin{aligned} & \hline 194, \\ & 194 \end{aligned}$ | $\begin{aligned} & \hline 188, \\ & 194 \end{aligned}$ | $\begin{aligned} & \hline 194, \\ & 194 \end{aligned}$ | $\begin{aligned} & \hline 194, \\ & 200 \\ & \hline \end{aligned}$ | $\begin{aligned} & \hline 194, \\ & 200 \end{aligned}$ |  |  |
| D2S2110 | $\begin{gathered} 90.8 \\ 2 \end{gathered}$ | $\begin{aligned} & 139, \\ & 141 \end{aligned}$ | $\begin{aligned} & \hline 133, \\ & 139 \end{aligned}$ | $\begin{array}{\|l\|} \hline 133, \\ 141 \\ \hline \end{array}$ | $\begin{array}{\|l\|} \hline 133, \\ 141 \end{array}$ | $\begin{aligned} & 139 \\ & 141 \end{aligned}$ | $\begin{array}{\|l\|} \hline 133, \\ 139 \\ \hline \end{array}$ | $\begin{aligned} & 133, \\ & 139 \end{aligned}$ | $\begin{aligned} & \hline 133, \\ & 141 \end{aligned}$ | $\begin{aligned} & 139, \\ & 13, \end{aligned}$ | $\begin{gathered} 133, \\ 139 \end{gathered}$ | $\begin{aligned} & \hline 139, \\ & 139 \end{aligned}$ | $\begin{gathered} 133, \\ 141 \end{gathered}$ | $\begin{aligned} & \hline 133, \\ & 139 \end{aligned}$ | $\begin{aligned} & \hline 139, \\ & 14 \end{aligned}$ | $\begin{aligned} & \hline 139, \\ & 141 \end{aligned}$ | $\begin{aligned} & 133, \\ & 141 \end{aligned}$ |  |  |
| D2S286 | $\begin{gathered} 94.0 \\ 5 \end{gathered}$ | $\begin{aligned} & 136, \\ & 144 \end{aligned}$ | $\begin{aligned} & 136, \\ & 136 \end{aligned}$ | $\begin{aligned} & 136, \\ & 144 \end{aligned}$ | $\begin{aligned} & 136 \\ & 144 \end{aligned}$ | $\begin{aligned} & 136, \\ & 144 \end{aligned}$ | $\begin{aligned} & 136, \\ & 136 \end{aligned}$ | $\begin{aligned} & 136, \\ & 136 \end{aligned}$ |  | $\begin{aligned} & 136 \\ & 144 \end{aligned}$ | $\begin{aligned} & 136, \\ & 136 \end{aligned}$ | $\begin{gathered} 136, \\ 136 \end{gathered}$ | $\begin{aligned} & 144, \\ & 136 \end{aligned}$ | $\begin{aligned} & 130, \\ & 136 \end{aligned}$ | $\begin{aligned} & 136, \\ & 144 \end{aligned}$ | $\begin{aligned} & 136, \\ & 136 \end{aligned}$ | $\begin{aligned} & 136, \\ & 136 \end{aligned}$ | $\begin{aligned} & 136, \\ & 144 \end{aligned}$ | $\begin{aligned} & 136, \\ & 144 \end{aligned}$ |
| D2S1777 | $99.4$ | $\begin{aligned} & 200, \\ & 200 \end{aligned}$ | $\begin{aligned} & 200, \\ & 204 \end{aligned}$ | $\begin{aligned} & 200, \\ & 204 \end{aligned}$ | $\begin{array}{\|l\|} \hline 200, \\ 204 \\ \hline \end{array}$ | $\begin{aligned} & 200, \\ & 200 \end{aligned}$ | $\begin{aligned} & 200, \\ & 200 \end{aligned}$ |  |  | $\begin{array}{\|l\|} \hline 200, \\ 200 \\ \hline \end{array}$ | $\begin{aligned} & 204, \\ & 208 \end{aligned}$ | $\begin{aligned} & 200, \\ & 208 \end{aligned}$ | $\begin{aligned} & 200, \\ & 204 \end{aligned}$ | $\begin{aligned} & 200, \\ & 200 \end{aligned}$ | $\begin{array}{\|l\|} \hline 200, \\ 200 \\ \hline \end{array}$ | $\begin{aligned} & 200, \\ & 204 \end{aligned}$ | $\begin{aligned} & 200, \\ & 200 \end{aligned}$ |  |  |
| D2S329 | $\begin{gathered} 101 . \\ 02 \end{gathered}$ | $\begin{aligned} & 244, \\ & 244 \end{aligned}$ | $\begin{aligned} & 236, \\ & 238 \end{aligned}$ | $\begin{aligned} & 238, \\ & 244 \\ & \hline \end{aligned}$ | $\begin{aligned} & 238, \\ & 244 \end{aligned}$ | $\begin{aligned} & 236 \\ & 244 \end{aligned}$ | $\begin{array}{\|l\|} \hline 238, \\ 244 \\ \hline \end{array}$ | $\begin{aligned} & 238, \\ & 244 \end{aligned}$ | $\begin{array}{\|l\|} \hline 238, \\ 244 \\ \hline \end{array}$ | $\begin{array}{\|c\|} \hline 242, \\ 244 \\ \hline \end{array}$ | $\begin{aligned} & 244, \\ & 244 \end{aligned}$ | $\begin{gathered} 242, \\ 244 \end{gathered}$ | $\begin{aligned} & 244, \\ & 244 \end{aligned}$ | $\begin{aligned} & 242, \\ & 248 \end{aligned}$ | $\begin{aligned} & 244, \\ & 244 \end{aligned}$ | $\begin{aligned} & 242, \\ & 242 \end{aligned}$ | $\begin{aligned} & 242, \\ & 244 \end{aligned}$ |  |  |
| D2S2333 | $\begin{gathered} 103 . \\ 16 \end{gathered}$ | $\begin{aligned} & 254, \\ & 260 \end{aligned}$ | $\begin{aligned} & 254, \\ & 256 \end{aligned}$ | $\begin{aligned} & 254, \\ & 256 \\ & \hline \end{aligned}$ | $\begin{aligned} & 254, \\ & 260 \end{aligned}$ | $\begin{aligned} & 254, \\ & 254 \end{aligned}$ | $\begin{array}{\|l\|} \hline 256, \\ 260 \\ \hline \end{array}$ | $\begin{aligned} & 256, \\ & 260 \end{aligned}$ | $\begin{array}{\|l\|} \hline 254, \\ 256 \\ \hline \end{array}$ | $\begin{aligned} & 254, \\ & 256 \end{aligned}$ | $\begin{aligned} & 248, \\ & 254 \end{aligned}$ | $\begin{aligned} & 254, \\ & 256 \end{aligned}$ | $\begin{aligned} & 248, \\ & 254 \end{aligned}$ | $\begin{aligned} & 254, \\ & 256 \end{aligned}$ | $\begin{aligned} & 254, \\ & 256 \end{aligned}$ | $\begin{aligned} & \hline 254, \\ & 258 \end{aligned}$ | $\begin{aligned} & 256, \\ & 258 \end{aligned}$ | $\begin{aligned} & 254, \\ & 258 \end{aligned}$ | $\begin{aligned} & 254, \\ & 254 \end{aligned}$ |
| D2S160 | $\begin{gathered} 122 . \\ 96 \end{gathered}$ | $\begin{aligned} & 212, \\ & 214 \end{aligned}$ | $\begin{aligned} & 212, \\ & 214 \end{aligned}$ | $\begin{aligned} & \hline 214, \\ & 214 \\ & \hline \end{aligned}$ | $\begin{aligned} & 212, \\ & 214 \end{aligned}$ | $\begin{aligned} & 212, \\ & 214 \end{aligned}$ | $\begin{array}{\|l\|} \hline 212, \\ 214 \\ \hline \end{array}$ | $\begin{aligned} & 212, \\ & 214 \end{aligned}$ | $\begin{array}{\|l\|} \hline 214, \\ 214 \\ \hline \end{array}$ | $\begin{aligned} & 212, \\ & 214 \end{aligned}$ | $\begin{aligned} & 208, \\ & 214 \end{aligned}$ | $\begin{aligned} & 208, \\ & 212 \end{aligned}$ | $\begin{aligned} & 208, \\ & 214 \end{aligned}$ | $\begin{aligned} & 212, \\ & 212 \end{aligned}$ | $\begin{aligned} & 212, \\ & 214 \end{aligned}$ | $\begin{gathered} 210, \\ 210 \end{gathered}$ | $\begin{aligned} & 210, \\ & 212 \end{aligned}$ |  | $\begin{aligned} & 210, \\ & 214 \end{aligned}$ |
| D2S1334 | $\begin{gathered} 145 . \\ 08 . \end{gathered}$ | $\begin{aligned} & 286, \\ & 290 \end{aligned}$ | $\begin{aligned} & 286, \\ & 298 \end{aligned}$ | $\begin{aligned} & 290, \\ & 298 \end{aligned}$ | $\begin{gathered} 298, \\ 86 \end{gathered}$ | $\begin{gathered} 28, \\ 286 \end{gathered}$ | $\begin{aligned} & 286, \\ & 286 \end{aligned}$ |  |  | $\begin{array}{\|l\|} \hline 290, \\ 294 \\ \hline \end{array}$ | $\begin{aligned} & 286, \\ & 298 \end{aligned}$ | $\begin{aligned} & 286 \\ & 294 \end{aligned}$ | $\begin{aligned} & 286, \\ & 290 \end{aligned}$ | $\begin{aligned} & 286, \\ & 298 \end{aligned}$ | $\begin{aligned} & 290, \\ & 294 \end{aligned}$ | $\begin{aligned} & 286, \\ & 294 \end{aligned}$ |  |  |  |


| D2S151 | $\begin{gathered} 152 . \\ 04 \end{gathered}$ | $\begin{aligned} & 221, \\ & 227 \end{aligned}$ | $\begin{aligned} & 221, \\ & 221 \end{aligned}$ | $\begin{aligned} & 221, \\ & 227 \end{aligned}$ | $\begin{aligned} & 221, \\ & 221 \end{aligned}$ | $\begin{aligned} & 221, \\ & 221 \end{aligned}$ | $\begin{aligned} & 221, \\ & 221 \end{aligned}$ | $\begin{aligned} & 221, \\ & 227 \end{aligned}$ | $\begin{aligned} & 221, \\ & 227 \end{aligned}$ | $\begin{aligned} & 221, \\ & 227 \end{aligned}$ | $\begin{aligned} & 225, \\ & 225 \end{aligned}$ | $\begin{aligned} & 221, \\ & 225 \end{aligned}$ | $\begin{aligned} & 225, \\ & 227 \end{aligned}$ | $\begin{aligned} & 221, \\ & 225 \end{aligned}$ |  | $\begin{aligned} & 221, \\ & 227 \end{aligned}$ |  | $\begin{aligned} & 227, \\ & 231 \end{aligned}$ | $\begin{aligned} & 221, \\ & 227 \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D2S142 | $\begin{gathered} 161 . \\ 26 \end{gathered}$ | $\begin{aligned} & 254, \\ & 254 \end{aligned}$ | $\begin{aligned} & 260, \\ & 262 \end{aligned}$ | $\begin{aligned} & 254, \\ & 262 \end{aligned}$ | $\begin{aligned} & 254, \\ & 260 \end{aligned}$ | $\begin{aligned} & 254, \\ & 262 \end{aligned}$ | $\begin{aligned} & 254, \\ & 262 \end{aligned}$ | $\begin{aligned} & 254, \\ & 260 \end{aligned}$ | $\begin{aligned} & 254, \\ & 260 \end{aligned}$ | $\begin{aligned} & 254, \\ & 254 \end{aligned}$ | $\begin{aligned} & \hline 260, \\ & 260 \end{aligned}$ | $\begin{aligned} & 254, \\ & 260 \end{aligned}$ |  | $\begin{aligned} & 254, \\ & 264 \end{aligned}$ | $\begin{aligned} & 254, \\ & 262 \end{aligned}$ | $\begin{aligned} & 258, \\ & 264 \end{aligned}$ | $\begin{aligned} & 254, \\ & 264 \end{aligned}$ | $\begin{aligned} & 258, \\ & 262 \end{aligned}$ | $\begin{aligned} & 258, \\ & 262 \end{aligned}$ |
| D2S117 | $\begin{gathered} 194 . \\ 45 \end{gathered}$ | $\begin{aligned} & 204, \\ & 206 \end{aligned}$ |  | $\begin{aligned} & 206, \\ & 206 \end{aligned}$ | $\begin{aligned} & 206, \\ & 206 \end{aligned}$ | $\begin{aligned} & 206 \\ & 206 \end{aligned}$ | $\begin{aligned} & 204, \\ & 208 \end{aligned}$ | $\begin{aligned} & 206, \\ & 208 \end{aligned}$ | $\begin{aligned} & 206 \\ & 206 \end{aligned}$ | $\begin{aligned} & 192, \\ & 204 \end{aligned}$ | $\begin{gathered} 188, \\ 192 \end{gathered}$ | $\begin{gathered} 192, \\ 192 \end{gathered}$ | $\begin{aligned} & 192, \\ & 204 \end{aligned}$ | $\begin{aligned} & 192, \\ & 204 \end{aligned}$ | $\begin{aligned} & 192, \\ & 192 \end{aligned}$ | $\begin{aligned} & \hline 206, \\ & 208 \end{aligned}$ | $\begin{aligned} & 192, \\ & 208 \end{aligned}$ | $\begin{aligned} & 192, \\ & 206 \\ & \hline \end{aligned}$ | $\begin{aligned} & 192, \\ & 206 \\ & \hline \end{aligned}$ |
| D2S325 | $\begin{gathered} 204 . \\ 53 \end{gathered}$ | $\begin{aligned} & \hline 200, \\ & 206 \\ & \hline \end{aligned}$ |  | $\begin{aligned} & 206, \\ & 206 \end{aligned}$ | $\begin{aligned} & 204, \\ & 206 \end{aligned}$ | $\begin{aligned} & 206 \\ & 206 \end{aligned}$ | $\begin{aligned} & 200, \\ & 204 \end{aligned}$ | $\begin{aligned} & 204, \\ & 206 \end{aligned}$ | $\begin{aligned} & 206, \\ & 206 \end{aligned}$ | $\begin{aligned} & 200, \\ & 206 \end{aligned}$ | $\begin{aligned} & 198, \\ & 198 \\ & \hline \end{aligned}$ | $\begin{aligned} & 198, \\ & 206 \end{aligned}$ | $\begin{aligned} & \hline 198, \\ & 200 \end{aligned}$ | $\begin{aligned} & 200, \\ & 200 \end{aligned}$ | $\begin{aligned} & \hline 200, \\ & 200 \end{aligned}$ | $\begin{aligned} & 198, \\ & 200 \end{aligned}$ | $\begin{aligned} & 200, \\ & 200 \end{aligned}$ | $\begin{aligned} & 200, \\ & 200 \end{aligned}$ | $\begin{aligned} & 200, \\ & 200 \end{aligned}$ |
| D2S2382 | $\begin{gathered} 213 . \\ 49 \end{gathered}$ | $\begin{aligned} & 251, \\ & 261 \end{aligned}$ | $\begin{aligned} & 251, \\ & 259 \end{aligned}$ | $\begin{aligned} & 259, \\ & 261 \end{aligned}$ | $\begin{aligned} & 259, \\ & 261 \end{aligned}$ |  | $\begin{aligned} & 251, \\ & 251 \end{aligned}$ | $\begin{aligned} & 259, \\ & 261 \end{aligned}$ | $\begin{aligned} & 259, \\ & 261 \end{aligned}$ | $\begin{aligned} & \hline 261, \\ & 273 \end{aligned}$ | $\begin{gathered} 279, \\ 279 \end{gathered}$ | $\begin{aligned} & 261, \\ & 279 \end{aligned}$ | $\begin{aligned} & 273, \\ & 279 \end{aligned}$ | $\begin{aligned} & 273 \\ & 285 \end{aligned}$ | $\begin{aligned} & 251, \\ & 285 \end{aligned}$ | $\begin{aligned} & 251, \\ & 256 \end{aligned}$ |  |  | $\begin{aligned} & 255 \\ & 285 \end{aligned}$ |
| D2S434 | $\begin{gathered} 215 \\ 78 \end{gathered}$ | $\begin{aligned} & 270, \\ & 274 \end{aligned}$ | $\begin{aligned} & 266, \\ & 278 \end{aligned}$ | $\begin{aligned} & 266, \\ & 274 \end{aligned}$ | $\begin{aligned} & 266, \\ & 274 \end{aligned}$ | $\begin{aligned} & 274, \\ & 278 \end{aligned}$ | $\begin{aligned} & 270, \\ & 278 \end{aligned}$ |  |  | $\begin{aligned} & 274, \\ & 274 \end{aligned}$ | $\begin{aligned} & 270, \\ & 282 \end{aligned}$ | $\begin{aligned} & 274, \\ & 282 \end{aligned}$ | $\begin{aligned} & 274, \\ & 282 \end{aligned}$ | $\begin{aligned} & 274, \\ & 274 \end{aligned}$ | $\begin{aligned} & 270, \\ & 274 \end{aligned}$ | $\begin{aligned} & \hline 270, \\ & 274 \end{aligned}$ | $\begin{aligned} & 274, \\ & 274 \end{aligned}$ |  |  |
| D2S126 | $\begin{gathered} 221 . \\ 13 \end{gathered}$ | $\begin{aligned} & 141, \\ & 151 \end{aligned}$ |  | $\begin{aligned} & 151, \\ & 153 \end{aligned}$ | $\begin{gathered} 141, \\ 153 \end{gathered}$ | $\begin{gathered} 141, \\ 151 \end{gathered}$ | $\begin{gathered} 141, \\ 151 \end{gathered}$ | $\begin{aligned} & 151, \\ & 153 \end{aligned}$ | $\begin{aligned} & \hline 141, \\ & 153 \\ & \hline \end{aligned}$ | $\begin{gathered} 141, \\ 151 \end{gathered}$ | $\begin{gathered} 153, \\ 155 \end{gathered}$ | $\begin{aligned} & 151, \\ & 155 \end{aligned}$ | $\begin{aligned} & 151, \\ & 153 \end{aligned}$ | $\begin{aligned} & 151, \\ & 155 \end{aligned}$ | $\begin{aligned} & 141, \\ & 151 \end{aligned}$ | $\begin{aligned} & 153, \\ & 155 \end{aligned}$ | $\begin{aligned} & 151, \\ & 155 \end{aligned}$ | $\begin{aligned} & 151, \\ & 155 \end{aligned}$ | $\begin{aligned} & 151, \\ & 155 \end{aligned}$ |
| D2S2968 | $\begin{gathered} 251 . \\ 94 \end{gathered}$ | $\begin{aligned} & 183, \\ & 187 \end{aligned}$ | $\begin{aligned} & 183, \\ & 187 \end{aligned}$ | $\begin{aligned} & 183, \\ & 187 \end{aligned}$ | $\begin{gathered} 187, \\ 187 \end{gathered}$ | $\begin{aligned} & 183, \\ & 187 \end{aligned}$ | $\begin{aligned} & 183, \\ & 187 \end{aligned}$ |  |  | $\begin{gathered} 183, \\ 183 \end{gathered}$ | $\begin{gathered} 183, \\ 183 \end{gathered}$ | $\begin{gathered} 183, \\ 183 \end{gathered}$ | $\begin{aligned} & 183, \\ & 183 \end{aligned}$ | $\begin{gathered} 183, \\ 183 \end{gathered}$ | $\begin{aligned} & 183, \\ & 187 \end{aligned}$ | $\begin{gathered} 183, \\ 179 \end{gathered}$ | $\begin{aligned} & 179, \\ & 183 \end{aligned}$ |  |  |
| D2S125 | $\begin{gathered} 260 . \\ 63 \end{gathered}$ | $\begin{gathered} 98,9 \\ 8 \end{gathered}$ | $\begin{gathered} 98,1 \\ 04 \end{gathered}$ | $\begin{gathered} 98,9 \\ 8 \end{gathered}$ | $\begin{gathered} 98,9 \\ 8 \end{gathered}$ | $\begin{gathered} 98,1 \\ 04 \end{gathered}$ | $\begin{gathered} 98,1 \\ 04 \end{gathered}$ | $\begin{gathered} 98,9 \\ 8 \end{gathered}$ | $\begin{gathered} 98,1 \\ 04 \end{gathered}$ | $\begin{aligned} & 104 \\ & 106 \end{aligned}$ |  | $\begin{gathered} \hline 98,1 \\ 04 \end{gathered}$ | $\begin{gathered} 98,1 \\ 06 \end{gathered}$ | $\begin{gathered} 98,1 \\ 06 \end{gathered}$ | $\begin{gathered} \hline 98,1 \\ 04 \end{gathered}$ | $\begin{gathered} 92,9 \\ 4 \end{gathered}$ | $\begin{gathered} \hline 94,1 \\ 04 \end{gathered}$ |  | $\begin{gathered} 92,1 \\ 04 \end{gathered}$ |
| D3S1304 | $\begin{gathered} 22.3 \\ 3 \end{gathered}$ | $\begin{aligned} & 263, \\ & 263 \end{aligned}$ | $\begin{aligned} & 267, \\ & 267 \end{aligned}$ | $\begin{aligned} & 263, \\ & 267 \end{aligned}$ | $\begin{aligned} & 263, \\ & 267 \end{aligned}$ | $\begin{aligned} & 263, \\ & 267 \end{aligned}$ | $\begin{aligned} & 263, \\ & 267 \end{aligned}$ |  |  | $\begin{aligned} & 263, \\ & 263 \end{aligned}$ | $\begin{aligned} & 265 \\ & 267 \end{aligned}$ | $\begin{aligned} & 263, \\ & 267 \end{aligned}$ | $\begin{aligned} & 263 \\ & 265 \end{aligned}$ | $\begin{aligned} & 263, \\ & 263 \end{aligned}$ | $\begin{aligned} & 263, \\ & 267 \end{aligned}$ | $\begin{aligned} & 253, \\ & 267 \end{aligned}$ | $\begin{aligned} & 253, \\ & 267 \end{aligned}$ |  |  |
| D3S1263 | $\begin{gathered} 36.1 \\ 0 \end{gathered}$ | $\begin{aligned} & 233, \\ & 239 \end{aligned}$ |  | $\begin{aligned} & 239, \\ & 247 \end{aligned}$ |  | $\begin{aligned} & 233, \\ & 239 \end{aligned}$ | $\begin{aligned} & 239, \\ & 247 \end{aligned}$ | $\begin{aligned} & 239, \\ & 247 \end{aligned}$ | $\begin{aligned} & 239, \\ & 247 \end{aligned}$ | $\begin{aligned} & 233, \\ & 239 \end{aligned}$ | $\begin{aligned} & 233, \\ & 237 \end{aligned}$ | $\begin{aligned} & 233, \\ & 239 \end{aligned}$ | $\begin{aligned} & 233, \\ & 237 \end{aligned}$ | $\begin{aligned} & 233, \\ & 239 \end{aligned}$ | $\begin{aligned} & 237, \\ & 239 \end{aligned}$ | $\begin{gathered} 231, \\ 235 \end{gathered}$ | $\begin{aligned} & 235 \\ & 237 \end{aligned}$ | $\begin{aligned} & 231, \\ & 239 \end{aligned}$ | $\begin{aligned} & 231, \\ & 239 \end{aligned}$ |
| D3S2338 | $\begin{gathered} 42.1 \\ 0 \end{gathered}$ | $\begin{aligned} & 179 \\ & 179 \end{aligned}$ | $\begin{aligned} & 183, \\ & 189 \end{aligned}$ | $\begin{aligned} & 179, \\ & 189 \end{aligned}$ | $\begin{aligned} & 179, \\ & 183 \end{aligned}$ | $\begin{gathered} 179, \\ 183 \end{gathered}$ | $\begin{aligned} & 179 \\ & 189 \end{aligned}$ | $\begin{aligned} & 179, \\ & 189 \end{aligned}$ | $\begin{aligned} & 179, \\ & 189 \end{aligned}$ | $\begin{gathered} 179 \\ 179 \end{gathered}$ | $\begin{gathered} 189 \\ 189 \end{gathered}$ | $\begin{gathered} 179 \\ 189 \end{gathered}$ | $\begin{gathered} 179 \\ 189 \end{gathered}$ |  | $\begin{aligned} & 179 \\ & 195 \end{aligned}$ |  | $\begin{aligned} & 193 \\ & 195 \end{aligned}$ | $\begin{aligned} & 179, \\ & 191 \end{aligned}$ |  |
| D3S3038 | $\begin{gathered} 44.8 \\ 1 \end{gathered}$ | $\begin{aligned} & 199, \\ & 203 \end{aligned}$ | $\begin{aligned} & 195, \\ & 203 \end{aligned}$ | $\begin{gathered} 195, \\ 199 \end{gathered}$ | $\begin{aligned} & 203, \\ & 203 \end{aligned}$ | $\begin{aligned} & 203, \\ & 203 \end{aligned}$ | $\begin{aligned} & 195, \\ & 203 \end{aligned}$ |  |  | $\begin{aligned} & 199, \\ & 215 \end{aligned}$ | $\begin{gathered} 199, \\ 199 \end{gathered}$ | $\begin{gathered} 199, \\ 199 \end{gathered}$ | $\begin{aligned} & 199, \\ & 215 \end{aligned}$ | $\begin{aligned} & 199, \\ & 203 \end{aligned}$ | $\begin{aligned} & 199 \\ & 215 \end{aligned}$ | $\begin{gathered} 199, \\ 199 \end{gathered}$ | $\begin{aligned} & 199, \\ & 215 \end{aligned}$ |  |  |
| D3S1266 | $\begin{gathered} 52.6 \\ 0 \end{gathered}$ | $\begin{aligned} & 291, \\ & 291 \end{aligned}$ | $\begin{aligned} & 289, \\ & 291 \end{aligned}$ | $\begin{aligned} & 289, \\ & 291 \end{aligned}$ | $\begin{aligned} & 291, \\ & 291 \end{aligned}$ | $\begin{aligned} & 291, \\ & 291 \end{aligned}$ | $\begin{aligned} & 289, \\ & 291 \end{aligned}$ | $\begin{aligned} & 289, \\ & 291 \end{aligned}$ | $\begin{aligned} & 289, \\ & 291 \end{aligned}$ | $\begin{aligned} & 291, \\ & 297 \end{aligned}$ | $\begin{gathered} 289, \\ 297 \end{gathered}$ | $\begin{aligned} & 297, \\ & 297 \end{aligned}$ | $\begin{aligned} & 291, \\ & 297 \end{aligned}$ |  | $\begin{aligned} & 291, \\ & 291 \end{aligned}$ | $\begin{aligned} & 291, \\ & 297 \end{aligned}$ | $\begin{aligned} & 291, \\ & 291 \end{aligned}$ | $\begin{aligned} & 291, \\ & 297 \end{aligned}$ | $\begin{aligned} & 291, \\ & 297 \end{aligned}$ |
| D3S1277 | $\begin{gathered} 61.5 \\ 2 \end{gathered}$ | $\begin{aligned} & 274, \\ & 274 \end{aligned}$ | $\begin{aligned} & 266 \\ & 268 \end{aligned}$ | $\begin{aligned} & 268 \\ & 274 \end{aligned}$ | $\begin{aligned} & 268 \\ & 274 \end{aligned}$ | $\begin{aligned} & 268 \\ & 274 \end{aligned}$ | $\begin{aligned} & 266 \\ & 274 \end{aligned}$ | $\begin{aligned} & 266 \\ & 274 \end{aligned}$ | $\begin{aligned} & 266 \\ & 274 \end{aligned}$ |  | $\begin{gathered} 268 \\ 268 \end{gathered}$ | $\begin{aligned} & 268 \\ & 274 \end{aligned}$ | $\begin{aligned} & 268 \\ & 274 \end{aligned}$ |  | $\begin{aligned} & 274 \\ & 274 \end{aligned}$ |  | $\begin{aligned} & 264, \\ & 274 \end{aligned}$ | $\begin{aligned} & 274, \\ & 274 \end{aligned}$ | $\begin{aligned} & 274, \\ & 274 \end{aligned}$ |


| D3S1289 | $\begin{gathered} \hline 71.4 \\ 1 \\ \hline \end{gathered}$ | $\begin{aligned} & 211, \\ & 215 \end{aligned}$ | $\begin{aligned} & 205 \\ & 215 \end{aligned}$ | $\begin{aligned} & 205, \\ & 211 \end{aligned}$ | $\begin{aligned} & 211, \\ & 215 \end{aligned}$ | $\begin{aligned} & 211, \\ & 215 \end{aligned}$ | $\begin{aligned} & 205, \\ & 215 \end{aligned}$ | $\begin{aligned} & 205, \\ & 211 \end{aligned}$ | $\begin{aligned} & 205 \\ & 215 \end{aligned}$ | $\begin{aligned} & 209, \\ & 217 \end{aligned}$ | $\begin{gathered} 209, \\ 209 \end{gathered}$ | $\begin{gathered} 209, \\ 209 \end{gathered}$ | $\begin{gathered} 209, \\ 217 \end{gathered}$ | $\begin{aligned} & 211, \\ & 215 \end{aligned}$ | $\begin{aligned} & 211, \\ & 217 \end{aligned}$ |  | $\begin{aligned} & 211, \\ & 217 \end{aligned}$ | $\begin{aligned} & 211, \\ & 217 \end{aligned}$ | $\begin{gathered} 217, \\ 217 \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D3S1300 | $\begin{gathered} 80.3 \\ 2 \end{gathered}$ | $\begin{aligned} & 229, \\ & 241 \end{aligned}$ | $\begin{aligned} & 235, \\ & 237 \end{aligned}$ | $\begin{aligned} & 235, \\ & 241 \end{aligned}$ | $\begin{aligned} & 239, \\ & 237 \end{aligned}$ | $\begin{aligned} & 239, \\ & 237 \end{aligned}$ | $\begin{aligned} & 237, \\ & 241 \end{aligned}$ | $\begin{aligned} & 239, \\ & 235 \end{aligned}$ |  | $\begin{aligned} & 235, \\ & 237 \end{aligned}$ | $\begin{aligned} & 217, \\ & 217 \end{aligned}$ |  | $\begin{aligned} & 217, \\ & 235 \end{aligned}$ | $\begin{aligned} & 229, \\ & 241 \end{aligned}$ | $\begin{aligned} & 229, \\ & 235 \end{aligned}$ | $\begin{aligned} & 237, \\ & 237 \end{aligned}$ | $\begin{aligned} & 235, \\ & 237 \end{aligned}$ | $\begin{aligned} & 235, \\ & 237 \end{aligned}$ | $\begin{aligned} & 235, \\ & 237 \end{aligned}$ |
| D3S4542 | $\begin{gathered} 89.9 \\ 1 \end{gathered}$ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| D3S1285 | $\begin{gathered} 91.1 \\ 8 \end{gathered}$ | $\begin{aligned} & 232, \\ & 232 \end{aligned}$ | $\begin{aligned} & 238, \\ & 238 \end{aligned}$ |  | $\begin{aligned} & 232, \\ & 238 \end{aligned}$ | $\begin{aligned} & 232, \\ & 238 \end{aligned}$ | $\begin{aligned} & 232, \\ & 238 \end{aligned}$ | $\begin{aligned} & 232, \\ & 238 \end{aligned}$ | $\begin{aligned} & 232, \\ & 238 \end{aligned}$ | $\begin{aligned} & 232, \\ & 238 \end{aligned}$ | $\begin{gathered} 238, \\ 238 \end{gathered}$ | $\begin{aligned} & 238 \\ & 238 \end{aligned}$ | $\begin{aligned} & 232, \\ & 238 \end{aligned}$ | $\begin{aligned} & 232, \\ & 232 \end{aligned}$ | $\begin{aligned} & 232, \\ & 240 \end{aligned}$ | $\begin{aligned} & 232, \\ & 238 \end{aligned}$ |  | $\begin{aligned} & 232, \\ & 240 \end{aligned}$ | $\begin{aligned} & 232, \\ & 240 \end{aligned}$ |
| D3S2406 | $\begin{gathered} 102 . \\ 64 \end{gathered}$ | $\begin{aligned} & 346, \\ & 350 \end{aligned}$ | $\begin{aligned} & 314, \\ & 318 \end{aligned}$ | $\begin{aligned} & 314, \\ & 350 \end{aligned}$ | $\begin{aligned} & 314, \\ & 350 \end{aligned}$ | $\begin{aligned} & 318, \\ & 250 \end{aligned}$ | $\begin{aligned} & \hline 318, \\ & 350 \end{aligned}$ |  |  | $\begin{aligned} & 346, \\ & 350 \end{aligned}$ | $\begin{aligned} & 306, \\ & 330 \end{aligned}$ | $\begin{aligned} & 330, \\ & 346 \end{aligned}$ | $\begin{aligned} & 330, \\ & 350 \end{aligned}$ | $\begin{aligned} & 334, \\ & 350 \end{aligned}$ | $\begin{aligned} & 334, \\ & 346 \end{aligned}$ | $\begin{aligned} & \hline 306, \\ & 330 \end{aligned}$ | $\begin{aligned} & 330, \\ & 334 \end{aligned}$ |  |  |
| D3S3681 | $\begin{gathered} 109 . \\ 22 \end{gathered}$ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| D3S1271 | $\begin{gathered} 117 . \\ 76 \end{gathered}$ | $\begin{aligned} & 156, \\ & 158 \end{aligned}$ | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ | $\begin{aligned} & 156, \\ & 158 \end{aligned}$ | $\begin{aligned} & 156 \\ & 158 \end{aligned}$ | $\begin{aligned} & 156 \\ & 158 \end{aligned}$ | $\begin{aligned} & 156, \\ & 158 \end{aligned}$ | $\begin{aligned} & 156 \\ & 156 \end{aligned}$ | $\begin{aligned} & 156 \\ & 156 \end{aligned}$ | $\begin{aligned} & 156, \\ & 158 \end{aligned}$ | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ | $\begin{aligned} & 156 \\ & 156 \end{aligned}$ | $\begin{aligned} & \hline 156 \\ & 158 \end{aligned}$ |  | $\begin{aligned} & 156, \\ & 158 \end{aligned}$ | $\begin{gathered} 154, \\ 156 \end{gathered}$ | $\begin{aligned} & 154, \\ & 156 \end{aligned}$ | $\begin{aligned} & 154, \\ & 158 \end{aligned}$ |  |
| D3S3045 | $\begin{gathered} 124 . \\ 64 \end{gathered}$ | $\begin{aligned} & 187, \\ & 195 \end{aligned}$ | $\begin{aligned} & 183, \\ & 191 \end{aligned}$ | $\begin{aligned} & 191, \\ & 195 \end{aligned}$ | $\begin{gathered} 191, \\ 195 \end{gathered}$ | $\begin{gathered} 183, \\ 195 \end{gathered}$ | $\begin{gathered} 183 \\ 195 \end{gathered}$ |  |  | $\begin{gathered} 191, \\ 195 \end{gathered}$ | $\begin{gathered} 179, \\ 195 \end{gathered}$ | $\begin{gathered} 191, \\ 195 \end{gathered}$ | $\begin{gathered} 195, \\ 195 \end{gathered}$ | $\begin{gathered} 195, \\ 191 \end{gathered}$ | $\begin{aligned} & 187, \\ & 195 \end{aligned}$ | $\begin{aligned} & 203, \\ & 203 \end{aligned}$ | $\begin{aligned} & 187, \\ & 203 \end{aligned}$ |  |  |
| D3S1278 | $\begin{gathered} 129 . \\ 73 \end{gathered}$ | $\begin{aligned} & 203, \\ & 217 \end{aligned}$ | $\begin{aligned} & 209, \\ & 225 \end{aligned}$ | $\begin{aligned} & 203, \\ & 225 \end{aligned}$ | $\begin{aligned} & 203, \\ & 225 \end{aligned}$ | $\begin{aligned} & \hline 203, \\ & 209 \end{aligned}$ | $\begin{aligned} & \hline 203, \\ & 209 \end{aligned}$ | $\begin{aligned} & 217, \\ & 225 \end{aligned}$ | $\begin{aligned} & 203, \\ & 225 \end{aligned}$ | $\begin{aligned} & 203, \\ & 227 \end{aligned}$ | $\begin{aligned} & 207, \\ & 207 \end{aligned}$ | $\begin{aligned} & 207, \\ & 227 \end{aligned}$ | $\begin{aligned} & 203, \\ & 207 \end{aligned}$ |  | $\begin{aligned} & 203, \\ & 217 \end{aligned}$ | $\begin{aligned} & 221, \\ & 221 \end{aligned}$ | $\begin{aligned} & 217, \\ & 221 \end{aligned}$ | $\begin{aligned} & 203, \\ & 221 \end{aligned}$ | $\begin{aligned} & 203, \\ & 221 \end{aligned}$ |
| D3S1303 | $\begin{gathered} 136 . \\ 32 \end{gathered}$ | $\begin{aligned} & 196, \\ & 202 \end{aligned}$ | $\begin{aligned} & 202, \\ & 202 \end{aligned}$ | $\begin{aligned} & 202, \\ & 202 \end{aligned}$ | $\begin{aligned} & 202, \\ & 202 \end{aligned}$ | $\begin{aligned} & 202, \\ & 202 \end{aligned}$ | $\begin{aligned} & 202, \\ & 202 \end{aligned}$ |  |  | $\begin{aligned} & 202, \\ & 210 \end{aligned}$ | $\begin{aligned} & 196, \\ & 202 \end{aligned}$ | $\begin{aligned} & 202, \\ & 210 \end{aligned}$ | $\begin{aligned} & 202, \\ & 202 \end{aligned}$ | $\begin{aligned} & 202, \\ & 210 \end{aligned}$ | $\begin{aligned} & 196, \\ & 202 \end{aligned}$ | $\begin{aligned} & 206 \\ & 214 \end{aligned}$ | $\begin{aligned} & 196 \\ & 214 \end{aligned}$ |  |  |
| D3S1267 | $\begin{gathered} 139 . \\ 12 \end{gathered}$ | $\begin{aligned} & 153, \\ & 167 \end{aligned}$ | $\begin{gathered} 145 \\ 149 \end{gathered}$ | $\begin{aligned} & 145, \\ & 153 \end{aligned}$ | $\begin{gathered} 149 \\ 153 \end{gathered}$ | $\begin{gathered} 145 \\ 167 \end{gathered}$ |  | $\begin{gathered} 149 \\ 167 \end{gathered}$ | $\begin{aligned} & 149 \\ & 167 \end{aligned}$ | $\begin{gathered} 149, \\ 153 \end{gathered}$ | $\begin{gathered} 155 \\ 157 \end{gathered}$ | $\begin{gathered} 149 \\ 155 \end{gathered}$ | $\begin{aligned} & 153, \\ & 155 \end{aligned}$ | $\begin{gathered} 149, \\ 153 \end{gathered}$ | $\begin{aligned} & 153, \\ & 167 \end{aligned}$ | $\begin{aligned} & 147, \\ & 149 \end{aligned}$ | $\begin{aligned} & 149 \\ & 167 \end{aligned}$ | $\begin{aligned} & 147, \\ & 167 \end{aligned}$ | $\begin{array}{r} 147 \\ 153 \end{array}$ |
| D3S1292 | $\begin{gathered} 146 . \\ 60 \end{gathered}$ | $\begin{aligned} & 160, \\ & 166 \end{aligned}$ | $\begin{aligned} & 144, \\ & 156 \end{aligned}$ | $\begin{aligned} & 156, \\ & 166 \end{aligned}$ | $\begin{gathered} 144, \\ 160 \end{gathered}$ | $\begin{aligned} & 156, \\ & 160 \end{aligned}$ | $\begin{gathered} 156, \\ 166 \end{gathered}$ | $\begin{aligned} & 156, \\ & 160 \end{aligned}$ | $\begin{aligned} & 144, \\ & 160 \end{aligned}$ | $\begin{gathered} 160, \\ 166 \end{gathered}$ | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ | $\begin{aligned} & 156, \\ & 160 \end{aligned}$ | $\begin{aligned} & 156, \\ & 166 \end{aligned}$ | $\begin{aligned} & 156, \\ & 166 \end{aligned}$ | $\begin{aligned} & 148, \\ & 160 \end{aligned}$ | $\begin{aligned} & 144, \\ & 156 \end{aligned}$ | $\begin{aligned} & 156 \\ & 160 \end{aligned}$ | $\begin{aligned} & 144, \\ & 160 \end{aligned}$ | $\begin{aligned} & 148, \\ & 156 \end{aligned}$ |
| D3S1569 | $\begin{gathered} 158 . \\ 38 \end{gathered}$ | $\begin{aligned} & 283, \\ & 287 \end{aligned}$ | $\begin{aligned} & 277, \\ & 291 \end{aligned}$ | $\begin{aligned} & 283, \\ & 291 \end{aligned}$ | $\begin{aligned} & 277, \\ & 287 \end{aligned}$ | $\begin{aligned} & 277, \\ & 283 \end{aligned}$ | $\begin{aligned} & 283, \\ & 291 \end{aligned}$ | $\begin{aligned} & 283, \\ & 291 \end{aligned}$ | $\begin{aligned} & 277, \\ & 287 \end{aligned}$ | $\begin{aligned} & 277, \\ & 291 \end{aligned}$ | $\begin{aligned} & 281, \\ & 287 \end{aligned}$ | $\begin{aligned} & 277, \\ & 287 \end{aligned}$ | $\begin{aligned} & 281, \\ & 291 \end{aligned}$ |  | $\begin{aligned} & 277, \\ & 287 \end{aligned}$ |  | $\begin{aligned} & 277, \\ & 277 \end{aligned}$ | $\begin{aligned} & 277, \\ & 277 \end{aligned}$ | $\begin{aligned} & 277, \\ & 289 \end{aligned}$ |
| D3S3053 | $\begin{gathered} 181 . \\ 87 \end{gathered}$ | $\begin{aligned} & 234, \\ & 242 \end{aligned}$ | $\begin{aligned} & 226, \\ & 234 \end{aligned}$ | $\begin{aligned} & 226, \\ & 242 \end{aligned}$ | $\begin{aligned} & 226, \\ & 242 \end{aligned}$ | $\begin{aligned} & 226, \\ & 234 \end{aligned}$ | $\begin{aligned} & 226, \\ & 242 \end{aligned}$ |  |  | $\begin{aligned} & 226, \\ & 242 \end{aligned}$ | $\begin{gathered} 226, \\ 238 \end{gathered}$ | $\begin{aligned} & 226, \\ & 238 \end{aligned}$ | $\begin{aligned} & 226, \\ & 226 \end{aligned}$ | $\begin{aligned} & 234, \\ & 238 \end{aligned}$ | $\begin{aligned} & 226, \\ & 238 \end{aligned}$ | $\begin{aligned} & 226, \\ & 238 \end{aligned}$ | $\begin{aligned} & 238 \\ & 238 \end{aligned}$ | $\begin{aligned} & 234, \\ & 238 \end{aligned}$ |  |
| D3S1565 | $\begin{gathered} 186 . \\ 04 \end{gathered}$ | $\begin{aligned} & 239, \\ & 241 \end{aligned}$ | $\begin{aligned} & 239 \\ & 245 \end{aligned}$ | $\begin{aligned} & 239, \\ & 241 \end{aligned}$ | $\begin{aligned} & 239 \\ & 241 \end{aligned}$ | $\begin{aligned} & 239, \\ & 239 \end{aligned}$ | $\begin{aligned} & 239, \\ & 241 \end{aligned}$ | $\begin{aligned} & 239, \\ & 241 \end{aligned}$ | $\begin{aligned} & 239, \\ & 239 \end{aligned}$ | $\begin{aligned} & 239, \\ & 241 \end{aligned}$ | $\begin{aligned} & 241, \\ & 241 \end{aligned}$ | $\begin{aligned} & 239 \\ & 241 \end{aligned}$ | $\begin{aligned} & 239, \\ & 241 \end{aligned}$ | $\begin{aligned} & 239, \\ & 241 \end{aligned}$ | $\begin{aligned} & 239, \\ & 241 \end{aligned}$ | $\begin{aligned} & 241, \\ & 241 \end{aligned}$ | $\begin{aligned} & 241, \\ & 241 \end{aligned}$ | $\begin{aligned} & 239 \\ & 241 \end{aligned}$ | $\begin{aligned} & 241, \\ & 241 \end{aligned}$ |


| D3S1262 | $\begin{gathered} 201 . \\ 14 \end{gathered}$ | $\begin{aligned} & 112, \\ & 112 \end{aligned}$ | $\begin{aligned} & 118 \\ & 128 \end{aligned}$ | $\begin{aligned} & 112, \\ & 128 \end{aligned}$ | $\begin{aligned} & 112, \\ & 128 \end{aligned}$ | $\begin{aligned} & 112, \\ & 128 \end{aligned}$ | $\begin{aligned} & 112, \\ & 128 \end{aligned}$ |  |  | $\begin{aligned} & 116 \\ & 116 \end{aligned}$ | $\begin{aligned} & 116, \\ & 122 \end{aligned}$ | $\begin{aligned} & 116, \\ & 122 \end{aligned}$ | $\begin{aligned} & 116, \\ & 116 \end{aligned}$ | $\begin{aligned} & 112, \\ & 116 \end{aligned}$ | $\begin{aligned} & 116, \\ & 124 \end{aligned}$ | $\begin{aligned} & 116, \\ & 124 \end{aligned}$ | $\begin{aligned} & 112, \\ & 124 \end{aligned}$ |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D3S1580 | $\begin{gathered} 207 . \\ 73 \end{gathered}$ | $\begin{aligned} & 139, \\ & 155 \end{aligned}$ | $\begin{aligned} & 139 \\ & 143 \end{aligned}$ | $\begin{aligned} & 139, \\ & 155 \end{aligned}$ | $\begin{gathered} 139, \\ 155 \end{gathered}$ | $\begin{gathered} 139, \\ 155 \end{gathered}$ | $\begin{aligned} & \hline 139, \\ & 155 \end{aligned}$ | $\begin{aligned} & 143, \\ & 155 \end{aligned}$ |  | $\begin{gathered} 145 \\ 145 \end{gathered}$ | $\begin{aligned} & 153, \\ & 155 \end{aligned}$ | $\begin{gathered} 145, \\ 155 \end{gathered}$ | $\begin{gathered} 145, \\ 153 \end{gathered}$ | $\begin{aligned} & 139, \\ & 155 \end{aligned}$ | $\begin{aligned} & 145, \\ & 155 \end{aligned}$ | $\begin{aligned} & 143, \\ & 157 \end{aligned}$ | $\begin{aligned} & 143, \\ & 155 \end{aligned}$ | $\begin{aligned} & 143, \\ & 145 \end{aligned}$ | $\begin{aligned} & 143, \\ & 155 \end{aligned}$ |
| D3S3023 | $\begin{aligned} & \hline \text { (deC } \\ & \text { ode) } \end{aligned}$ | $\begin{aligned} & 234, \\ & 234 \end{aligned}$ | $\begin{aligned} & 234, \\ & 246 \end{aligned}$ | $\begin{aligned} & 234, \\ & 234 \end{aligned}$ | $\begin{aligned} & 234, \\ & 234 \end{aligned}$ | $\begin{aligned} & 234, \\ & 246 \end{aligned}$ | $\begin{aligned} & 234, \\ & 246 \end{aligned}$ |  |  | $\begin{aligned} & 234, \\ & 234 \end{aligned}$ | $\begin{aligned} & 243, \\ & 246 \end{aligned}$ | $\begin{aligned} & 234, \\ & 246 \end{aligned}$ | $\begin{aligned} & 234, \\ & 246 \end{aligned}$ | $\begin{aligned} & 234, \\ & 234 \end{aligned}$ | $\begin{aligned} & 234, \\ & 324 \end{aligned}$ | $\begin{aligned} & 234, \\ & 240 \end{aligned}$ | $\begin{aligned} & 234, \\ & 240 \end{aligned}$ |  |  |
| D4S412 | 4.74 | $\begin{aligned} & 245 \\ & 245 \end{aligned}$ | $\begin{aligned} & 237, \\ & 237 \end{aligned}$ | $\begin{aligned} & 237, \\ & 245 \end{aligned}$ | $\begin{aligned} & 237, \\ & 245 \end{aligned}$ | $\begin{aligned} & 237, \\ & 245 \end{aligned}$ | $\begin{aligned} & 237, \\ & 245 \end{aligned}$ | $\begin{aligned} & 237, \\ & 245 \end{aligned}$ | $\begin{aligned} & 237, \\ & 245 \end{aligned}$ | $\begin{aligned} & 243, \\ & 245 \end{aligned}$ | $\begin{aligned} & 245 \\ & 245 \end{aligned}$ | $\begin{aligned} & 245 \\ & 245 \end{aligned}$ |  |  | $\begin{aligned} & 245, \\ & 245 \end{aligned}$ | $\begin{aligned} & 245, \\ & 247 \end{aligned}$ | $\begin{aligned} & 245, \\ & 247 \end{aligned}$ | $\begin{aligned} & 245, \\ & 245 \end{aligned}$ | $\begin{aligned} & 245, \\ & 247 \end{aligned}$ |
| D4S403 | $\begin{gathered} 25.9 \\ 0 \end{gathered}$ | $\begin{aligned} & 225, \\ & 227 \end{aligned}$ | $\begin{aligned} & 217, \\ & 219 \end{aligned}$ | $\begin{aligned} & 219, \\ & 225 \end{aligned}$ | $\begin{aligned} & 219, \\ & 225 \end{aligned}$ | $\begin{aligned} & 217, \\ & 227 \end{aligned}$ | $\begin{aligned} & 217, \\ & 225 \end{aligned}$ |  |  | $\begin{aligned} & 217, \\ & 227 \end{aligned}$ | $\begin{aligned} & 217, \\ & 227 \end{aligned}$ | $\begin{aligned} & 217, \\ & 217 \end{aligned}$ | $\begin{aligned} & 227, \\ & 227 \end{aligned}$ | $\begin{aligned} & 217, \\ & 225 \end{aligned}$ | $\begin{aligned} & 217, \\ & 227 \end{aligned}$ | $\begin{aligned} & 225, \\ & 227 \end{aligned}$ | $\begin{aligned} & 227, \\ & 227 \end{aligned}$ |  |  |
| D4S3248 | $\begin{gathered} 75.5 \\ 2 \end{gathered}$ | $\begin{aligned} & 241, \\ & 245 \end{aligned}$ | $\begin{aligned} & 241, \\ & 237 \end{aligned}$ | $\begin{aligned} & 241, \\ & 241 \end{aligned}$ | $\begin{aligned} & 241, \\ & 245 \end{aligned}$ | $\begin{aligned} & 237, \\ & 245 \end{aligned}$ | $\begin{aligned} & 237, \\ & 241 \end{aligned}$ |  |  | $\begin{aligned} & 241, \\ & 241 \end{aligned}$ | $\begin{aligned} & 237, \\ & 237 \end{aligned}$ | $\begin{aligned} & 241, \\ & 237 \end{aligned}$ | $\begin{aligned} & 241, \\ & 237 \end{aligned}$ | $\begin{aligned} & 237, \\ & 245 \end{aligned}$ | $\begin{aligned} & 241, \\ & 245 \end{aligned}$ | $\begin{aligned} & 237, \\ & 245 \end{aligned}$ | $\begin{aligned} & 237, \\ & 241 \end{aligned}$ |  |  |
| D4S3243 | $\begin{gathered} 88.3 \\ 5 \end{gathered}$ | $\begin{aligned} & 174, \\ & 174 \end{aligned}$ | $\begin{aligned} & 170, \\ & 174 \end{aligned}$ | $\begin{aligned} & 170, \\ & 174 \end{aligned}$ | $\begin{gathered} 170, \\ 174 \end{gathered}$ | $\begin{gathered} 174, \\ 174 \end{gathered}$ | $\begin{aligned} & 174, \\ & 174 \end{aligned}$ |  |  | $\begin{gathered} 162, \\ 170 \end{gathered}$ | $\begin{gathered} 162, \\ 174 \end{gathered}$ | $\begin{aligned} & 170, \\ & 174 \end{aligned}$ | $\begin{aligned} & 162, \\ & 170 \end{aligned}$ | $\begin{aligned} & 174, \\ & 174 \end{aligned}$ | $\begin{aligned} & 174, \\ & 174 \end{aligned}$ | $\begin{aligned} & 174, \\ & 178 \end{aligned}$ | $\begin{aligned} & 178, \\ & 174 \end{aligned}$ |  |  |
| D4S1534 | $\begin{gathered} 95.0 \\ 9 \end{gathered}$ | $\begin{aligned} & 146, \\ & 156 \end{aligned}$ | $\begin{aligned} & 158, \\ & 156 \end{aligned}$ | $\begin{aligned} & 146, \\ & 148 \end{aligned}$ | $\begin{aligned} & 148, \\ & 156 \end{aligned}$ | $\begin{gathered} 146, \\ 156 \end{gathered}$ | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ | $\begin{aligned} & 148, \\ & 156 \end{aligned}$ | $\begin{aligned} & 146, \\ & 148 \end{aligned}$ | $\begin{gathered} 146, \\ 156 \end{gathered}$ | $\begin{gathered} 146, \\ 146 \end{gathered}$ | $\begin{aligned} & \hline 146 \\ & 156 \end{aligned}$ |  | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ | $\begin{aligned} & 146 \\ & 156 \end{aligned}$ | $\begin{aligned} & 154, \\ & 158 \end{aligned}$ | $\begin{aligned} & 154, \\ & 156 \end{aligned}$ | $\begin{aligned} & 156, \\ & 158 \end{aligned}$ | $\begin{aligned} & 154, \\ & 156 \end{aligned}$ |
| D4S414 | $\begin{gathered} 100 . \\ 75 \end{gathered}$ | $\begin{aligned} & 231, \\ & 236 \end{aligned}$ | $\begin{aligned} & 236, \\ & 241 \end{aligned}$ | $\begin{aligned} & 231, \\ & 241 \end{aligned}$ | $\begin{aligned} & 231, \\ & 241 \end{aligned}$ | $\begin{aligned} & 236, \\ & 241 \end{aligned}$ | $\begin{aligned} & 236 \\ & 236 \end{aligned}$ | $\begin{aligned} & 236, \\ & 241 \end{aligned}$ | $\begin{aligned} & 231, \\ & 241 \end{aligned}$ | $\begin{aligned} & 241, \\ & 246 \end{aligned}$ | $\begin{aligned} & 234, \\ & 236 \end{aligned}$ |  | $\begin{aligned} & 234, \\ & 241 \end{aligned}$ | $\begin{aligned} & 231, \\ & 236 \end{aligned}$ | $\begin{aligned} & 231, \\ & 236 \end{aligned}$ | $\begin{aligned} & 236 \\ & 238 \end{aligned}$ | $\begin{aligned} & 236, \\ & 238 \end{aligned}$ | $\begin{aligned} & 236 \\ & 236 \end{aligned}$ | $\begin{aligned} & 236, \\ & 238 \end{aligned}$ |
| D4S1647 | $\begin{gathered} 104 . \\ 94 \end{gathered}$ | $\begin{aligned} & 140 \\ & 148 \end{aligned}$ | $\begin{aligned} & 148 \\ & 148 \end{aligned}$ | $\begin{aligned} & 148, \\ & 148 \end{aligned}$ | $\begin{aligned} & 148 \\ & 148 \end{aligned}$ | $\begin{gathered} 148, \\ 148 \end{gathered}$ | $\begin{aligned} & 140 \\ & 148 \end{aligned}$ |  |  | $\begin{aligned} & 144, \\ & 148 \end{aligned}$ | $\begin{aligned} & 144, \\ & 132 \end{aligned}$ | $\begin{aligned} & 144 \\ & 144 \end{aligned}$ | $\begin{aligned} & 144, \\ & 132 \end{aligned}$ | $\begin{aligned} & 140 \\ & 148 \end{aligned}$ | $\begin{aligned} & 140, \\ & 148 \end{aligned}$ | $\begin{aligned} & 132, \\ & 140 \end{aligned}$ | $\begin{aligned} & 140, \\ & 140 \end{aligned}$ |  |  |
| D4S1572 | $\begin{gathered} 107 . \\ 95 \end{gathered}$ | $\begin{aligned} & 147, \\ & 147 \end{aligned}$ | $\begin{gathered} 141, \\ 143 \end{gathered}$ | $\begin{gathered} 143, \\ 147 \end{gathered}$ | $\begin{gathered} 143, \\ 147 \end{gathered}$ | $\begin{gathered} 141 \\ 147 \end{gathered}$ | $\begin{aligned} & 141, \\ & 147 \end{aligned}$ | $\begin{gathered} 143, \\ 147 \end{gathered}$ | $\begin{aligned} & 143, \\ & 147 \end{aligned}$ | $\begin{gathered} 141, \\ 147 \end{gathered}$ | $\begin{aligned} & 147, \\ & 149 \end{aligned}$ | $\begin{aligned} & 141, \\ & 147 \end{aligned}$ | $\begin{aligned} & 141, \\ & 149 \end{aligned}$ | $\begin{aligned} & 147, \\ & 147 \end{aligned}$ | $\begin{aligned} & 147, \\ & 147 \end{aligned}$ | $\begin{aligned} & 147 \\ & 147 \end{aligned}$ | $\begin{aligned} & 147, \\ & 147 \end{aligned}$ | $\begin{aligned} & 147 \\ & 147 \end{aligned}$ | $\begin{aligned} & 147, \\ & 147 \end{aligned}$ |
| D4S407 | $\begin{gathered} 117 . \\ 06 \end{gathered}$ | $\begin{aligned} & 117, \\ & 125 \end{aligned}$ | $\begin{aligned} & 111, \\ & 121 \end{aligned}$ | $\begin{aligned} & 111, \\ & 117 \end{aligned}$ | $\begin{gathered} 111, \\ 117 \end{gathered}$ | $\begin{aligned} & 117, \\ & 121 \end{aligned}$ | $\begin{aligned} & 121, \\ & 125 \end{aligned}$ | $\begin{aligned} & 111, \\ & 117 \end{aligned}$ | $\begin{aligned} & 111, \\ & 117 \end{aligned}$ | $\begin{aligned} & 127, \\ & 127 \end{aligned}$ | $\begin{gathered} 117, \\ 129 \end{gathered}$ | $\begin{aligned} & 127, \\ & 129 \end{aligned}$ | $\begin{aligned} & 117, \\ & 127 \end{aligned}$ | $\begin{aligned} & 117, \\ & 125 \end{aligned}$ | $\begin{aligned} & 125, \\ & 127 \end{aligned}$ | $\begin{aligned} & 119, \\ & 125 \end{aligned}$ | $\begin{aligned} & 125, \\ & 125 \end{aligned}$ | $\begin{aligned} & 125 \\ & 125 \end{aligned}$ | $\begin{aligned} & 125, \\ & 125 \end{aligned}$ |
| D4S2989 | $\begin{aligned} & 203, \\ & 211 \end{aligned}$ | $\begin{aligned} & 207, \\ & 209 \end{aligned}$ | $\begin{aligned} & 207, \\ & 211 \end{aligned}$ | $\begin{aligned} & 207, \\ & 211 \end{aligned}$ | $\begin{gathered} 207, \\ 211 \end{gathered}$ | $\begin{aligned} & 209, \\ & 211 \end{aligned}$ | $\begin{aligned} & 203, \\ & 209 \end{aligned}$ | $\begin{aligned} & 207, \\ & 211 \end{aligned}$ |  | $\begin{aligned} & 205, \\ & 209 \end{aligned}$ | $\begin{gathered} 201, \\ 207 \end{gathered}$ | $\begin{aligned} & 205, \\ & 207 \end{aligned}$ | $\begin{gathered} 201, \\ 205 \end{gathered}$ | $\begin{aligned} & 203, \\ & 211 \end{aligned}$ | $\begin{aligned} & 203, \\ & 209 \end{aligned}$ | $\begin{aligned} & \hline 201, \\ & 213 \end{aligned}$ | $\begin{aligned} & 203, \\ & 213 \end{aligned}$ | $\begin{aligned} & 203, \\ & 213 \end{aligned}$ | $\begin{aligned} & 203, \\ & 213 \end{aligned}$ |
| D4S406 | $\begin{gathered} 117 . \\ 06 \end{gathered}$ | $\begin{aligned} & 234, \\ & 246 \end{aligned}$ | $\begin{aligned} & 248, \\ & 250 \end{aligned}$ | $\begin{aligned} & 234, \\ & 248 \end{aligned}$ | $\begin{aligned} & 234, \\ & 248 \end{aligned}$ | $\begin{aligned} & 234, \\ & 250 \end{aligned}$ | $\begin{aligned} & 246, \\ & 250 \end{aligned}$ | $\begin{aligned} & 234, \\ & 248 \end{aligned}$ | $\begin{aligned} & 234, \\ & 248 \end{aligned}$ | $\begin{aligned} & 250, \\ & 254 \end{aligned}$ | $\begin{aligned} & \hline 234, \\ & 252 \end{aligned}$ | $\begin{aligned} & 234, \\ & 250 \end{aligned}$ | $\begin{aligned} & 250, \\ & 252 \end{aligned}$ | $\begin{aligned} & 234, \\ & 246 \end{aligned}$ | $\begin{aligned} & 246, \\ & 254 \end{aligned}$ | $\begin{aligned} & 248, \\ & 250 \end{aligned}$ | $\begin{aligned} & 246, \\ & 248 \end{aligned}$ | $\begin{aligned} & 246 \\ & 248 \end{aligned}$ | $\begin{aligned} & 246, \\ & 248 \end{aligned}$ |
| D4S402 | $\begin{gathered} 124 . \\ 45 \end{gathered}$ | $\begin{gathered} 289, \\ 309 \end{gathered}$ | $\begin{aligned} & 287, \\ & 319 \end{aligned}$ | $\begin{aligned} & 309 \\ & 319 \end{aligned}$ | $\begin{gathered} 309, \\ 319 \end{gathered}$ |  | $\begin{gathered} 289, \\ 319 \end{gathered}$ | $\begin{aligned} & 309 \\ & 319 \end{aligned}$ | $\begin{gathered} 309, \\ 319 \end{gathered}$ | $\begin{gathered} 289, \\ 309 \end{gathered}$ | $\begin{aligned} & 287, \\ & 315 \end{aligned}$ |  | $\begin{gathered} 289 \\ 315 \end{gathered}$ | $\begin{aligned} & 289, \\ & 309 \end{aligned}$ | $\begin{aligned} & 289, \\ & 319 \end{aligned}$ | $\begin{gathered} 297 \\ 315 \end{gathered}$ | $\begin{aligned} & 289 \\ & 315 \end{aligned}$ | $\begin{aligned} & 289 \\ & 315 \end{aligned}$ | $\begin{gathered} 289 \\ 315 \end{gathered}$ |


| D4S1575 | $\begin{gathered} 132 . \\ 05 \end{gathered}$ | $\begin{aligned} & \hline 221, \\ & 223 \end{aligned}$ | $\begin{aligned} & 223, \\ & 225 \end{aligned}$ | $\begin{aligned} & 223, \\ & 225 \end{aligned}$ | $\begin{aligned} & 221, \\ & 223 \end{aligned}$ | $\begin{aligned} & \hline 223, \\ & 223 \end{aligned}$ | $\begin{array}{\|l\|} \hline 221, \\ 225 \end{array}$ |  |  |  | $\begin{aligned} & 219, \\ & 221 \end{aligned}$ | $\begin{aligned} & 221, \\ & 221 \end{aligned}$ | $\begin{aligned} & 221, \\ & 223 \end{aligned}$ | $\begin{aligned} & 221, \\ & 221 \end{aligned}$ | $\begin{aligned} & 219, \\ & 221 \end{aligned}$ | $\begin{aligned} & 221, \\ & 221 \end{aligned}$ | $\begin{aligned} & 221, \\ & 221 \end{aligned}$ | $\begin{aligned} & \hline 219, \\ & 221 \end{aligned}$ | $\begin{aligned} & 219, \\ & 221 \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D4S424 | $\begin{gathered} 144 . \\ 56 \end{gathered}$ | $\begin{aligned} & 188, \\ & 190 \end{aligned}$ | $\begin{aligned} & \hline 180, \\ & 190 \end{aligned}$ | $\begin{aligned} & \hline 188, \\ & 190 \end{aligned}$ | $\begin{aligned} & 188, \\ & 190 \end{aligned}$ | $\begin{aligned} & \hline 180, \\ & 188 \end{aligned}$ | $\begin{aligned} & 180, \\ & 190 \end{aligned}$ | $\begin{aligned} & \hline 190, \\ & 190 \end{aligned}$ | $\begin{aligned} & 190, \\ & 190 \end{aligned}$ | $\begin{aligned} & \hline 188, \\ & 190 \end{aligned}$ | $\begin{aligned} & \hline 182, \\ & 190 \end{aligned}$ | $\begin{aligned} & 190, \\ & 190 \end{aligned}$ | $\begin{aligned} & \hline 190, \\ & 190 \end{aligned}$ | $\begin{aligned} & \hline 199, \\ & 190 \end{aligned}$ | $\begin{aligned} & \hline 199, \\ & 190 \end{aligned}$ | $\begin{aligned} & 190, \\ & 190 \end{aligned}$ | $\begin{aligned} & 188, \\ & 190 \end{aligned}$ | $\begin{aligned} & \hline 190, \\ & 190 \end{aligned}$ | $\begin{aligned} & \hline 190, \\ & 190 \end{aligned}$ |
| D4S1629 | $\begin{gathered} 157 . \\ 99 \end{gathered}$ | $\begin{aligned} & 141, \\ & 149 \end{aligned}$ | $\begin{aligned} & \hline 145, \\ & 157 \end{aligned}$ | $\begin{aligned} & 141, \\ & 145 \end{aligned}$ | $\begin{aligned} & \hline 145, \\ & 149 \end{aligned}$ | $\begin{aligned} & 141, \\ & 147 \end{aligned}$ | $\begin{aligned} & \hline 149, \\ & 157 \end{aligned}$ |  |  | $\begin{aligned} & \hline 141, \\ & 149 \end{aligned}$ | $\begin{gathered} 145, \\ 153 \end{gathered}$ | $\begin{aligned} & 145, \\ & 149 \end{aligned}$ | $\begin{aligned} & \hline 141, \\ & 153 \end{aligned}$ | $\begin{aligned} & 145, \\ & 153 \end{aligned}$ | $\begin{aligned} & \hline 141, \\ & 149 \end{aligned}$ | $\begin{aligned} & \hline 141, \\ & 153 \end{aligned}$ | $\begin{aligned} & \hline 141, \\ & 153 \end{aligned}$ |  |  |
| D4S413 | $\begin{gathered} 157 . \\ 99 \end{gathered}$ | $\begin{aligned} & 128, \\ & 166 \end{aligned}$ | $\begin{aligned} & \hline 130, \\ & 140 \end{aligned}$ | $\begin{aligned} & \hline 140, \\ & 166 \end{aligned}$ | $\begin{gathered} 128, \\ 140 \end{gathered}$ | $\begin{aligned} & \hline 130, \\ & 166 \end{aligned}$ | $\begin{aligned} & 128, \\ & 130 \end{aligned}$ | $\begin{aligned} & 128, \\ & 140 \end{aligned}$ | $\begin{aligned} & 128, \\ & 140 \end{aligned}$ | $\begin{gathered} 128, \\ 166 \end{gathered}$ |  |  |  | $\begin{aligned} & 128, \\ & 166 \end{aligned}$ | $\begin{aligned} & \hline 128, \\ & 166 \end{aligned}$ | $\begin{gathered} \hline 134, \\ 140 \end{gathered}$ | $\begin{aligned} & \hline 134, \\ & 166 \end{aligned}$ | $\begin{aligned} & 128, \\ & 140 \end{aligned}$ | $\begin{aligned} & 128, \\ & 134 \end{aligned}$ |
| D4S1539 | $\begin{gathered} 176 . \\ 19 \end{gathered}$ | $\begin{aligned} & 225, \\ & 227 \\ & \hline \end{aligned}$ | $\begin{array}{\|l\|} \hline 223, \\ 225 \\ \hline \end{array}$ | $\begin{aligned} & \hline 223, \\ & 225 \\ & \hline \end{aligned}$ | $\begin{aligned} & 223, \\ & 227 \\ & \hline \end{aligned}$ | $\begin{aligned} & 225, \\ & 225 \\ & \hline \end{aligned}$ | $\begin{array}{\|l} \hline 225, \\ 225 \\ \hline \end{array}$ | $\begin{aligned} & 223, \\ & 227 \\ & \hline \end{aligned}$ | $\begin{aligned} & 223, \\ & 227 \\ & \hline \end{aligned}$ | $\begin{aligned} & 225, \\ & 227 \\ & \hline \end{aligned}$ | $\begin{aligned} & 225, \\ & 227 \\ & \hline \end{aligned}$ | $\begin{array}{\|l\|} \hline 225, \\ 227 \\ \hline \end{array}$ | $\begin{aligned} & \hline 225, \\ & 225 \\ & \hline \end{aligned}$ | $\begin{aligned} & 223, \\ & 227 \\ & \hline \end{aligned}$ | $\begin{aligned} & \hline 225, \\ & 227 \\ & \hline \end{aligned}$ | $\begin{aligned} & 225, \\ & 225 \\ & \hline \end{aligned}$ | $\begin{aligned} & 225, \\ & 225 \\ & \hline \end{aligned}$ | $\begin{aligned} & 225, \\ & 227 \\ & \hline \end{aligned}$ | $\begin{aligned} & 225, \\ & 225 \\ & \hline \end{aligned}$ |
| D4S415 | $\begin{gathered} 181 . \\ 36 \end{gathered}$ | $\begin{aligned} & \hline 174, \\ & 196 \end{aligned}$ | $\begin{aligned} & \hline 174, \\ & 194 \end{aligned}$ | $\begin{aligned} & \hline 194, \\ & 196 \\ & \hline \end{aligned}$ | $\begin{aligned} & \hline 194, \\ & 196 \end{aligned}$ | $\begin{aligned} & \hline 174, \\ & 174 \end{aligned}$ | $\begin{array}{\|l\|} \hline 174, \\ 174 \\ \hline \end{array}$ | $\begin{aligned} & \hline 194, \\ & 196 \end{aligned}$ | $\begin{aligned} & \hline 194, \\ & 196 \end{aligned}$ | $\begin{aligned} & \hline 174, \\ & 196 \end{aligned}$ | $\begin{aligned} & \hline 174, \\ & 198 \end{aligned}$ | $\begin{array}{\|l\|} \hline 196, \\ 198 \\ \hline \end{array}$ | $\begin{aligned} & \hline 174, \\ & 198 \end{aligned}$ | $\begin{aligned} & \hline 196, \\ & 196 \end{aligned}$ | $\begin{aligned} & \hline 174, \\ & 196 \\ & \hline \end{aligned}$ | $\begin{aligned} & 194, \\ & 194 \end{aligned}$ | $\begin{aligned} & \hline 174, \\ & 194 \end{aligned}$ | $\begin{aligned} & \hline 194, \\ & 196 \end{aligned}$ | $\begin{aligned} & \hline 194, \\ & 196 \end{aligned}$ |
| D4S2417 | $\begin{gathered} 181 . \\ 93 \end{gathered}$ | $\begin{aligned} & 255, \\ & 255 \end{aligned}$ | $\begin{aligned} & 263, \\ & 263 \end{aligned}$ | $\begin{aligned} & 255, \\ & 263 \end{aligned}$ | $\begin{aligned} & 255, \\ & 263 \end{aligned}$ | $\begin{aligned} & 255, \\ & 263 \end{aligned}$ | $\begin{array}{\|l\|} \hline 255, \\ 263 \\ \hline \end{array}$ | $\begin{aligned} & 255, \\ & 263 \end{aligned}$ | $\begin{aligned} & 255, \\ & 263 \end{aligned}$ | $\begin{aligned} & 255, \\ & 255 \end{aligned}$ | $\begin{aligned} & 263, \\ & 263 \end{aligned}$ | $\begin{aligned} & 255, \\ & 263 \end{aligned}$ | $\begin{aligned} & \hline 255, \\ & 263 \end{aligned}$ | $\begin{aligned} & 259, \\ & 263 \end{aligned}$ | $\begin{aligned} & 255, \\ & 255 \end{aligned}$ | $\begin{aligned} & 255, \\ & 259 \end{aligned}$ | $\begin{aligned} & 255, \\ & 259 \end{aligned}$ |  |  |
| D4S2417 | $\begin{gathered} 181 . \\ 92 \end{gathered}$ | $\begin{aligned} & 255, \\ & 255 \end{aligned}$ | $\begin{array}{\|l\|} \hline 263, \\ 263 \\ \hline \end{array}$ | $\begin{aligned} & \hline 255, \\ & 263 \end{aligned}$ | $\begin{aligned} & 255, \\ & 263 \end{aligned}$ | $\begin{aligned} & 255, \\ & 263 \end{aligned}$ | $\begin{aligned} & \hline 255, \\ & 263 \end{aligned}$ |  |  | $\begin{aligned} & 255, \\ & 255 \end{aligned}$ | $\begin{aligned} & 263, \\ & 263 \end{aligned}$ | $\begin{aligned} & 255, \\ & 263 \end{aligned}$ | $\begin{aligned} & \hline 255, \\ & 263 \end{aligned}$ | $\begin{aligned} & 259, \\ & 263 \end{aligned}$ | $\begin{aligned} & 255, \\ & 255 \end{aligned}$ | $\begin{aligned} & 255, \\ & 259 \end{aligned}$ | $\begin{aligned} & 255, \\ & 259 \end{aligned}$ |  |  |
| D4S1535 | $\begin{gathered} 195 . \\ 06 \end{gathered}$ | $\begin{aligned} & 185, \\ & 185 \end{aligned}$ | $\begin{aligned} & 183, \\ & 185 \end{aligned}$ | $\begin{aligned} & \hline 183, \\ & 185 \end{aligned}$ | $\begin{aligned} & 183, \\ & 185 \end{aligned}$ | $\begin{gathered} 185 \\ 185 \end{gathered}$ | $\begin{aligned} & \hline 185, \\ & 185 \end{aligned}$ | $\begin{aligned} & \hline 183, \\ & 185 \end{aligned}$ | $\begin{aligned} & 183, \\ & 185 \end{aligned}$ | $\begin{aligned} & \hline 185, \\ & 185 \end{aligned}$ | $\begin{aligned} & 185, \\ & 185 \end{aligned}$ | $\begin{aligned} & \hline 185, \\ & 187 \end{aligned}$ | $\begin{aligned} & 185, \\ & 187 \end{aligned}$ |  |  |  | $\begin{aligned} & 187, \\ & 191 \end{aligned}$ |  | $\begin{gathered} 187, \\ 191 \end{gathered}$ |
| D4S408 | $\begin{gathered} 195 . \\ 06 \end{gathered}$ | $\begin{aligned} & 229, \\ & 229 \end{aligned}$ | $\begin{array}{\|l\|} \hline 233, \\ 242 \\ \hline \end{array}$ | $\begin{aligned} & 22, \\ & 243 \end{aligned}$ | $\begin{aligned} & 229, \\ & 243 \end{aligned}$ | $\begin{aligned} & 229, \\ & 233 \end{aligned}$ | $\begin{aligned} & 229, \\ & 233 \end{aligned}$ |  |  | $\begin{aligned} & 229, \\ & 229 \end{aligned}$ | $\begin{aligned} & 229, \\ & 233 \end{aligned}$ | $\begin{aligned} & 229, \\ & 229 \end{aligned}$ | $\begin{aligned} & 229, \\ & 229 \end{aligned}$ | $\begin{aligned} & 233, \\ & 233 \end{aligned}$ | $\begin{aligned} & 229, \\ & 233 \end{aligned}$ | $\begin{aligned} & 229, \\ & 239 \end{aligned}$ | $\begin{aligned} & 229, \\ & 233 \end{aligned}$ |  |  |
| D4S426 | $\begin{gathered} 206 . \\ 98 \end{gathered}$ | $\begin{aligned} & 179, \\ & 181 \end{aligned}$ | $\begin{aligned} & 177, \\ & 191 \end{aligned}$ | $\begin{aligned} & 177, \\ & 181 \end{aligned}$ | $\begin{gathered} 181, \\ 191 \end{gathered}$ | $\begin{aligned} & 181, \\ & 191 \end{aligned}$ | $\begin{array}{\|l\|} \hline 179, \\ 191 \end{array}$ | $\begin{aligned} & 177, \\ & 179 \end{aligned}$ | $\begin{aligned} & 177, \\ & 181 \end{aligned}$ | $\begin{aligned} & \text { 181, } \\ & 191 \end{aligned}$ | $\begin{aligned} & 179, \\ & 191 \end{aligned}$ |  | $\begin{aligned} & \hline 191, \\ & 191 \end{aligned}$ | $\begin{aligned} & 189, \\ & 191 \end{aligned}$ |  | $\begin{aligned} & 181, \\ & 187 \end{aligned}$ | $\begin{aligned} & 187, \\ & 189 \end{aligned}$ | $\begin{aligned} & 187, \\ & 189 \end{aligned}$ | $\begin{aligned} & 187, \\ & 189 \end{aligned}$ |
| D5S1981 | 1.72 | $\begin{aligned} & 262, \\ & 266 \end{aligned}$ | $\begin{aligned} & 262, \\ & 268 \end{aligned}$ |  | $\begin{aligned} & 262, \\ & 266 \end{aligned}$ | $\begin{aligned} & 262, \\ & 268 \end{aligned}$ | $\begin{array}{\|l\|} \hline 266, \\ 268 \\ \hline \end{array}$ | $\begin{aligned} & 262, \\ & 268 \end{aligned}$ | $\begin{aligned} & 262, \\ & 266 \end{aligned}$ | $\begin{aligned} & 268, \\ & 272 \end{aligned}$ | $\begin{gathered} 262, \\ 266 \end{gathered}$ | $\begin{aligned} & 262, \\ & 268 \end{aligned}$ | $\begin{aligned} & 262, \\ & 268 \end{aligned}$ | $\begin{aligned} & 262, \\ & 272 \end{aligned}$ | $\begin{aligned} & 262, \\ & 266 \end{aligned}$ | $\begin{aligned} & 268, \\ & 278 \end{aligned}$ | $\begin{aligned} & 266, \\ & 268 \end{aligned}$ | $\begin{aligned} & 266, \\ & 278 \end{aligned}$ | $\begin{aligned} & 266, \\ & 278 \end{aligned}$ |
| D5S406 | $\begin{gathered} 11.8 \\ 5 \end{gathered}$ | $\begin{aligned} & 178, \\ & 1 \end{aligned}$ | $\begin{array}{\|l\|} \hline 168, \\ 178 \\ \hline \end{array}$ |  | $\begin{aligned} & 168, \\ & 178 \end{aligned}$ | $\begin{aligned} & \hline 178, \\ & 180 \end{aligned}$ | $\begin{array}{\|l\|} \hline 178, \\ 178 \\ \hline \end{array}$ | $\begin{aligned} & 178, \end{aligned}$ | $\begin{aligned} & 168, \\ & 178 \end{aligned}$ | $\begin{aligned} & \hline 178, \\ & 180 \end{aligned}$ | $\begin{aligned} & \hline 168, \\ & 180 \end{aligned}$ | $\begin{array}{\|l\|} \hline 168, \\ 178 \\ \hline \end{array}$ | $\begin{aligned} & \hline 178, \\ & 180 \end{aligned}$ | $\begin{aligned} & 178, \\ & 180 \end{aligned}$ | $\begin{aligned} & 178, \\ & 180 \end{aligned}$ | $\begin{aligned} & \hline 168, \\ & 180 \end{aligned}$ | $\begin{aligned} & 178, \\ & 180 \end{aligned}$ | $\begin{aligned} & 178, \\ & 180 \end{aligned}$ | $\begin{aligned} & 168, \\ & 178 \end{aligned}$ |
| D5S630 | $\begin{gathered} 19.6 \\ 7 \end{gathered}$ | $\begin{aligned} & 231, \\ & 248 \end{aligned}$ | $\begin{aligned} & 254, \\ & 258 \end{aligned}$ | $\begin{aligned} & 248, \\ & 254 \end{aligned}$ | $\begin{aligned} & 248, \\ & 254 \end{aligned}$ | $\begin{aligned} & 231, \\ & 258 \end{aligned}$ | $\begin{array}{\|l\|} \hline 248, \\ 254 \\ \hline \end{array}$ | $\begin{aligned} & 231, \\ & 258 \end{aligned}$ | $\begin{aligned} & 248, \\ & 254 \end{aligned}$ | $\begin{aligned} & 248, \\ & 258 \end{aligned}$ | $\begin{aligned} & 248, \\ & 254 \end{aligned}$ | $\begin{array}{r} 248, \\ 254 \end{array}$ | $\begin{aligned} & 248, \\ & 248 \end{aligned}$ | $\begin{aligned} & 231, \\ & 248 \end{aligned}$ | $\begin{aligned} & 231, \\ & 248 \end{aligned}$ | $\begin{gathered} 248, \\ 262 \end{gathered}$ | $\begin{aligned} & 248, \\ & 248 \end{aligned}$ | $\begin{aligned} & 231, \\ & 248 \end{aligned}$ | $\begin{aligned} & 248, \\ & 248 \end{aligned}$ |
| D5S817 | $\begin{gathered} 22.8 \\ 8 \end{gathered}$ | $\begin{aligned} & 264, \\ & 268 \end{aligned}$ | $\begin{aligned} & 264, \\ & 268 \end{aligned}$ | $\begin{aligned} & 268, \\ & 268 \end{aligned}$ | $\begin{aligned} & 268 \\ & 268 \end{aligned}$ | $\begin{aligned} & 264 \\ & 264 \end{aligned}$ | $\begin{aligned} & 268, \\ & 268 \end{aligned}$ |  |  | $\begin{aligned} & 268, \\ & 272 \end{aligned}$ | $\begin{aligned} & 260, \\ & 268 \end{aligned}$ | $\begin{aligned} & 260, \\ & 268 \end{aligned}$ | $\begin{aligned} & 268, \\ & 268 \end{aligned}$ | $\begin{aligned} & 264, \\ & 268 \end{aligned}$ | $\begin{aligned} & 264, \\ & 268 \end{aligned}$ | $\begin{aligned} & 260, \\ & 272 \end{aligned}$ | $\begin{aligned} & 268, \\ & 272 \end{aligned}$ |  |  |


| D5S416 | $\begin{gathered} 28.7 \\ 6 \end{gathered}$ | $\begin{aligned} & 284, \\ & 286 \end{aligned}$ | $\begin{aligned} & 292, \\ & 292 \end{aligned}$ |  | $\begin{aligned} & 284, \\ & 292 \end{aligned}$ | $\begin{aligned} & 286, \\ & 292 \end{aligned}$ | $\begin{aligned} & 284, \\ & 292 \end{aligned}$ | $\begin{aligned} & 286, \\ & 292 \end{aligned}$ | $\begin{aligned} & 284, \\ & 292 \end{aligned}$ | $\begin{aligned} & 282, \\ & 284 \end{aligned}$ | $\begin{aligned} & 284, \\ & 288 \end{aligned}$ | $\begin{aligned} & 284, \\ & 284 \end{aligned}$ | $\begin{aligned} & 284, \\ & 286 \end{aligned}$ | $\begin{aligned} & 284, \\ & 286 \end{aligned}$ | $\begin{aligned} & 284, \\ & 286 \end{aligned}$ | $\begin{aligned} & 284, \\ & 290 \end{aligned}$ | $\begin{aligned} & 284, \\ & 284 \end{aligned}$ |  | $\begin{aligned} & 284, \\ & 284 \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D5S419 | $\begin{gathered} 39.9 \\ 9 \end{gathered}$ | $\begin{aligned} & 212, \\ & 222 \end{aligned}$ | $\begin{aligned} & 218, \\ & 222 \end{aligned}$ | $\begin{aligned} & 212, \\ & 218 \end{aligned}$ | $\begin{aligned} & 212, \\ & 218 \end{aligned}$ |  | $\begin{aligned} & 222, \\ & 222 \end{aligned}$ | $\begin{aligned} & 218, \\ & 222 \end{aligned}$ | $\begin{aligned} & 212, \\ & 222 \end{aligned}$ | $\begin{aligned} & 206, \\ & 212 \end{aligned}$ | $\begin{aligned} & 222, \\ & 224 \end{aligned}$ | $\begin{aligned} & 212, \\ & 222 \end{aligned}$ | $\begin{aligned} & 212, \\ & 224 \end{aligned}$ |  | $\begin{aligned} & 206, \\ & 216 \end{aligned}$ |  |  | $\begin{aligned} & 216, \\ & 216 \end{aligned}$ | $\begin{aligned} & 206, \\ & 216 \end{aligned}$ |
| D5S1470 | $\begin{gathered} 45.3 \\ 4 \end{gathered}$ | $\begin{aligned} & 173, \\ & 197 \end{aligned}$ | $\begin{aligned} & 177, \\ & 197 \end{aligned}$ | $\begin{gathered} 197, \\ 197 \end{gathered}$ | $\begin{gathered} 197, \\ 197 \end{gathered}$ | $\begin{aligned} & 173 \\ & 197 \end{aligned}$ | $\begin{aligned} & 197, \\ & 177 \end{aligned}$ |  |  | $\begin{gathered} 177, \\ 197 \end{gathered}$ | $\begin{gathered} 181, \\ 197 \end{gathered}$ | $\begin{gathered} 197, \\ 197 \end{gathered}$ | $\begin{aligned} & 181, \\ & 197 \end{aligned}$ | $\begin{aligned} & 173, \\ & 181 \end{aligned}$ | $\begin{aligned} & 177, \\ & 181 \end{aligned}$ | $\begin{gathered} 189 \\ 193 \end{gathered}$ | $\begin{aligned} & \hline 181, \\ & 189 \end{aligned}$ |  |  |
| D5S426 | $\begin{gathered} 51.9 \\ 9 \end{gathered}$ | $\begin{gathered} 183, \\ 193 \end{gathered}$ | $\begin{aligned} & 183, \\ & 199 \end{aligned}$ | $\begin{aligned} & 193, \\ & 199 \end{aligned}$ | $\begin{gathered} 193, \\ 199 \end{gathered}$ | $\begin{gathered} 183, \\ 199 \end{gathered}$ | $\begin{aligned} & \hline 183, \\ & 193 \end{aligned}$ | $\begin{gathered} 183, \\ 199 \end{gathered}$ | $\begin{aligned} & 183, \\ & 199 \end{aligned}$ | $\begin{aligned} & 193, \\ & 203 \end{aligned}$ | $\begin{aligned} & 201, \\ & 201 \end{aligned}$ | $\begin{aligned} & 193, \\ & 201 \end{aligned}$ | $\begin{aligned} & 193, \\ & 201 \end{aligned}$ | $\begin{aligned} & 183, \\ & 201 \end{aligned}$ | $\begin{aligned} & 201, \\ & 203 \end{aligned}$ | $\begin{gathered} 183, \\ 199 \end{gathered}$ | $\begin{aligned} & 199, \\ & 201 \end{aligned}$ | $\begin{aligned} & 183, \\ & 203 \end{aligned}$ | $\begin{aligned} & 199, \\ & 201 \end{aligned}$ |
| D5S418 | $\begin{gathered} 58.5 \\ 5 \end{gathered}$ | $\begin{aligned} & 215, \\ & 217 \end{aligned}$ | $\begin{aligned} & 213, \\ & 215 \end{aligned}$ | $\begin{aligned} & 213, \\ & 215 \end{aligned}$ | $\begin{aligned} & 213, \\ & 215 \end{aligned}$ | $\begin{aligned} & 213, \\ & 217 \end{aligned}$ | $\begin{aligned} & 215, \\ & 215 \end{aligned}$ | $\begin{aligned} & 213, \\ & 217 \end{aligned}$ | $\begin{aligned} & 215, \\ & 215 \end{aligned}$ | $\begin{aligned} & 215, \\ & 221 \end{aligned}$ | $\begin{aligned} & 215, \\ & 219 \end{aligned}$ | $\begin{aligned} & 215 \\ & 215 \end{aligned}$ | $\begin{aligned} & 215, \\ & 219 \end{aligned}$ | $\begin{aligned} & 213, \\ & 217 \end{aligned}$ | $\begin{aligned} & 213, \\ & 221 \end{aligned}$ | $\begin{aligned} & 215, \\ & 219 \end{aligned}$ | $\begin{aligned} & 213, \\ & 215 \end{aligned}$ | $\begin{aligned} & 219, \\ & 221 \end{aligned}$ | $\begin{aligned} & 213, \\ & 219 \end{aligned}$ |
| D5S2500 | $\begin{gathered} 69.2 \\ 3 \end{gathered}$ | $\begin{aligned} & 157, \\ & 161 \end{aligned}$ | $\begin{aligned} & 153, \\ & 153 \end{aligned}$ | $\begin{aligned} & 153, \\ & 161 \end{aligned}$ | $\begin{aligned} & 153, \\ & 161 \end{aligned}$ | $\begin{aligned} & 153, \\ & 157 \end{aligned}$ | $\begin{aligned} & 153, \\ & 161 \end{aligned}$ | $\begin{aligned} & 153, \\ & 161 \end{aligned}$ | $\begin{aligned} & 153, \\ & 157 \end{aligned}$ | $\begin{gathered} 161 . \\ 173 \end{gathered}$ | $\begin{aligned} & 161, \\ & 161 \end{aligned}$ | $\begin{gathered} 161, \\ 161 \end{gathered}$ | $\begin{aligned} & 161, \\ & 161 \end{aligned}$ | $\begin{aligned} & 149, \\ & 173 \end{aligned}$ | $\begin{aligned} & 149, \\ & 173 \end{aligned}$ | $\begin{aligned} & 153, \\ & 153 \end{aligned}$ | $\begin{aligned} & 153, \\ & 149 \end{aligned}$ |  |  |
| D5S2072 | $\begin{gathered} 73.3 \\ 5 \end{gathered}$ | $\begin{aligned} & 159 \\ & 163 \end{aligned}$ | $\begin{aligned} & 159, \\ & 163 \end{aligned}$ | $\begin{aligned} & 163, \\ & 163 \end{aligned}$ | $\begin{aligned} & 163, \\ & 163 \end{aligned}$ | $\begin{aligned} & 163, \\ & 163 \end{aligned}$ | $\begin{aligned} & 159, \\ & 163 \end{aligned}$ |  |  | $\begin{gathered} 159, \\ 163 \end{gathered}$ | $\begin{gathered} 161, \\ 163 \end{gathered}$ | $\begin{aligned} & 161, \\ & 163 \end{aligned}$ | $\begin{aligned} & 161, \\ & 163 \end{aligned}$ | $\begin{aligned} & 159, \\ & 163 \end{aligned}$ | $\begin{aligned} & 159, \\ & 163 \end{aligned}$ | $\begin{aligned} & 159 \\ & 167 \end{aligned}$ | $\begin{aligned} & 159, \\ & 163 \end{aligned}$ |  |  |
| D5S647 | $\begin{gathered} 74.0 \\ 7 \end{gathered}$ | $\begin{aligned} & 140, \\ & 158 \end{aligned}$ | $\begin{aligned} & 140, \\ & 142 \end{aligned}$ | $\begin{aligned} & 142, \\ & 158 \end{aligned}$ | $\begin{aligned} & 142, \\ & 158 \end{aligned}$ | $\begin{aligned} & 142, \\ & 158 \end{aligned}$ | $\begin{aligned} & \hline 140, \\ & 158 \end{aligned}$ |  |  | $\begin{aligned} & 138 \\ & 158 \end{aligned}$ | $\begin{aligned} & 134, \\ & 136 \end{aligned}$ | $\begin{aligned} & 134, \\ & 158 \end{aligned}$ | $\begin{aligned} & 136, \\ & 158 \end{aligned}$ | $\begin{aligned} & 136, \\ & 158 \end{aligned}$ | $\begin{aligned} & 136, \\ & 156 \end{aligned}$ | $\begin{aligned} & 138, \\ & 142 \end{aligned}$ | $\begin{aligned} & 138, \\ & 156 \end{aligned}$ |  |  |
| D5S2036 | $\begin{gathered} 74.6 \\ 8 \end{gathered}$ | $\begin{aligned} & 113, \\ & 123 \end{aligned}$ | $\begin{aligned} & 121, \\ & 123 \end{aligned}$ | $\begin{aligned} & 113, \\ & 121 \end{aligned}$ | $\begin{aligned} & 113, \\ & 121 \end{aligned}$ | $\begin{aligned} & 113, \\ & 121 \end{aligned}$ | $\begin{aligned} & 113, \\ & 123 \end{aligned}$ | $\begin{aligned} & 113, \\ & 123 \end{aligned}$ | $\begin{aligned} & 113, \\ & 123 \end{aligned}$ | $\begin{gathered} 113, \\ 113 \end{gathered}$ |  | $\begin{aligned} & 113, \\ & 123 \end{aligned}$ |  | $\begin{aligned} & 113, \\ & 121 \end{aligned}$ | $\begin{aligned} & 113, \\ & 121 \end{aligned}$ | $\begin{aligned} & 113, \\ & 123 \end{aligned}$ | $\begin{aligned} & 113, \\ & 121 \end{aligned}$ |  |  |
| D5S2003 | $\begin{gathered} 78.3 \\ 1 \end{gathered}$ | $\begin{aligned} & 185, \\ & 189 \end{aligned}$ | $\begin{aligned} & 187, \\ & 177 \end{aligned}$ |  |  |  |  |  |  |  |  |  |  |  |  | $\begin{aligned} & 175 \\ & 189 \end{aligned}$ |  |  |  |
| D5S424 | $\begin{gathered} 81.9 \\ 5 \end{gathered}$ | $\begin{aligned} & 130, \\ & 130 \end{aligned}$ | $\begin{aligned} & 128, \\ & 130 \end{aligned}$ | $\begin{aligned} & 130, \\ & 130 \end{aligned}$ | $\begin{gathered} 130, \\ 130 \end{gathered}$ |  | $\begin{aligned} & 128, \\ & 130 \end{aligned}$ | $\begin{aligned} & 130, \\ & 130 \end{aligned}$ | $\begin{aligned} & 128, \\ & 130 \end{aligned}$ | $\begin{gathered} 130, \\ 130 \end{gathered}$ | $\begin{gathered} 124, \\ 130 \end{gathered}$ | $\begin{gathered} 130, \\ 130 \end{gathered}$ | $\begin{aligned} & 124, \\ & 130 \end{aligned}$ | $\begin{aligned} & 126, \\ & 130 \end{aligned}$ | $\begin{aligned} & 126, \\ & 130 \end{aligned}$ | $\begin{aligned} & 130, \\ & 132 \end{aligned}$ | $\begin{aligned} & 126, \\ & 132 \end{aligned}$ | $\begin{aligned} & 130, \\ & 130 \end{aligned}$ | $\begin{gathered} 130, \\ 130 \end{gathered}$ |
| D5S641 | $\begin{gathered} 92.3 \\ 8 \end{gathered}$ | $\begin{aligned} & 267, \\ & 269 \end{aligned}$ | $\begin{aligned} & 267, \\ & 269 \end{aligned}$ | $\begin{aligned} & 267, \\ & 269 \end{aligned}$ | $\begin{aligned} & 267, \\ & 269 \end{aligned}$ | $\begin{aligned} & 267, \\ & 269 \end{aligned}$ | $\begin{gathered} 267, \\ 269 \end{gathered}$ | $\begin{aligned} & 267, \\ & 267 \end{aligned}$ | $\begin{aligned} & 269, \\ & 269 \end{aligned}$ | $\begin{aligned} & 267, \\ & 269 \end{aligned}$ | $\begin{aligned} & 265, \\ & 267 \end{aligned}$ | $\begin{aligned} & 265, \\ & 267 \end{aligned}$ | $\begin{aligned} & 267, \\ & 267 \end{aligned}$ | $\begin{aligned} & 267, \\ & 275 \end{aligned}$ | $\begin{aligned} & 265 \\ & 275 \end{aligned}$ | $\begin{aligned} & 267, \\ & 271 \end{aligned}$ | $\begin{aligned} & 265, \\ & 267 \end{aligned}$ | $\begin{aligned} & 271, \\ & 275 \end{aligned}$ | $\begin{aligned} & 271, \\ & 275 \end{aligned}$ |
| D5S428 | $\begin{gathered} 95.4 \\ 0 \end{gathered}$ | $\begin{aligned} & 245, \\ & 247 \end{aligned}$ | $\begin{aligned} & 241, \\ & 247 \end{aligned}$ | $\begin{aligned} & 245, \\ & 241 \end{aligned}$ | $\begin{aligned} & 245, \\ & 247 \end{aligned}$ | $\begin{aligned} & \hline 241, \\ & 245 \end{aligned}$ | $\begin{aligned} & 247, \\ & 247 \end{aligned}$ |  | $\begin{aligned} & 247 \\ & 247 \end{aligned}$ | $\begin{aligned} & 245, \\ & 247 \end{aligned}$ | $\begin{aligned} & 241, \\ & 249 \end{aligned}$ | $\begin{aligned} & 241, \\ & 245 \end{aligned}$ | $\begin{aligned} & 245, \\ & 249 \end{aligned}$ | $\begin{aligned} & 245, \\ & 249 \end{aligned}$ |  | $\begin{aligned} & 241, \\ & 247 \end{aligned}$ | $\begin{aligned} & 241, \\ & 241 \end{aligned}$ | $\begin{aligned} & 247, \\ & 249 \end{aligned}$ | $\begin{aligned} & 247, \\ & 249 \end{aligned}$ |
| D5S1725 | $\begin{gathered} 97.8 \\ 2 \end{gathered}$ | $\begin{aligned} & 188, \\ & 192 \end{aligned}$ | $\begin{aligned} & 188, \\ & 200 \end{aligned}$ | $\begin{aligned} & 188, \\ & 188 \end{aligned}$ | $\begin{aligned} & 192, \\ & 200 \end{aligned}$ | $\begin{aligned} & 188, \\ & 192 \end{aligned}$ | $\begin{aligned} & 188, \\ & 200 \end{aligned}$ |  |  | $\begin{aligned} & 188, \\ & 192 \end{aligned}$ | $\begin{aligned} & 196, \\ & 204 \end{aligned}$ | $\begin{aligned} & 192, \\ & 204 \end{aligned}$ | $\begin{aligned} & 192, \\ & 196 \end{aligned}$ | $\begin{aligned} & 192, \\ & 208 \end{aligned}$ | $\begin{aligned} & 188, \\ & 208 \end{aligned}$ | $\begin{aligned} & 188, \\ & 188 \end{aligned}$ | $\begin{aligned} & 188, \\ & 188 \end{aligned}$ |  |  |
| D5S644 | $\begin{gathered} 104 \\ 76 \end{gathered}$ | $\begin{gathered} 97,9 \\ 9 \end{gathered}$ | $\begin{gathered} 97,1 \\ 07 \end{gathered}$ | $\begin{gathered} 97,9 \\ 9 \end{gathered}$ | $\begin{gathered} 97,1 \\ 07 \end{gathered}$ | $\begin{gathered} 97,9 \\ 7 \end{gathered}$ | $\begin{gathered} 99,1 \\ 07 \end{gathered}$ | $\begin{gathered} 97,9 \\ 7 \end{gathered}$ | $\begin{gathered} 99,1 \\ 07 \end{gathered}$ | $\begin{gathered} 95,9 \\ 7 \end{gathered}$ | $\begin{gathered} 101, \\ 107 \end{gathered}$ | $\begin{gathered} 97,1 \\ 01 \end{gathered}$ | $\begin{gathered} 97,1 \\ 07 \end{gathered}$ | $\begin{gathered} 97,9 \\ 9 \end{gathered}$ |  | $\begin{gathered} 95,9 \\ 9 \end{gathered}$ | $\begin{gathered} 83,9 \\ 9 \end{gathered}$ | $\begin{gathered} 95,9 \\ 9 \end{gathered}$ | $\begin{gathered} 95,9 \\ 9 \end{gathered}$ |


| D5S2027 | $\begin{gathered} 119 . \\ 50 \end{gathered}$ | $\begin{aligned} & 149, \\ & 157 \end{aligned}$ | $\begin{aligned} & 151, \\ & 157 \end{aligned}$ | $\begin{aligned} & 149, \\ & 157 \end{aligned}$ | $\begin{aligned} & 149, \\ & 151 \end{aligned}$ | $\begin{aligned} & 157, \\ & 157 \end{aligned}$ | $\begin{aligned} & 151, \\ & 157 \end{aligned}$ | $\begin{aligned} & 157, \\ & 157 \end{aligned}$ | $\begin{aligned} & 149, \\ & 151 \end{aligned}$ | $\begin{aligned} & 157, \\ & 157 \end{aligned}$ | $\begin{aligned} & 157, \\ & 157 \end{aligned}$ | $\begin{aligned} & 157, \\ & 157 \end{aligned}$ | $\begin{aligned} & 157, \\ & 157 \end{aligned}$ | $\begin{aligned} & 149, \\ & 157 \end{aligned}$ | $\begin{aligned} & 151, \\ & 157 \end{aligned}$ | $\begin{aligned} & 151, \\ & 157 \end{aligned}$ | $\begin{aligned} & 151, \\ & 157 \end{aligned}$ | $\begin{aligned} & 151, \\ & 157 \end{aligned}$ | $\begin{aligned} & 151, \\ & 157 \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D5S1505 | $\begin{gathered} 129 . \\ 83 \end{gathered}$ | $\begin{aligned} & 255, \\ & 259 \end{aligned}$ | $\begin{aligned} & 267, \\ & 259 \end{aligned}$ | $\begin{aligned} & 255, \\ & 267 \end{aligned}$ | $\begin{aligned} & 255, \\ & 259 \end{aligned}$ | $\begin{aligned} & 255, \\ & 267 \end{aligned}$ | $\begin{aligned} & 259, \\ & 259 \end{aligned}$ |  |  | $\begin{aligned} & 251, \\ & 259 \end{aligned}$ | $\begin{aligned} & 267, \\ & 271 \end{aligned}$ | $\begin{aligned} & 251, \\ & 267 \end{aligned}$ | $\begin{aligned} & 259, \\ & 267 \end{aligned}$ | $\begin{aligned} & 255, \\ & 259 \end{aligned}$ | $\begin{aligned} & 251, \\ & 259 \end{aligned}$ | $\begin{aligned} & 259, \\ & 271 \end{aligned}$ | $\begin{aligned} & 259, \\ & 259 \end{aligned}$ |  |  |
| D5S471 | $\begin{gathered} 129 . \\ 83 \end{gathered}$ | $\begin{aligned} & 242, \\ & 248 \end{aligned}$ | $\begin{aligned} & 242, \\ & 246 \end{aligned}$ | $\begin{aligned} & 242, \\ & 246 \end{aligned}$ | $\begin{aligned} & 242, \\ & 246 \end{aligned}$ | $\begin{aligned} & 242, \\ & 246 \end{aligned}$ | $\begin{aligned} & 246, \\ & 248 \end{aligned}$ | $\begin{aligned} & \hline 246, \\ & 248 \end{aligned}$ | $\begin{aligned} & 242, \\ & 246 \end{aligned}$ | $\begin{aligned} & \hline 236, \\ & 248 \end{aligned}$ | $\begin{aligned} & 236, \\ & 248 \end{aligned}$ | $\begin{aligned} & 236, \\ & 248 \end{aligned}$ | $\begin{aligned} & \hline 236, \\ & 248 \end{aligned}$ | $\begin{aligned} & 242, \\ & 248 \end{aligned}$ | $\begin{aligned} & 236, \\ & 246 \end{aligned}$ | $\begin{aligned} & 236, \\ & 246 \end{aligned}$ | $\begin{aligned} & 236, \\ & 246 \end{aligned}$ | $\begin{aligned} & 236, \\ & 246 \end{aligned}$ | $\begin{aligned} & 236, \\ & 246 \end{aligned}$ |
| D5S2115 | $\begin{gathered} 138 . \\ 64 \end{gathered}$ | $\begin{aligned} & 271, \\ & 273 \end{aligned}$ | $\begin{aligned} & \hline 259, \\ & 271 \end{aligned}$ | $\begin{aligned} & 271, \\ & 273 \end{aligned}$ | $\begin{aligned} & 259, \\ & 273 \end{aligned}$ |  | $\begin{aligned} & 271, \\ & 273 \end{aligned}$ | $\begin{aligned} & 271, \\ & 271 \end{aligned}$ | $\begin{aligned} & 259, \\ & 273 \end{aligned}$ | $\begin{aligned} & \hline 271, \\ & 273 \end{aligned}$ | $\begin{aligned} & 271, \\ & 271 \end{aligned}$ | $\begin{aligned} & 271, \\ & 273 \end{aligned}$ | $\begin{aligned} & \hline 271, \\ & 271 \end{aligned}$ | $\begin{aligned} & 273, \\ & 279 \end{aligned}$ | $\begin{aligned} & 275, \\ & 279 \end{aligned}$ | $\begin{aligned} & 271, \\ & 271 \end{aligned}$ | $\begin{aligned} & 271, \\ & 275 \end{aligned}$ | $\begin{aligned} & 271, \\ & 279 \end{aligned}$ | $\begin{aligned} & 271, \\ & 279 \end{aligned}$ |
| D5S436 | $\begin{gathered} 147 . \\ 49 \end{gathered}$ | $\begin{aligned} & 238, \\ & 248 \end{aligned}$ | $\begin{aligned} & 236, \\ & 246 \end{aligned}$ | $\begin{aligned} & 246, \\ & 248 \end{aligned}$ | $\begin{aligned} & 236, \\ & 238 \end{aligned}$ | $\begin{aligned} & 236, \\ & 238 \end{aligned}$ | $\begin{aligned} & 236, \\ & 248 \end{aligned}$ | $\begin{aligned} & 246, \\ & 248 \end{aligned}$ | $\begin{aligned} & 236, \\ & 248 \end{aligned}$ | $\begin{aligned} & \hline 238, \\ & 248 \end{aligned}$ | $\begin{aligned} & 236, \\ & 248 \end{aligned}$ | $\begin{aligned} & \hline 248 \\ & 248 \end{aligned}$ | $\begin{aligned} & 236, \\ & 238 \end{aligned}$ | $\begin{aligned} & 236, \\ & 246 \end{aligned}$ | $\begin{aligned} & 236, \\ & 246 \end{aligned}$ | $\begin{aligned} & 236, \\ & 240 \end{aligned}$ | $\begin{aligned} & 236, \\ & 240 \end{aligned}$ | $\begin{aligned} & 236, \\ & 240 \end{aligned}$ | $\begin{aligned} & 236, \\ & 240 \end{aligned}$ |
| D5S1480 | $\begin{gathered} 147 . \\ 49 \end{gathered}$ | $\begin{aligned} & 218, \\ & 236 \end{aligned}$ | $\begin{aligned} & 239, \\ & 236 \end{aligned}$ | $\begin{aligned} & 236, \\ & 239 \end{aligned}$ | $\begin{aligned} & 236, \\ & 236 \end{aligned}$ | $\begin{aligned} & 218, \\ & 236 \end{aligned}$ | $\begin{aligned} & 236 \\ & 236 \end{aligned}$ |  |  | $\begin{aligned} & 218, \\ & 236 \end{aligned}$ | $\begin{aligned} & \hline 218, \\ & 218 \end{aligned}$ | $\begin{aligned} & 218, \\ & 236 \end{aligned}$ | $\begin{aligned} & 218, \\ & 218 \end{aligned}$ | $\begin{aligned} & 218, \\ & 236 \end{aligned}$ | $\begin{aligned} & 218, \\ & 236 \end{aligned}$ | $\begin{aligned} & 239, \\ & 239 \end{aligned}$ | $\begin{aligned} & 218, \\ & 239 \end{aligned}$ |  |  |
| D5S410 | $\begin{gathered} 156 . \\ 47 \end{gathered}$ | $\begin{aligned} & \hline 161, \\ & 169 \end{aligned}$ |  | $\begin{aligned} & \hline 161, \\ & 163 \end{aligned}$ | $\begin{gathered} \hline 161, \\ 169 \end{gathered}$ | $\begin{gathered} 161, \\ 169 \end{gathered}$ | $\begin{gathered} 161, \\ 163 \end{gathered}$ | $\begin{aligned} & 163, \\ & 169 \end{aligned}$ | $\begin{aligned} & \hline 161, \\ & 163 \end{aligned}$ | $\begin{gathered} 163, \\ 169 \end{gathered}$ | $\begin{gathered} 159, \\ 169 \end{gathered}$ | $\begin{aligned} & 159, \\ & 163 \end{aligned}$ | $\begin{aligned} & \hline 169, \\ & 169 \end{aligned}$ | $\begin{aligned} & 163, \\ & 163 \end{aligned}$ | $\begin{aligned} & 163, \\ & 163 \end{aligned}$ | $\begin{gathered} 159, \\ 163 \end{gathered}$ | $\begin{aligned} & 159, \\ & 163 \end{aligned}$ | $\begin{aligned} & 163, \\ & 163 \end{aligned}$ | $\begin{aligned} & \hline 159, \\ & 163 \end{aligned}$ |
| D5S400 | $\begin{gathered} 174 . \\ 80 \end{gathered}$ | $\begin{aligned} & 228, \\ & 234 \end{aligned}$ | $\begin{aligned} & 228, \\ & 230 \end{aligned}$ | $\begin{aligned} & 228, \\ & 230 \end{aligned}$ | $\begin{aligned} & 228, \\ & 230 \end{aligned}$ | $\begin{aligned} & 228, \\ & 234 \end{aligned}$ |  | $\begin{aligned} & 228, \\ & 228 \end{aligned}$ | $\begin{aligned} & 228, \\ & 230 \end{aligned}$ | $\begin{aligned} & 228, \\ & 228 \end{aligned}$ | $\begin{aligned} & 224, \\ & 230 \end{aligned}$ | $\begin{aligned} & 224, \\ & 228 \end{aligned}$ |  | $\begin{aligned} & 228, \\ & 234 \end{aligned}$ | $\begin{aligned} & 228, \\ & 230 \end{aligned}$ | $\begin{aligned} & 224, \\ & 228 \end{aligned}$ | $\begin{aligned} & 228, \\ & 228 \end{aligned}$ | $\begin{aligned} & 228, \\ & 228 \end{aligned}$ | $\begin{aligned} & 228, \\ & 228 \end{aligned}$ |
| D5S211 | $\begin{gathered} 182 . \\ 89 \end{gathered}$ | $\begin{aligned} & 192, \\ & 200 \end{aligned}$ | $\begin{aligned} & 192, \\ & 192 \end{aligned}$ | $\begin{aligned} & 192, \\ & 192 \end{aligned}$ | $\begin{aligned} & 192, \\ & 200 \end{aligned}$ | $\begin{aligned} & 192, \\ & 192 \end{aligned}$ | $\begin{aligned} & 192, \\ & 200 \end{aligned}$ |  |  | $\begin{aligned} & 192, \\ & 200 \end{aligned}$ | $\begin{aligned} & \hline 200, \\ & 200 \end{aligned}$ | $\begin{aligned} & 200, \\ & 200 \end{aligned}$ | $\begin{gathered} \hline 200, \\ 192 \end{gathered}$ | $\begin{aligned} & 192, \\ & 202 \end{aligned}$ | $\begin{aligned} & 192, \\ & 202 \end{aligned}$ | $\begin{aligned} & 200, \\ & 202 \end{aligned}$ | $\begin{aligned} & 202, \\ & 202 \end{aligned}$ |  |  |
| D5S408 | $\begin{gathered} 195 . \\ 49 \end{gathered}$ | $\begin{aligned} & 257 \\ & 257 \end{aligned}$ | $\begin{aligned} & 257, \\ & 257 \end{aligned}$ |  | $\begin{aligned} & 257, \\ & 257 \end{aligned}$ | $\begin{aligned} & 257, \\ & 257 \end{aligned}$ | $\begin{aligned} & 257, \\ & 257 \end{aligned}$ |  |  | $\begin{aligned} & 257, \\ & 257 \end{aligned}$ | $\begin{aligned} & 255, \\ & 257 \end{aligned}$ | $\begin{aligned} & 255, \\ & 257 \end{aligned}$ | $\begin{aligned} & 255, \\ & 257 \end{aligned}$ | $\begin{aligned} & 255, \\ & 257 \end{aligned}$ | $\begin{aligned} & 255, \\ & 257 \end{aligned}$ | $\begin{aligned} & 257, \\ & 259 \end{aligned}$ | $\begin{aligned} & 255 \\ & 257 \end{aligned}$ | $\begin{aligned} & 255, \\ & 259 \end{aligned}$ | $\begin{aligned} & 257, \\ & 257 \end{aligned}$ |
| D6S1574 | 9.81 | $\begin{aligned} & 166, \\ & 166 \end{aligned}$ | $\begin{aligned} & 174, \\ & 178 \end{aligned}$ | $\begin{gathered} 166, \\ 174 \end{gathered}$ | $\begin{gathered} 166 \\ 178 \end{gathered}$ | $\begin{gathered} 166, \\ 174 \end{gathered}$ | $\begin{gathered} 166 \\ 174 \end{gathered}$ | $\begin{aligned} & 166, \\ & 178 \end{aligned}$ | $\begin{gathered} 166 \\ 178 \end{gathered}$ | $\begin{gathered} 174, \\ 176 \end{gathered}$ | $\begin{gathered} 160, \\ 172 \end{gathered}$ | $\begin{gathered} 160, \\ 174 \end{gathered}$ | $\begin{gathered} 172, \\ 176 \end{gathered}$ | $\begin{aligned} & 166, \\ & 166 \end{aligned}$ | $\begin{aligned} & 166 \\ & 174 \end{aligned}$ | $\begin{gathered} 166, \\ 172 \end{gathered}$ | $\begin{aligned} & 166, \\ & 166 \end{aligned}$ | $\begin{aligned} & 166 \\ & 172 \end{aligned}$ | $\begin{gathered} 166 \\ 166 \end{gathered}$ |
| D6S470 | $\begin{gathered} 18.2 \\ 2 \end{gathered}$ | $\begin{aligned} & 126, \\ & 138 \end{aligned}$ | $\begin{aligned} & 128, \\ & 130 \end{aligned}$ | $\begin{aligned} & 126, \\ & 130 \end{aligned}$ | $\begin{aligned} & 128 \\ & 138 \end{aligned}$ | $\begin{gathered} 126, \\ 130 \end{gathered}$ |  | $\begin{aligned} & 128, \\ & 138 \end{aligned}$ | $\begin{aligned} & 126, \\ & 128 \end{aligned}$ | $\begin{aligned} & 128, \\ & 130 \end{aligned}$ | $\begin{gathered} 126, \\ 128 \end{gathered}$ | $\begin{gathered} 126, \\ 130 \end{gathered}$ | $\begin{gathered} 128, \\ 128 \end{gathered}$ | $\begin{aligned} & 126, \\ & 138 \end{aligned}$ | $\begin{aligned} & 130 \\ & 138 \end{aligned}$ | $\begin{gathered} 128, \\ 130 \end{gathered}$ | $\begin{aligned} & 130, \\ & 138 \end{aligned}$ | $\begin{aligned} & 128 \\ & 138 \end{aligned}$ | $\begin{aligned} & 130 \\ & 138 \end{aligned}$ |
| D6S2434 | $\begin{gathered} 25.0 \\ 8 \end{gathered}$ | $\begin{aligned} & 230, \\ & 230 \end{aligned}$ | $\begin{aligned} & 233, \\ & 227 \end{aligned}$ | $\begin{aligned} & 230, \\ & 233 \end{aligned}$ | $\begin{aligned} & 227, \\ & 230 \end{aligned}$ | $\begin{aligned} & 230, \\ & 233 \end{aligned}$ | $\begin{aligned} & 230, \\ & 233 \end{aligned}$ |  |  | $\begin{aligned} & 227, \\ & 230 \end{aligned}$ | $\begin{aligned} & 224, \\ & 230 \end{aligned}$ | $\begin{aligned} & 230, \\ & 230 \end{aligned}$ | $\begin{aligned} & 224, \\ & 227 \end{aligned}$ | $\begin{aligned} & 230, \\ & 230 \end{aligned}$ | $\begin{aligned} & 230, \\ & 233 \end{aligned}$ | $\begin{aligned} & 227, \\ & 233 \end{aligned}$ | $\begin{aligned} & 233, \\ & 233 \end{aligned}$ |  |  |
| D6S289 | $\begin{gathered} 29.9 \\ 3 \end{gathered}$ | $\begin{aligned} & 219, \\ & 221 \end{aligned}$ | $\begin{aligned} & 221, \\ & 223 \end{aligned}$ | $\begin{aligned} & 219, \\ & 221 \end{aligned}$ | $\begin{aligned} & 221, \\ & 223 \end{aligned}$ | $\begin{aligned} & 219, \\ & 221 \end{aligned}$ | $\begin{aligned} & 221, \\ & 221 \end{aligned}$ | $\begin{aligned} & 221, \\ & 223 \end{aligned}$ | $\begin{aligned} & 219, \\ & 223 \end{aligned}$ | $\begin{aligned} & 217, \\ & 229 \end{aligned}$ | $\begin{aligned} & 217, \\ & 223 \end{aligned}$ | $\begin{aligned} & 217, \\ & 229 \end{aligned}$ |  | $\begin{aligned} & 219, \\ & 221 \end{aligned}$ |  | $\begin{aligned} & 219, \\ & 219 \end{aligned}$ |  | $\begin{aligned} & 219, \\ & 221 \end{aligned}$ | $\begin{aligned} & 221, \\ & 219 \end{aligned}$ |
| D6S422 | $\begin{gathered} 35.6 \\ 6 \end{gathered}$ | $\begin{gathered} 191, \\ 197 \end{gathered}$ | $\begin{aligned} & 191, \\ & 207 \end{aligned}$ | $\begin{aligned} & 191, \\ & 197 \end{aligned}$ | $\begin{aligned} & 197, \\ & 207 \end{aligned}$ | $\begin{gathered} 191, \\ 191 \end{gathered}$ | $\begin{gathered} 191, \\ 197 \end{gathered}$ | $\begin{aligned} & 197, \\ & 207 \end{aligned}$ | $\begin{aligned} & 191, \\ & 207 \end{aligned}$ | $\begin{gathered} 191, \\ 197 \end{gathered}$ | $\begin{aligned} & 197, \\ & 211 \end{aligned}$ | $\begin{aligned} & 191, \\ & 211 \end{aligned}$ | $\begin{gathered} 197, \\ 197 \end{gathered}$ | $\begin{gathered} 191, \\ 197 \end{gathered}$ | $\begin{aligned} & 191, \\ & 195 \end{aligned}$ | $\begin{gathered} 191, \\ 195 \end{gathered}$ | $\begin{gathered} 191, \\ 195 \end{gathered}$ | $\begin{aligned} & 195 \\ & 195 \end{aligned}$ | $\begin{aligned} & 195 \\ & 195 \end{aligned}$ |


| D6S2439 | $\begin{gathered} 42.2 \\ 7 \end{gathered}$ | $\begin{aligned} & 250, \\ & 250 \end{aligned}$ | $\begin{aligned} & 230, \\ & 242 \end{aligned}$ | $\begin{aligned} & 242, \\ & 250 \end{aligned}$ | $\begin{aligned} & 230, \\ & 250 \end{aligned}$ | $\begin{aligned} & 242, \\ & 240 \end{aligned}$ | $\begin{aligned} & 242, \\ & 240 \end{aligned}$ |  |  | $\begin{aligned} & 250, \\ & 250 \end{aligned}$ | $\begin{aligned} & 234, \\ & 242 \end{aligned}$ | $\begin{aligned} & 242, \\ & 250 \end{aligned}$ | $\begin{aligned} & 250, \\ & 234 \end{aligned}$ | $\begin{aligned} & 242, \\ & 242 \end{aligned}$ | $\begin{aligned} & 242, \\ & 250 \end{aligned}$ | $\begin{aligned} & 238, \\ & 242 \end{aligned}$ | $\begin{aligned} & 238, \\ & 242 \end{aligned}$ |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D6S1051 | $\begin{gathered} 50.7 \\ 5 \end{gathered}$ | $\begin{aligned} & 231, \\ & 231 \end{aligned}$ | $\begin{aligned} & 231, \\ & 235 \end{aligned}$ | $\begin{aligned} & 231, \\ & 231 \end{aligned}$ | $\begin{aligned} & 231, \\ & 235 \end{aligned}$ | $\begin{aligned} & 231, \\ & 231 \end{aligned}$ | $\begin{gathered} 231, \\ 231 \end{gathered}$ | $\begin{aligned} & 231, \\ & 235 \end{aligned}$ | $\begin{aligned} & 231, \\ & 235 \end{aligned}$ | $\begin{aligned} & 227, \\ & 231 \end{aligned}$ | $\begin{aligned} & 227, \\ & 235 \end{aligned}$ | $\begin{aligned} & 227, \\ & 227 \end{aligned}$ | $\begin{aligned} & 231, \\ & 235 \end{aligned}$ | $\begin{aligned} & 231, \\ & 231 \end{aligned}$ | $\begin{aligned} & \hline 231, \\ & 231 \end{aligned}$ | $\begin{aligned} & 231, \\ & 235 \end{aligned}$ | $\begin{aligned} & 231, \\ & 235 \end{aligned}$ |  |  |
| D6S1610 | $\begin{gathered} 53.8 \\ 1 \end{gathered}$ | $\begin{aligned} & 133, \\ & 139 \end{aligned}$ | $\begin{aligned} & 133, \\ & 139 \end{aligned}$ | $\begin{aligned} & 139, \\ & 139 \end{aligned}$ | $\begin{aligned} & 133, \\ & 139 \end{aligned}$ | $\begin{aligned} & 133, \\ & 139 \end{aligned}$ | $\begin{gathered} 139, \\ 139 \end{gathered}$ | $\begin{aligned} & 133, \\ & 139 \end{aligned}$ |  | $\begin{aligned} & 133, \\ & 139 \end{aligned}$ | $\begin{gathered} 135 \\ 137 \end{gathered}$ | $\begin{aligned} & 133, \\ & 135 \end{aligned}$ | $\begin{aligned} & 137, \\ & 139 \end{aligned}$ | $\begin{aligned} & 133, \\ & 139 \end{aligned}$ | $\begin{aligned} & 133, \\ & 139 \end{aligned}$ | $\begin{aligned} & 133, \\ & 133 \end{aligned}$ | $\begin{aligned} & 133, \\ & 133 \end{aligned}$ | $\begin{aligned} & 133, \\ & 133 \end{aligned}$ | $\begin{aligned} & 133, \\ & 133 \end{aligned}$ |
| D6S1607 | $\begin{gathered} 59.3 \\ 4 \end{gathered}$ | $\begin{aligned} & 285, \\ & 279 \end{aligned}$ | $\begin{aligned} & 277, \\ & 279 \end{aligned}$ | $\begin{aligned} & 279, \\ & 279 \end{aligned}$ | $\begin{aligned} & 279, \\ & 279 \end{aligned}$ | $\begin{aligned} & 279, \\ & 285 \end{aligned}$ | $\begin{aligned} & 279, \\ & 279 \end{aligned}$ |  |  | $\begin{aligned} & 277, \\ & 279 \end{aligned}$ | $\begin{aligned} & 283, \\ & 283 \end{aligned}$ | $\begin{aligned} & 277, \\ & 283 \end{aligned}$ | $\begin{aligned} & 279, \\ & 283 \end{aligned}$ | $\begin{aligned} & 279, \\ & 285 \end{aligned}$ | $\begin{aligned} & 281, \\ & 285 \end{aligned}$ | $\begin{aligned} & 275, \\ & 287 \end{aligned}$ | $\begin{aligned} & 281, \\ & 287 \end{aligned}$ |  |  |
| D6S2410 | $\begin{gathered} 73.1 \\ 3 \end{gathered}$ | $\begin{aligned} & 244, \\ & 248 \end{aligned}$ | $\begin{aligned} & 240, \\ & 252 \end{aligned}$ | $\begin{aligned} & 244, \\ & 252 \end{aligned}$ | $\begin{aligned} & 240, \\ & 248 \end{aligned}$ | $\begin{aligned} & 248, \\ & 252 \end{aligned}$ | $\begin{aligned} & 244, \\ & 252 \end{aligned}$ |  |  | $\begin{aligned} & 244, \\ & 244 \end{aligned}$ | $\begin{aligned} & 240, \\ & 240 \end{aligned}$ | $\begin{aligned} & 240, \\ & 244 \end{aligned}$ | $\begin{aligned} & 240, \\ & 244 \end{aligned}$ | $\begin{aligned} & 244, \\ & 248 \end{aligned}$ | $\begin{aligned} & 244, \\ & 248 \end{aligned}$ | $\begin{aligned} & 236, \\ & 244 \end{aligned}$ | $\begin{aligned} & 240, \\ & 244 \end{aligned}$ |  |  |
| D6S257 | $\begin{gathered} 79.9 \\ 2 \end{gathered}$ | $\begin{aligned} & 174, \\ & 178 \end{aligned}$ | $\begin{gathered} 178, \\ 182 \end{gathered}$ | $\begin{aligned} & 178, \\ & 182 \end{aligned}$ | $\begin{gathered} 174, \\ 178 \end{gathered}$ | $\begin{aligned} & 174, \\ & 182 \end{aligned}$ | $\begin{aligned} & 178, \\ & 182 \end{aligned}$ | $\begin{aligned} & 174, \\ & 178 \end{aligned}$ | $\begin{aligned} & 174, \\ & 178 \end{aligned}$ | $\begin{aligned} & 176 \\ & 176 \end{aligned}$ | $\begin{aligned} & 176, \\ & 178 \end{aligned}$ | $\begin{aligned} & 176 \\ & 178 \end{aligned}$ | $\begin{aligned} & 176, \\ & 178 \end{aligned}$ | $\begin{aligned} & 174, \\ & 176 \end{aligned}$ | $\begin{aligned} & 174, \\ & 176 \end{aligned}$ | $\begin{gathered} 174, \\ 180 \end{gathered}$ | $\begin{aligned} & 174, \\ & 176 \end{aligned}$ | $\begin{aligned} & 174, \\ & 174 \end{aligned}$ | $\begin{aligned} & 174, \\ & 180 \end{aligned}$ |
| D6S460 | $\begin{gathered} 89.9 \\ 3 \end{gathered}$ |  |  | $\begin{gathered} 150, \\ 160 \end{gathered}$ | $\begin{gathered} 144, \\ 158 \end{gathered}$ | $\begin{aligned} & \hline 150, \\ & 160 \end{aligned}$ | $\begin{gathered} 150, \\ 158 \end{gathered}$ | $\begin{aligned} & 144, \\ & 158 \end{aligned}$ | $\begin{aligned} & 144, \\ & 158 \end{aligned}$ | $\begin{aligned} & 144, \\ & 144 \end{aligned}$ | $\begin{gathered} 144, \\ 152 \end{gathered}$ | $\begin{aligned} & 144, \\ & 144 \end{aligned}$ | $\begin{aligned} & 144, \\ & 152 \end{aligned}$ | $\begin{aligned} & 144, \\ & 158 \end{aligned}$ | $\begin{aligned} & 144, \\ & 158 \end{aligned}$ | $\begin{aligned} & 154, \\ & 158 \end{aligned}$ | $\begin{aligned} & 144, \\ & 154 \end{aligned}$ | $\begin{aligned} & 154, \\ & 158 \end{aligned}$ | $\begin{aligned} & 158, \\ & 158 \end{aligned}$ |
| D6S462 | $\begin{gathered} 99.0 \\ 1 \end{gathered}$ | $\begin{aligned} & 285, \\ & 287 \end{aligned}$ | $\begin{aligned} & 285, \\ & 285 \end{aligned}$ | $\begin{aligned} & 285, \\ & 287 \end{aligned}$ | $\begin{aligned} & 285, \\ & 285 \end{aligned}$ | $\begin{aligned} & 285, \\ & 287 \end{aligned}$ | $\begin{aligned} & 285, \\ & 285 \end{aligned}$ | $\begin{aligned} & 285 \\ & 285 \end{aligned}$ | $\begin{aligned} & 285, \\ & 285 \end{aligned}$ | $\begin{aligned} & 285, \\ & 287 \end{aligned}$ | $\begin{aligned} & 285, \\ & 287 \end{aligned}$ | $\begin{aligned} & 825, \\ & 287 \end{aligned}$ | $\begin{aligned} & 285, \\ & 287 \end{aligned}$ | $\begin{aligned} & 285, \\ & 285 \end{aligned}$ | $\begin{aligned} & 285 \\ & 285 \end{aligned}$ | $\begin{aligned} & 285, \\ & 287 \end{aligned}$ | $\begin{aligned} & 285 \\ & 285 \end{aligned}$ | $\begin{aligned} & 285, \\ & 285 \end{aligned}$ | $\begin{aligned} & 285, \\ & 287 \end{aligned}$ |
| D6S287 | $\begin{gathered} 121 . \\ 97 \end{gathered}$ | $\begin{aligned} & 159 \\ & 167 \end{aligned}$ | $\begin{aligned} & 161, \\ & 167 \end{aligned}$ | $\begin{aligned} & 167, \\ & 167 \end{aligned}$ | $\begin{gathered} 161, \\ 167 \end{gathered}$ | $\begin{aligned} & 159, \\ & 167 \end{aligned}$ | $\begin{gathered} 161, \\ 167 \end{gathered}$ | $\begin{aligned} & 167, \\ & 167 \end{aligned}$ | $\begin{aligned} & 161, \\ & 167 \end{aligned}$ | $\begin{aligned} & 161, \\ & 167 \end{aligned}$ | $\begin{aligned} & \hline 161, \\ & 163 \end{aligned}$ | $\begin{aligned} & 163, \\ & 167 \end{aligned}$ | $\begin{aligned} & \hline 161, \\ & 161 \end{aligned}$ | $\begin{aligned} & 159, \\ & 167 \end{aligned}$ | $\begin{aligned} & 159 \\ & 167 \end{aligned}$ | $\begin{gathered} 141, \\ 161 \end{gathered}$ | $\begin{aligned} & 141, \\ & 167 \end{aligned}$ | $\begin{aligned} & 159, \\ & 161 \end{aligned}$ | $\begin{aligned} & 159, \\ & 161 \end{aligned}$ |
| D6S1040 | $\begin{gathered} 128 . \\ 93 \end{gathered}$ | $\begin{aligned} & 273 \\ & 277 \end{aligned}$ | $\begin{aligned} & 269, \\ & 277 \end{aligned}$ | $\begin{aligned} & 269, \\ & 277 \end{aligned}$ | $\begin{aligned} & 273, \\ & 277 \end{aligned}$ | $\begin{aligned} & 269, \\ & 277 \end{aligned}$ | $\begin{aligned} & 273, \\ & 277 \end{aligned}$ |  |  | $\begin{aligned} & 269, \\ & 277 \end{aligned}$ | $\begin{aligned} & 269 \\ & 277 \end{aligned}$ | $\begin{aligned} & 277, \\ & 277 \end{aligned}$ | $\begin{gathered} 269, \\ 269 \end{gathered}$ | $\begin{aligned} & 277, \\ & 277 \end{aligned}$ | $\begin{aligned} & 277, \\ & 277 \end{aligned}$ | $\begin{aligned} & 277, \\ & 269 \end{aligned}$ | $\begin{aligned} & 269, \\ & 277 \end{aligned}$ |  |  |
| D6S262 | $\begin{gathered} 130 . \\ 00 \end{gathered}$ | $\begin{aligned} & 177, \\ & 179 \end{aligned}$ | $\begin{aligned} & 171, \\ & 179 \end{aligned}$ | $\begin{aligned} & 171, \\ & 179 \end{aligned}$ | $\begin{gathered} 171, \\ 179 \end{gathered}$ | $\begin{aligned} & 171, \\ & 179 \end{aligned}$ | $\begin{gathered} 177, \\ 179 \end{gathered}$ | $\begin{aligned} & 171, \\ & 177 \end{aligned}$ | $\begin{aligned} & 177, \\ & 179 \end{aligned}$ | $\begin{gathered} 177, \\ 179 \end{gathered}$ |  | $\begin{aligned} & 177 \\ & 183 \end{aligned}$ |  | $\begin{aligned} & 169, \\ & 179 \end{aligned}$ | $\begin{aligned} & 169 \\ & 179 \end{aligned}$ |  |  |  | $\begin{aligned} & 169 \\ & 179 \end{aligned}$ |
| D6S292 | $\begin{gathered} 136 . \\ 97 \end{gathered}$ |  |  | $\begin{aligned} & 149 \\ & 149 \end{aligned}$ | $\begin{aligned} & 151, \\ & 157 \end{aligned}$ |  | $\begin{aligned} & 151, \\ & 157 \end{aligned}$ | $\begin{aligned} & 149 \\ & 157 \end{aligned}$ | $\begin{aligned} & 151, \\ & 157 \end{aligned}$ | $\begin{aligned} & 157, \\ & 157 \end{aligned}$ | $\begin{gathered} 153, \\ 153 \end{gathered}$ | $\begin{aligned} & 153, \\ & 157 \end{aligned}$ | $\begin{aligned} & 153, \\ & 157 \end{aligned}$ | $\begin{aligned} & 149, \\ & 157 \end{aligned}$ | $\begin{aligned} & 149 \\ & 157 \end{aligned}$ |  | $\begin{aligned} & 153, \\ & 157 \end{aligned}$ | $\begin{aligned} & 149, \\ & 153 \end{aligned}$ | $\begin{aligned} & 149, \\ & 153 \end{aligned}$ |
| D6S308 | $\begin{gathered} 144 . \\ 46 \end{gathered}$ | $\begin{aligned} & 199, \\ & 199 \end{aligned}$ | $\begin{aligned} & 199, \\ & 199 \end{aligned}$ | $\begin{aligned} & 199, \\ & 199 \end{aligned}$ | $\begin{gathered} 199, \\ 199 \end{gathered}$ | $\begin{aligned} & 199, \\ & 199 \end{aligned}$ | $\begin{gathered} 199, \\ 199 \end{gathered}$ | $\begin{aligned} & 199, \\ & 199 \end{aligned}$ | $\begin{aligned} & \hline 199, \\ & 199 \\ & \hline \end{aligned}$ | $\begin{gathered} \hline 199, \\ 199 \end{gathered}$ |  | $\begin{gathered} 193, \\ 199 \end{gathered}$ | $\begin{gathered} 193, \\ 199 \end{gathered}$ | $\begin{gathered} 199, \\ 199 \end{gathered}$ | $\begin{aligned} & 199, \\ & 199 \end{aligned}$ | $\begin{aligned} & 197, \\ & 197 \end{aligned}$ | $\begin{aligned} & 197, \\ & 199 \end{aligned}$ | $\begin{aligned} & 197, \\ & 199 \end{aligned}$ | $\begin{gathered} 197, \\ 199 \end{gathered}$ |
| D6S441 | $\begin{gathered} 154 . \\ 10 \end{gathered}$ | $\begin{aligned} & 172, \\ & 180 \end{aligned}$ | $\begin{gathered} 166, \\ 180 \end{gathered}$ | $\begin{aligned} & 166, \\ & 172 \end{aligned}$ | $\begin{gathered} 172, \\ 180 \end{gathered}$ | $\begin{aligned} & 166, \\ & 172 \end{aligned}$ |  | $\begin{aligned} & 166, \\ & 180 \end{aligned}$ | $\begin{aligned} & 172, \\ & 180 \end{aligned}$ | $\begin{aligned} & 168, \\ & 172 \end{aligned}$ | $\begin{aligned} & 172, \\ & 174 \end{aligned}$ | $\begin{aligned} & 172, \\ & 172 \end{aligned}$ | $\begin{aligned} & 168, \\ & 172 \end{aligned}$ | $\begin{aligned} & 172, \\ & 180 \end{aligned}$ |  | $\begin{aligned} & 168, \\ & 172 \end{aligned}$ | $\begin{aligned} & 172, \\ & 180 \end{aligned}$ | $\begin{aligned} & 168, \\ & 172 \end{aligned}$ | $\begin{aligned} & 172, \\ & 172 \end{aligned}$ |
| D6S2436 | $\begin{gathered} 154 . \\ 64 \end{gathered}$ | $\begin{aligned} & 121, \\ & 141 \end{aligned}$ | $\begin{aligned} & 121, \\ & 145 \end{aligned}$ | $\begin{aligned} & 121, \\ & 141 \end{aligned}$ | $\begin{gathered} 141, \\ 145 \end{gathered}$ | $\begin{gathered} 131, \\ 141 \end{gathered}$ | $\begin{aligned} & 121, \\ & 145 \end{aligned}$ |  |  | $\begin{aligned} & 121, \\ & 139 \end{aligned}$ | $\begin{aligned} & 121, \\ & 149 \end{aligned}$ | $\begin{aligned} & 121, \\ & 133 \end{aligned}$ | $\begin{aligned} & 121, \\ & 121 \end{aligned}$ | $\begin{aligned} & 121, \\ & 141 \end{aligned}$ | $\begin{aligned} & 121, \\ & 141 \end{aligned}$ | $\begin{gathered} 121, \\ 149 \end{gathered}$ | $\begin{aligned} & 121, \\ & 149 \end{aligned}$ |  |  |


| D6S1581 | $\begin{gathered} 164 . \\ 78 \end{gathered}$ | $\begin{aligned} & 219, \\ & 219 \end{aligned}$ | $\begin{aligned} & 219, \\ & 221 \end{aligned}$ | $\begin{aligned} & 219, \\ & 219 \end{aligned}$ | $\begin{aligned} & 219, \\ & 221 \end{aligned}$ | $\begin{aligned} & 219, \\ & 219 \end{aligned}$ | $\begin{aligned} & 219, \\ & 221 \end{aligned}$ | $\begin{aligned} & 219, \\ & 219 \end{aligned}$ | $\begin{aligned} & 219, \\ & 221 \end{aligned}$ | $\begin{aligned} & 219, \\ & 227 \end{aligned}$ | $\begin{aligned} & 229, \\ & 229 \end{aligned}$ | $\begin{aligned} & 219, \\ & 229 \end{aligned}$ |  | $\begin{aligned} & 219, \\ & 219 \end{aligned}$ | $\begin{aligned} & 219, \\ & 219 \end{aligned}$ | $\begin{aligned} & 215, \\ & 219 \end{aligned}$ | $\begin{aligned} & 215, \\ & 219 \end{aligned}$ | $\begin{aligned} & 219, \\ & 219 \end{aligned}$ | $\begin{aligned} & 215, \\ & 219 \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D6S264 | $\begin{gathered} 179 . \\ 07 \end{gathered}$ | $\begin{aligned} & 114, \\ & 122 \end{aligned}$ | $\begin{aligned} & 108 \\ & 108 \end{aligned}$ | $\begin{aligned} & 108 \\ & 114 \end{aligned}$ | $\begin{aligned} & 108 \\ & 114 \end{aligned}$ | $\begin{aligned} & 108, \\ & 114 \end{aligned}$ |  | $\begin{aligned} & 108 \\ & 114 \end{aligned}$ | $\begin{aligned} & 108 \\ & 114 \end{aligned}$ | $\begin{aligned} & 114, \\ & 114 \end{aligned}$ | $\begin{aligned} & 114, \\ & 114 \end{aligned}$ | $\begin{aligned} & 114, \\ & 114 \end{aligned}$ | $\begin{aligned} & 114, \\ & 114 \end{aligned}$ | $\begin{aligned} & 114, \\ & 122 \end{aligned}$ | $\begin{aligned} & 114, \\ & 122 \end{aligned}$ | $\begin{aligned} & 108 \\ & 114 \end{aligned}$ | $\begin{aligned} & 108, \\ & 122 \end{aligned}$ | $\begin{aligned} & 114, \\ & 122 \end{aligned}$ | $\begin{aligned} & \hline 114, \\ & 122 \end{aligned}$ |
| D6S446 | $\begin{gathered} 189 . \\ 00 \end{gathered}$ | $\begin{aligned} & 201, \\ & 201 \end{aligned}$ | $\begin{aligned} & 203, \\ & 209 \end{aligned}$ | $\begin{aligned} & 201, \\ & 209 \end{aligned}$ | $\begin{aligned} & \hline 201, \\ & 203 \end{aligned}$ | $\begin{aligned} & \hline 201, \\ & 209 \end{aligned}$ | $\begin{aligned} & 201, \\ & 203 \end{aligned}$ | $\begin{aligned} & 201, \\ & 203 \end{aligned}$ | $\begin{aligned} & 201, \\ & 203 \end{aligned}$ | $\begin{aligned} & 201, \\ & 201 \end{aligned}$ | $\begin{aligned} & 201, \\ & 207 \end{aligned}$ | $\begin{aligned} & 201, \\ & 207 \end{aligned}$ |  |  | $\begin{aligned} & 201, \\ & 201 \end{aligned}$ | $\begin{aligned} & 201, \\ & 205 \end{aligned}$ |  | $\begin{aligned} & 201, \\ & 201 \end{aligned}$ | $\begin{aligned} & 201, \\ & 201 \end{aligned}$ |
| D6S281 | $\begin{gathered} 190 . \\ 14 \end{gathered}$ |  | $\begin{aligned} & 209, \\ & 209 \end{aligned}$ | $\begin{aligned} & 207, \\ & 209 \end{aligned}$ | $\begin{aligned} & 207, \\ & 209 \end{aligned}$ | $\begin{aligned} & 207, \\ & 209 \end{aligned}$ | $\begin{aligned} & 207, \\ & 209 \end{aligned}$ | $\begin{aligned} & 207, \\ & 209 \end{aligned}$ | $\begin{aligned} & 207, \\ & 209 \end{aligned}$ | $\begin{aligned} & 207, \\ & 207 \end{aligned}$ | $\begin{aligned} & 203, \\ & 207 \end{aligned}$ |  | $\begin{aligned} & 203, \\ & 207 \end{aligned}$ | $\begin{aligned} & 203, \\ & 207 \end{aligned}$ | $\begin{aligned} & 203, \\ & 207 \end{aligned}$ | $\begin{aligned} & 203, \\ & 221 \end{aligned}$ | $\begin{aligned} & \hline 203, \\ & 207 \\ & \hline \end{aligned}$ | $\begin{aligned} & \hline 203, \\ & 203 \end{aligned}$ | $\begin{aligned} & 203, \\ & 203 \end{aligned}$ |
| D7S517 | 7.44 | $\begin{aligned} & 247, \\ & 255 \end{aligned}$ | $\begin{aligned} & 245, \\ & 245 \end{aligned}$ | $\begin{aligned} & 245, \\ & 247 \end{aligned}$ | $\begin{aligned} & 245, \\ & 255 \end{aligned}$ | $\begin{aligned} & 245, \\ & 247 \end{aligned}$ | $\begin{aligned} & 245 \\ & 255 \end{aligned}$ | $\begin{aligned} & 245 \\ & 255 \end{aligned}$ | $\begin{aligned} & 245 \\ & 255 \end{aligned}$ | $\begin{gathered} 245, \\ 249 \end{gathered}$ | $\begin{aligned} & 251, \\ & 255 \end{aligned}$ |  | $\begin{aligned} & 245, \\ & 255 \end{aligned}$ | $\begin{aligned} & 245, \\ & 247 \end{aligned}$ | $\begin{aligned} & 247, \\ & 255 \end{aligned}$ | $\begin{aligned} & 251, \\ & 257 \end{aligned}$ | $\begin{aligned} & 247, \\ & 257 \end{aligned}$ | $\begin{aligned} & 247, \\ & 257 \end{aligned}$ | $\begin{aligned} & 255, \\ & 257 \end{aligned}$ |
| D7S513 | $\begin{gathered} 17.7 \\ 4 \end{gathered}$ | $\begin{aligned} & 183, \\ & 195 \end{aligned}$ | $\begin{aligned} & 181, \\ & 195 \end{aligned}$ | $\begin{aligned} & 181, \\ & 183 \end{aligned}$ | $\begin{gathered} 195 \\ 195 \end{gathered}$ | $\begin{gathered} 195, \\ 195 \end{gathered}$ | $\begin{gathered} 181, \\ 195 \end{gathered}$ |  |  | $\begin{gathered} 195, \\ 199 \end{gathered}$ | $\begin{aligned} & 195 \\ & 195 \end{aligned}$ | $\begin{gathered} 195, \\ 199 \end{gathered}$ | $\begin{aligned} & 195 \\ & 195 \end{aligned}$ | $\begin{aligned} & 183, \\ & 195 \end{aligned}$ | $\begin{aligned} & 183, \\ & 191 \end{aligned}$ | $\begin{aligned} & 181, \\ & 195 \end{aligned}$ | $\begin{aligned} & 183, \\ & 195 \end{aligned}$ |  |  |
| D7S3051 | $\begin{gathered} 29.2 \\ 8 \end{gathered}$ | $\begin{gathered} 144, \\ 170 \end{gathered}$ | $\begin{aligned} & 166 \\ & 166 \end{aligned}$ | $\begin{aligned} & 144, \\ & 166 \end{aligned}$ | $\begin{gathered} 166, \\ 170 \end{gathered}$ | $\begin{aligned} & 170, \\ & 166 \end{aligned}$ | $\begin{aligned} & 166, \\ & 170 \end{aligned}$ |  |  | $\begin{gathered} 170, \\ 170 \end{gathered}$ | $\begin{aligned} & \hline 144, \\ & 170 \end{aligned}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ | $\begin{aligned} & 170, \\ & 144 \end{aligned}$ | $\begin{aligned} & 144, \\ & 170 \end{aligned}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ |  |  |
| D7S493 | $\begin{gathered} 34.6 \\ 9 \end{gathered}$ | $\begin{aligned} & 210, \\ & 214 \end{aligned}$ | $\begin{aligned} & 214, \\ & 220 \end{aligned}$ | $\begin{aligned} & 210 \\ & 214 \end{aligned}$ | $\begin{aligned} & 214, \\ & 214 \end{aligned}$ | $\begin{aligned} & 214, \\ & 214 \end{aligned}$ | $\begin{aligned} & 214, \\ & 220 \end{aligned}$ | $\begin{aligned} & 214, \\ & 220 \end{aligned}$ | $\begin{aligned} & 214, \\ & 214 \end{aligned}$ | $\begin{aligned} & 194, \\ & 214 \end{aligned}$ | $\begin{aligned} & 210, \\ & 214 \end{aligned}$ | $\begin{aligned} & 194, \\ & 210 \end{aligned}$ | $\begin{aligned} & 194, \\ & 214 \end{aligned}$ | $\begin{aligned} & 210, \\ & 212 \end{aligned}$ | $\begin{aligned} & 210, \\ & 214 \end{aligned}$ | $\begin{aligned} & 210, \\ & 216 \end{aligned}$ | $\begin{aligned} & 210, \\ & 214 \end{aligned}$ | $\begin{aligned} & 210, \\ & 210 \end{aligned}$ | $\begin{aligned} & 210, \\ & 214 \end{aligned}$ |
| D7S516 | $\begin{gathered} 41.6 \\ 9 \end{gathered}$ | $\begin{aligned} & 266, \\ & 266 \end{aligned}$ | $\begin{aligned} & 256, \\ & 260 \end{aligned}$ | $\begin{aligned} & 260, \\ & 266 \end{aligned}$ | $\begin{aligned} & 256 \\ & 266 \end{aligned}$ | $\begin{aligned} & 256 \\ & 266 \end{aligned}$ | $\begin{aligned} & 260, \\ & 266 \end{aligned}$ | $\begin{aligned} & 260, \\ & 266 \end{aligned}$ | $\begin{aligned} & 256, \\ & 266 \end{aligned}$ | $\begin{aligned} & 254, \\ & 260 \end{aligned}$ | $\begin{aligned} & 254, \\ & 262 \end{aligned}$ | $\begin{aligned} & 254, \\ & 262 \end{aligned}$ | $\begin{aligned} & 254, \\ & 254 \end{aligned}$ | $\begin{aligned} & 260, \\ & 266 \end{aligned}$ | $\begin{aligned} & 266, \\ & 266 \end{aligned}$ | $\begin{aligned} & 260, \\ & 266 \end{aligned}$ | $\begin{aligned} & 260, \\ & 266 \end{aligned}$ | $\begin{aligned} & 260, \\ & 266 \end{aligned}$ | $\begin{aligned} & 260, \\ & 266 \end{aligned}$ |
| D7S484 | $\begin{gathered} 53.7 \\ 0 \end{gathered}$ | $\begin{gathered} 99,1 \\ 05 \end{gathered}$ | $\begin{aligned} & 105 \\ & 105 \end{aligned}$ |  | $\begin{gathered} 99,1 \\ 05 \end{gathered}$ | $\begin{gathered} 105 \\ 105 \end{gathered}$ | $\begin{gathered} 105 \\ 105 \end{gathered}$ | $\begin{gathered} 105 \\ 105 \end{gathered}$ | $\begin{gathered} 105 \\ 105 \end{gathered}$ | $\begin{gathered} 105 \\ 105 \end{gathered}$ | $\begin{gathered} 103, \\ 105 \end{gathered}$ |  | $\begin{gathered} 105 \\ 105 \end{gathered}$ | $\begin{gathered} 99,1 \\ 05 \end{gathered}$ | $\begin{gathered} 99,1 \\ 05 \end{gathered}$ | $\begin{gathered} 105 \\ 111 \end{gathered}$ | $\begin{aligned} & 105 \\ & 105 \end{aligned}$ |  | $\begin{gathered} 105 \\ 111 \end{gathered}$ |
| D7S2846 | $\begin{gathered} 57.7 \\ 9 \end{gathered}$ | $\begin{aligned} & 180, \\ & 184 \end{aligned}$ | $\begin{aligned} & 180, \\ & 184 \end{aligned}$ | $\begin{gathered} 180 \\ 184 \end{gathered}$ | $\begin{gathered} 180, \\ 180 \end{gathered}$ | 180 | $\begin{gathered} 180 \\ 184 \end{gathered}$ |  |  | $\begin{gathered} 180 \\ 184 \end{gathered}$ | $\begin{gathered} 188 \\ 188 \end{gathered}$ | $\begin{gathered} 180, \\ 188 \end{gathered}$ | $\begin{aligned} & 180 \\ & 188 \end{aligned}$ | $\begin{aligned} & 180, \\ & 188 \end{aligned}$ | $\begin{aligned} & 180, \\ & 188 \end{aligned}$ | $\begin{aligned} & 180 \\ & 180 \end{aligned}$ | $\begin{aligned} & 180, \\ & 188 \end{aligned}$ |  |  |
| D7S519 | $\begin{gathered} 69.0 \\ 3 \end{gathered}$ |  | $\begin{aligned} & 256, \\ & 272 \end{aligned}$ | $\begin{aligned} & 266 \\ & 272 \end{aligned}$ | $\begin{aligned} & 266, \\ & 272 \end{aligned}$ | $\begin{aligned} & 262, \\ & 272 \end{aligned}$ | $\begin{aligned} & 256, \\ & 262 \end{aligned}$ | $\begin{aligned} & 256, \\ & 266 \end{aligned}$ | $\begin{aligned} & 262, \\ & 272 \end{aligned}$ | $\begin{gathered} 262, \\ 266 \end{gathered}$ | $\begin{aligned} & 256 \\ & 262 \end{aligned}$ | $\begin{aligned} & 256 \\ & 266 \end{aligned}$ | $\begin{aligned} & 262, \\ & 266 \end{aligned}$ | $\begin{aligned} & 262, \\ & 266 \end{aligned}$ | $\begin{aligned} & 262, \\ & 266 \end{aligned}$ | $\begin{aligned} & 266 \\ & 272 \end{aligned}$ | $\begin{aligned} & 262, \\ & 266 \end{aligned}$ | $\begin{aligned} & 262, \\ & 266 \end{aligned}$ | $\begin{aligned} & 262, \\ & 272 \end{aligned}$ |
| D7S3046 | $\begin{gathered} 78.6 \\ 5 \end{gathered}$ | $\begin{aligned} & 338, \\ & 342 \end{aligned}$ | $\begin{aligned} & 330, \\ & 334 \end{aligned}$ | $\begin{gathered} 334, \\ 338 \end{gathered}$ | $\begin{aligned} & \hline 334, \\ & 338 \end{aligned}$ | $\begin{gathered} 334, \\ 338 \end{gathered}$ | $\begin{aligned} & 339, \\ & 338 \end{aligned}$ |  |  | $\begin{aligned} & 332, \\ & 338 \end{aligned}$ | $\begin{aligned} & 334, \\ & 338 \end{aligned}$ | $\begin{gathered} 334, \\ 338 \end{gathered}$ | $\begin{gathered} 334, \\ 338 \end{gathered}$ | $\begin{aligned} & 322, \\ & 338 \end{aligned}$ | $\begin{aligned} & 322, \\ & 338 \end{aligned}$ | $\begin{aligned} & 322, \\ & 346 \end{aligned}$ | $\begin{aligned} & 322, \\ & 346 \end{aligned}$ |  |  |
| D7S669 | $\begin{gathered} 90.4 \\ 2 \end{gathered}$ | $\begin{aligned} & 123, \\ & 137 \end{aligned}$ | $\begin{aligned} & 131, \\ & 139 \end{aligned}$ | $\begin{aligned} & 131, \\ & 137 \end{aligned}$ | $\begin{aligned} & 131, \\ & 137 \end{aligned}$ | $\begin{aligned} & 131, \\ & 137 \end{aligned}$ | $\begin{gathered} 137, \\ 139 \end{gathered}$ | $\begin{aligned} & 137, \\ & 139 \end{aligned}$ | $\begin{aligned} & 123, \\ & 131 \end{aligned}$ | $\begin{gathered} 123, \\ 137 \end{gathered}$ |  |  | $\begin{aligned} & 123, \\ & 137 \end{aligned}$ | $\begin{aligned} & 123, \\ & 137 \end{aligned}$ | $\begin{aligned} & 123, \\ & 137 \end{aligned}$ | $\begin{aligned} & 131, \\ & 141 \end{aligned}$ | $\begin{aligned} & 123, \\ & 141 \end{aligned}$ | $\begin{aligned} & 123, \\ & 141 \end{aligned}$ | $\begin{aligned} & 123, \\ & 131 \end{aligned}$ |
| D7S821 | $\begin{gathered} 109 . \\ 12 \end{gathered}$ | $\begin{aligned} & 258 \\ & 266 \end{aligned}$ | $\begin{aligned} & 254, \\ & 266 \end{aligned}$ | $\begin{aligned} & 258 \\ & 266 \end{aligned}$ | $\begin{aligned} & 266 \\ & 266 \end{aligned}$ | $\begin{aligned} & 266 \\ & 266 \end{aligned}$ | $\begin{aligned} & 254, \\ & 266 \end{aligned}$ |  |  | $\begin{aligned} & 262, \\ & 266 \end{aligned}$ | $\begin{aligned} & 258 \\ & 266 \end{aligned}$ | $\begin{aligned} & 262, \\ & 266 \end{aligned}$ | $\begin{aligned} & 258 \\ & 266 \end{aligned}$ | $\begin{aligned} & 250, \\ & 262 \end{aligned}$ | $\begin{aligned} & 250, \\ & 258 \end{aligned}$ | $\begin{aligned} & 258 \\ & 266 \end{aligned}$ | $\begin{aligned} & 258, \\ & 266 \end{aligned}$ |  |  |


| D7S530 | $\begin{gathered} 134 . \\ 55 \end{gathered}$ | $\begin{aligned} & 112, \\ & 114 \end{aligned}$ | $\begin{aligned} & 108 \\ & 114 \end{aligned}$ |  | $\begin{aligned} & 114, \\ & 144 \end{aligned}$ | $\begin{aligned} & 108, \\ & 112 \end{aligned}$ | $\begin{aligned} & 108, \\ & 114 \end{aligned}$ | $\begin{aligned} & 114, \\ & 114 \end{aligned}$ |  | $\begin{aligned} & 106 \\ & 112 \end{aligned}$ | $\begin{aligned} & 110, \\ & 114 \end{aligned}$ | $\begin{aligned} & 112, \\ & 114 \end{aligned}$ | $\begin{aligned} & 112, \\ & 114 \end{aligned}$ | $\begin{aligned} & 106, \\ & 112 \end{aligned}$ | $\begin{aligned} & 112, \\ & 112 \end{aligned}$ | $\begin{aligned} & 112, \\ & 112 \end{aligned}$ | $\begin{aligned} & 112, \\ & 112 \end{aligned}$ | $\begin{aligned} & 112, \\ & 112 \end{aligned}$ | $\begin{aligned} & 112, \\ & 112 \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D7S1804 | $\begin{gathered} 136 . \\ 95 \end{gathered}$ | $\begin{aligned} & 258, \\ & 266 \end{aligned}$ | $\begin{aligned} & 258, \\ & 262 \end{aligned}$ | $\begin{aligned} & 258, \\ & 266 \end{aligned}$ | $\begin{aligned} & 258, \\ & 262 \end{aligned}$ | $\begin{aligned} & 262, \\ & 266 \end{aligned}$ | $\begin{aligned} & 262, \\ & 266 \end{aligned}$ |  |  | $\begin{aligned} & 254, \\ & 266 \end{aligned}$ | $\begin{aligned} & 262, \\ & 258 \end{aligned}$ | $\begin{aligned} & 262, \\ & 266 \end{aligned}$ | $\begin{aligned} & 258, \\ & 266 \end{aligned}$ | $\begin{aligned} & 254, \\ & 266 \end{aligned}$ | $\begin{aligned} & 266, \\ & 266 \end{aligned}$ | $\begin{aligned} & 258, \\ & 278 \end{aligned}$ | $\begin{aligned} & 258, \\ & 266 \end{aligned}$ |  |  |
| D7S640 | $\begin{gathered} 137 . \\ 83 \end{gathered}$ | $\begin{aligned} & 116, \\ & 122 \end{aligned}$ | $\begin{aligned} & 122, \\ & 124 \end{aligned}$ | $\begin{aligned} & 122, \\ & 122 \end{aligned}$ | $\begin{aligned} & \hline 116, \\ & 122 \end{aligned}$ | $\begin{aligned} & 122, \\ & 124 \end{aligned}$ | $\begin{aligned} & 116, \\ & 124 \end{aligned}$ | $\begin{aligned} & 116, \\ & 122 \end{aligned}$ | $\begin{aligned} & 122, \\ & 122 \end{aligned}$ | $\begin{aligned} & 122, \\ & 128 \end{aligned}$ |  | $\begin{aligned} & 116, \\ & 128 \end{aligned}$ | $\begin{aligned} & 116, \\ & 128 \end{aligned}$ | $\begin{aligned} & 122, \\ & 128 \end{aligned}$ | $\begin{aligned} & 122, \\ & 128 \end{aligned}$ | $\begin{aligned} & \hline 116, \\ & 122 \end{aligned}$ | $\begin{aligned} & 122, \\ & 122 \end{aligned}$ | $\begin{aligned} & 122, \\ & 122 \end{aligned}$ | $\begin{aligned} & \hline 116, \\ & 122 \end{aligned}$ |
| D7S684 | $\begin{gathered} 147 . \\ 22 \end{gathered}$ | $\begin{aligned} & 171, \\ & 175 \end{aligned}$ |  | $\begin{aligned} & 171, \\ & 171 \end{aligned}$ | $\begin{aligned} & 175, \\ & 175 \end{aligned}$ | $\begin{gathered} 171, \\ 175 \end{gathered}$ | $\begin{aligned} & 171, \\ & 171 \end{aligned}$ | $\begin{aligned} & 171, \\ & 175 \end{aligned}$ | $\begin{gathered} 175, \\ 175 \end{gathered}$ | $\begin{gathered} 171, \\ 183 \end{gathered}$ | $\begin{aligned} & 171, \\ & 179 \end{aligned}$ | $\begin{gathered} 171, \\ 183 \end{gathered}$ | $\begin{aligned} & 179, \\ & 183 \end{aligned}$ | $\begin{aligned} & 171, \\ & 183 \end{aligned}$ |  | $\begin{aligned} & 171, \\ & 171 \end{aligned}$ | $\begin{aligned} & 171, \\ & 175 \end{aligned}$ | $\begin{aligned} & 171, \\ & 175 \end{aligned}$ | $\begin{gathered} 171, \\ 175 \end{gathered}$ |
| D7S661 | $\begin{gathered} 155 . \\ 10 \end{gathered}$ | $\begin{aligned} & 260, \\ & 268 \end{aligned}$ | $\begin{aligned} & 266, \\ & 274 \end{aligned}$ |  | $\begin{aligned} & 260, \\ & 274 \end{aligned}$ | $\begin{aligned} & 260, \\ & 266 \end{aligned}$ | $\begin{aligned} & 266, \\ & 268 \end{aligned}$ | $\begin{aligned} & 268, \\ & 274 \end{aligned}$ | $\begin{aligned} & 260, \\ & 274 \end{aligned}$ |  | $\begin{aligned} & 270, \\ & 272 \end{aligned}$ | $\begin{aligned} & 269, \\ & 272 \end{aligned}$ | $\begin{aligned} & 264, \\ & 270 \end{aligned}$ | $\begin{aligned} & 264, \\ & 268 \end{aligned}$ | $\begin{aligned} & 260, \\ & 264 \end{aligned}$ | $\begin{aligned} & \hline 266, \\ & 268 \end{aligned}$ | $\begin{aligned} & 260, \\ & 266 \end{aligned}$ | $\begin{aligned} & 260, \\ & 266 \end{aligned}$ | $\begin{aligned} & 260, \\ & 268 \end{aligned}$ |
| $\begin{aligned} & \hline \text { D7S1823/ } \\ & \text { D7S3058 } \end{aligned}$ | $\begin{gathered} 173 . \\ 71 \end{gathered}$ | $\begin{aligned} & 211, \\ & 223 \end{aligned}$ | $\begin{aligned} & 211, \\ & 227 \end{aligned}$ | $\begin{aligned} & 211, \\ & 211 \end{aligned}$ | $\begin{aligned} & 223, \\ & 227 \end{aligned}$ | $\begin{aligned} & 211, \\ & 223 \end{aligned}$ | $\begin{aligned} & 223, \\ & 227 \end{aligned}$ | $\begin{aligned} & 211, \\ & 211 \end{aligned}$ |  | $\begin{aligned} & 223, \\ & 223 \end{aligned}$ | $\begin{aligned} & 227, \\ & 231 \end{aligned}$ | $\begin{aligned} & 223, \\ & 227 \end{aligned}$ | $\begin{aligned} & 223, \\ & 231 \end{aligned}$ | $\begin{aligned} & 223, \\ & 223 \end{aligned}$ | $\begin{aligned} & 223, \\ & 223 \end{aligned}$ | $\begin{aligned} & 211, \\ & 231 \end{aligned}$ | $\begin{aligned} & 211, \\ & 223 \end{aligned}$ |  |  |
| D8S264 | 0.73 | $\begin{aligned} & 131, \\ & 131 \end{aligned}$ | $\begin{aligned} & 129, \\ & 141 \end{aligned}$ |  | $\begin{aligned} & 131, \\ & 141 \end{aligned}$ | $\begin{gathered} \hline 131, \\ 141 \end{gathered}$ | $\begin{aligned} & 129, \\ & 131 \end{aligned}$ | $\begin{aligned} & 129, \\ & 131 \end{aligned}$ | $\begin{aligned} & 129, \\ & 131 \end{aligned}$ | $\begin{gathered} 131, \\ 131 \end{gathered}$ | $\begin{gathered} 129, \\ 139 \end{gathered}$ |  | $\begin{aligned} & 129, \\ & 131 \end{aligned}$ | $\begin{aligned} & 131, \\ & 133 \end{aligned}$ | $\begin{aligned} & 131, \\ & 133 \end{aligned}$ | $\begin{gathered} 137, \\ 139 \end{gathered}$ | $\begin{aligned} & 131, \\ & 139 \end{aligned}$ | $\begin{aligned} & 131, \\ & 139 \end{aligned}$ | $\begin{aligned} & 131, \\ & 137 \end{aligned}$ |
| D8S1130 | $\begin{gathered} 22.4 \\ 1 \end{gathered}$ | $\begin{aligned} & 132, \\ & 144 \end{aligned}$ | $\begin{aligned} & 144, \\ & 160 \end{aligned}$ | $\begin{aligned} & 144, \\ & 132 \end{aligned}$ | $\begin{gathered} 132, \\ 160 \end{gathered}$ | $\begin{gathered} 132, \\ 160 \end{gathered}$ | $\begin{aligned} & 132, \\ & 144 \end{aligned}$ |  |  | $\begin{gathered} 132, \\ 144 \end{gathered}$ | $\begin{aligned} & 140, \\ & 152 \end{aligned}$ | $\begin{aligned} & 144, \\ & 152 \end{aligned}$ | $\begin{aligned} & 132, \\ & 152 \end{aligned}$ | $\begin{aligned} & 144, \\ & 144 \end{aligned}$ | $\begin{aligned} & 144, \\ & 144 \end{aligned}$ | $\begin{aligned} & 140 \\ & 148 \end{aligned}$ | $\begin{aligned} & 144, \\ & 148 \end{aligned}$ |  |  |
| D8S549 | $\begin{gathered} 31.7 \\ 3 \end{gathered}$ |  | $\begin{aligned} & 168, \\ & 170 \end{aligned}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ | $\begin{aligned} & 168, \\ & 170 \end{aligned}$ | $\begin{aligned} & \hline 168, \\ & 170 \end{aligned}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ | $\begin{aligned} & \hline 168, \\ & 172 \end{aligned}$ | $\begin{aligned} & 168, \\ & 170 \end{aligned}$ | $\begin{aligned} & 168, \\ & 170 \end{aligned}$ |  | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ | $\begin{gathered} 170, \\ 170 \end{gathered}$ |
| D8S258 | $\begin{gathered} 41.5 \\ 5 \end{gathered}$ | $\begin{aligned} & 152, \\ & 152 \end{aligned}$ | $\begin{aligned} & 148, \\ & 150 \end{aligned}$ | $\begin{aligned} & 150, \\ & 152 \end{aligned}$ | $\begin{aligned} & 148 \\ & 152 \end{aligned}$ | $\begin{aligned} & 148, \\ & 152 \end{aligned}$ | $\begin{aligned} & 150 \\ & 152 \end{aligned}$ | $\begin{aligned} & 150, \\ & 152 \end{aligned}$ | $\begin{aligned} & 150, \\ & 152 \end{aligned}$ | $\begin{gathered} 150, \\ 152 \end{gathered}$ | $\begin{aligned} & 148, \\ & 152 \end{aligned}$ | $\begin{gathered} 150, \\ 152 \end{gathered}$ | $\begin{aligned} & 152, \\ & 152 \end{aligned}$ | $\begin{aligned} & 150, \\ & 152 \end{aligned}$ | $\begin{aligned} & 148, \\ & 150 \end{aligned}$ | $\begin{aligned} & 148, \\ & 152 \end{aligned}$ | $\begin{aligned} & 148, \\ & 152 \end{aligned}$ | $\begin{aligned} & 148, \\ & 150 \end{aligned}$ | $\begin{gathered} 148, \\ 150 \end{gathered}$ |
| D8S136 | $\begin{gathered} 43.9 \\ 6 \end{gathered}$ | $\begin{gathered} 74,7 \\ 4 \end{gathered}$ | $\begin{gathered} 82,8 \\ 2 \end{gathered}$ | $\begin{gathered} 74,8 \\ 2 \end{gathered}$ | $\begin{gathered} 74,8 \\ 2 \end{gathered}$ | $\begin{gathered} 74,8 \\ 2 \end{gathered}$ | $\begin{gathered} 74,8 \\ 2 \end{gathered}$ |  |  | $\begin{gathered} 74,7 \\ 4 \end{gathered}$ | $\begin{gathered} 74,8 \\ 2 \end{gathered}$ | $\begin{gathered} 74,8 \\ 2 \end{gathered}$ | $\begin{gathered} 74,8 \\ 2 \end{gathered}$ | $\begin{gathered} 74,7 \\ 4 \end{gathered}$ | $\begin{gathered} 74,7 \\ 4 \end{gathered}$ | $\begin{gathered} 82,8 \\ 6 \end{gathered}$ | $\begin{gathered} 74,8 \\ 6 \end{gathered}$ |  |  |
| D8S1771 | $\begin{gathered} 50.0 \\ 5 \end{gathered}$ | $\begin{aligned} & 226 \\ & 232 \end{aligned}$ | $\begin{aligned} & 224, \\ & 226 \end{aligned}$ | $\begin{aligned} & 224, \\ & 226 \end{aligned}$ | $\begin{aligned} & 226 \\ & 232 \end{aligned}$ | $\begin{aligned} & 226 \\ & 232 \end{aligned}$ | $\begin{aligned} & 224, \\ & 226 \end{aligned}$ | $\begin{aligned} & 226, \\ & 232 \end{aligned}$ | $\begin{aligned} & 224, \\ & 226 \end{aligned}$ | $\begin{aligned} & 226 \\ & 228 \end{aligned}$ | $\begin{aligned} & 226 \\ & 228 \end{aligned}$ | $\begin{aligned} & 226 \\ & 228 \end{aligned}$ |  | $\begin{aligned} & 226, \\ & 228 \end{aligned}$ |  | $\begin{aligned} & 226 \\ & 226 \end{aligned}$ | $\begin{aligned} & 226, \\ & 232 \end{aligned}$ | $\begin{aligned} & 226, \\ & 226 \end{aligned}$ | $\begin{aligned} & 226 \\ & 226 \end{aligned}$ |
| D8S505 | $\begin{gathered} 60.8 \\ 7 \end{gathered}$ | $\begin{aligned} & 207, \\ & 211 \end{aligned}$ | $\begin{aligned} & 203, \\ & 213 \end{aligned}$ | $\begin{aligned} & 211, \\ & 213 \end{aligned}$ | $\begin{aligned} & 203, \\ & 211 \end{aligned}$ |  | $\begin{aligned} & 211, \\ & 213 \end{aligned}$ | $\begin{aligned} & 207, \\ & 213 \end{aligned}$ | $\begin{aligned} & 211, \\ & 213 \end{aligned}$ |  | $\begin{aligned} & 203, \\ & 205 \end{aligned}$ | $\begin{aligned} & 205, \\ & 207 \end{aligned}$ | $\begin{aligned} & 205, \\ & 211 \end{aligned}$ | $\begin{aligned} & 207, \\ & 211 \end{aligned}$ | $\begin{aligned} & 207, \\ & 207 \end{aligned}$ | $\begin{aligned} & 205, \\ & 207 \end{aligned}$ | $\begin{aligned} & 205, \\ & 207 \end{aligned}$ | $\begin{aligned} & 207, \\ & 207 \end{aligned}$ | $\begin{gathered} 207, \\ 207 \end{gathered}$ |
| D8S532 | $\begin{gathered} 64.7 \\ 5 \end{gathered}$ | $\begin{aligned} & 239, \\ & 253 \end{aligned}$ | $\begin{aligned} & 251, \\ & 247 \end{aligned}$ | $\begin{aligned} & 247, \\ & 253 \end{aligned}$ | $\begin{aligned} & 251, \\ & 253 \end{aligned}$ | $\begin{aligned} & 239, \\ & 251 \end{aligned}$ | $\begin{aligned} & 247, \\ & 253 \end{aligned}$ |  |  | $\begin{aligned} & 241, \\ & 251 \end{aligned}$ | $\begin{aligned} & 243, \\ & 251 \end{aligned}$ | $\begin{aligned} & 241, \\ & 243 \end{aligned}$ | $\begin{aligned} & 243, \\ & 251 \end{aligned}$ | $\begin{aligned} & 241, \\ & 251 \end{aligned}$ | $\begin{aligned} & 239, \\ & 241 \end{aligned}$ | $\begin{aligned} & 245, \\ & 251 \end{aligned}$ | $\begin{aligned} & 239, \\ & 251 \end{aligned}$ |  |  |
| D8S285 | $\begin{gathered} 71.0 \\ 0 \end{gathered}$ | $\begin{aligned} & 118, \\ & 120 \end{aligned}$ | $\begin{aligned} & 108 \\ & 122 \end{aligned}$ | $\begin{aligned} & 120, \\ & 122 \end{aligned}$ | $\begin{gathered} 108, \\ 120 \end{gathered}$ | $\begin{aligned} & 120, \\ & 122 \end{aligned}$ | $\begin{aligned} & 120, \\ & 122 \end{aligned}$ |  |  | $\begin{gathered} 108, \\ 120 \end{gathered}$ | $\begin{gathered} 108 \\ 118 \end{gathered}$ | $\begin{aligned} & 108 \\ & 118 \end{aligned}$ | $\begin{gathered} 120, \\ 118 \end{gathered}$ | $\begin{aligned} & 108, \\ & 120 \end{aligned}$ | $\begin{aligned} & 108, \\ & 118 \end{aligned}$ | $\begin{aligned} & 120, \\ & 120 \end{aligned}$ | $\begin{aligned} & 120, \\ & 118 \end{aligned}$ |  |  |


| D8S260 | $\begin{gathered} 79.3 \\ 6 \end{gathered}$ | $\begin{aligned} & 209, \\ & 205 \end{aligned}$ | $\begin{aligned} & 203, \\ & 207 \end{aligned}$ | $\begin{aligned} & 205, \\ & 203 \end{aligned}$ | $\begin{gathered} 205, \\ 207 \end{gathered}$ | $\begin{gathered} 203, \\ 205 \end{gathered}$ | $\begin{aligned} & 203, \\ & 205 \end{aligned}$ |  |  | $\begin{aligned} & 199, \\ & 203 \end{aligned}$ | $\begin{aligned} & 187, \\ & 205 \end{aligned}$ | $\begin{aligned} & 199, \\ & 205 \end{aligned}$ | $\begin{gathered} 187, \\ 199 \end{gathered}$ | $\begin{aligned} & 199, \\ & 205 \end{aligned}$ | $\begin{aligned} & 209, \\ & 203 \end{aligned}$ | $\begin{aligned} & 199, \\ & 205 \end{aligned}$ | $\begin{aligned} & 205, \\ & 209 \end{aligned}$ |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D8S2324 | $\begin{gathered} 94.0 \\ 8 \end{gathered}$ | $\begin{aligned} & 200, \\ & 216 \end{aligned}$ | $\begin{aligned} & 204, \\ & 204 \end{aligned}$ | $\begin{aligned} & 204, \\ & 216 \end{aligned}$ | $\begin{gathered} \hline 204, \\ 216 \end{gathered}$ | $\begin{gathered} 204, \\ 216 \end{gathered}$ | $\begin{aligned} & 200, \\ & 204 \end{aligned}$ |  |  | $\begin{aligned} & \hline 208, \\ & 216 \end{aligned}$ | $\begin{aligned} & 196, \\ & 212 \end{aligned}$ | $\begin{aligned} & 196, \\ & 216 \end{aligned}$ | $\begin{aligned} & 208, \\ & 212 \end{aligned}$ | $\begin{aligned} & 204, \\ & 208 \end{aligned}$ | $\begin{aligned} & 200, \\ & 204 \end{aligned}$ | $\begin{aligned} & 200, \\ & 204 \end{aligned}$ | $\begin{aligned} & 200, \\ & 204 \end{aligned}$ |  |  |
| $\begin{gathered} \text { GAAT1A } \\ 4 \end{gathered}$ | $\begin{gathered} 110 . \\ 20 \end{gathered}$ | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ | $\begin{aligned} & 140, \\ & 152 \end{aligned}$ | $\begin{aligned} & 140, \\ & 156 \end{aligned}$ | $\begin{gathered} 152, \\ 156 \end{gathered}$ | $\begin{aligned} & 152, \\ & 156 \end{aligned}$ | $\begin{gathered} 152, \\ 156 \end{gathered}$ |  |  | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ | $\begin{aligned} & 156, \\ & 148 \end{aligned}$ | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ | $\begin{aligned} & 156, \\ & 148 \end{aligned}$ | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ | $\begin{aligned} & 152, \\ & 156 \end{aligned}$ | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ |  |  |
| D8S1784 | $\begin{gathered} 118 . \\ 15 \end{gathered}$ |  | $\begin{aligned} & 163, \\ & 167 \end{aligned}$ | $\begin{aligned} & 163, \\ & 167 \end{aligned}$ | $\begin{gathered} 163, \\ 163 \end{gathered}$ | $\begin{gathered} 163, \\ 163 \end{gathered}$ | $\begin{gathered} 163, \\ 163 \end{gathered}$ | $\begin{aligned} & 163, \\ & 167 \end{aligned}$ | $\begin{aligned} & 163, \\ & 167 \end{aligned}$ | $\begin{gathered} 163, \\ 163 \end{gathered}$ | $\begin{gathered} 157, \\ 163 \end{gathered}$ | $\begin{gathered} 157, \\ 163 \end{gathered}$ | $\begin{gathered} 163, \\ 163 \end{gathered}$ | $\begin{gathered} 163, \\ 163 \end{gathered}$ | $\begin{aligned} & 163, \\ & 163 \end{aligned}$ | $\begin{aligned} & 157, \\ & 165 \end{aligned}$ | $\begin{aligned} & 163, \\ & 165 \end{aligned}$ |  | $\begin{gathered} 163, \\ 165 \end{gathered}$ |
| D8S514 | $\begin{gathered} 130 . \\ 00 \end{gathered}$ | $\begin{aligned} & 215, \\ & 217 \end{aligned}$ | $\begin{aligned} & 213, \\ & 215 \end{aligned}$ | $\begin{aligned} & 215 \\ & 215 \end{aligned}$ | $\begin{aligned} & 213, \\ & 217 \end{aligned}$ | $\begin{aligned} & 213, \\ & 217 \end{aligned}$ | $\begin{aligned} & 213, \\ & 215 \end{aligned}$ | $\begin{aligned} & 215 \\ & 217 \end{aligned}$ | $\begin{aligned} & 215, \\ & 217 \end{aligned}$ | $\begin{aligned} & 217, \\ & 223 \end{aligned}$ | $\begin{aligned} & 215, \\ & 223 \end{aligned}$ |  | $\begin{aligned} & 215, \\ & 223 \end{aligned}$ | $\begin{aligned} & 215, \\ & 215 \end{aligned}$ | $\begin{aligned} & 215 \\ & 215 \end{aligned}$ | $\begin{aligned} & 213, \\ & 217 \end{aligned}$ | $\begin{aligned} & 213, \\ & 215 \end{aligned}$ | $\begin{aligned} & 213, \\ & 215 \end{aligned}$ | $\begin{aligned} & 213, \\ & 215 \end{aligned}$ |
| D8S1179 | $\begin{gathered} 135 . \\ 08 \end{gathered}$ | $\begin{gathered} 182, \\ 190 \end{gathered}$ | $\begin{aligned} & 182, \\ & 182 \end{aligned}$ | $\begin{aligned} & 182, \\ & 182 \end{aligned}$ | $\begin{aligned} & 182, \\ & 190 \end{aligned}$ | $\begin{gathered} 182, \\ 190 \end{gathered}$ | $\begin{aligned} & 182, \\ & 182 \end{aligned}$ |  |  | $\begin{gathered} 186, \\ 190 \end{gathered}$ | $\begin{aligned} & 174, \\ & 178 \end{aligned}$ | $\begin{gathered} 174, \\ 190 \end{gathered}$ | $\begin{aligned} & 178, \\ & 186 \end{aligned}$ | $\begin{aligned} & 182, \\ & 182 \end{aligned}$ | $\begin{aligned} & 182, \\ & 182 \end{aligned}$ | $\begin{aligned} & 182, \\ & 182 \end{aligned}$ | $\begin{aligned} & 182, \\ & 182 \end{aligned}$ |  |  |
| D8S284 | $\begin{gathered} 143 . \\ 82 \end{gathered}$ | $\begin{aligned} & 273 \\ & 275 \end{aligned}$ | $\begin{aligned} & 243, \\ & 253 \end{aligned}$ | $\begin{aligned} & 253, \\ & 273 \end{aligned}$ | $\begin{aligned} & 243, \\ & 273 \end{aligned}$ | $\begin{aligned} & 243, \\ & 273 \end{aligned}$ | $\begin{aligned} & 243, \\ & 273 \end{aligned}$ | $\begin{aligned} & 253, \\ & 275 \end{aligned}$ |  | $\begin{aligned} & 243, \\ & 257 \end{aligned}$ | $\begin{aligned} & 253, \\ & 255 \end{aligned}$ | $\begin{aligned} & 243, \\ & 253 \end{aligned}$ | $\begin{aligned} & 255, \\ & 257 \end{aligned}$ | $\begin{aligned} & 243, \\ & 273 \end{aligned}$ | $\begin{aligned} & 243, \\ & 273 \end{aligned}$ | $\begin{aligned} & 243, \\ & 253 \end{aligned}$ | $\begin{aligned} & \hline 253, \\ & 273 \end{aligned}$ | $\begin{aligned} & 243, \\ & 253 \end{aligned}$ | $\begin{aligned} & 243, \\ & 253 \end{aligned}$ |
| D8S256 | $\begin{gathered} 148 . \\ 12 \end{gathered}$ | $\begin{aligned} & 210, \\ & 220 \end{aligned}$ | $\begin{aligned} & 210, \\ & 228 \end{aligned}$ | $\begin{aligned} & 210, \\ & 210 \end{aligned}$ | $\begin{aligned} & 210, \\ & 228 \end{aligned}$ | $\begin{aligned} & 210, \\ & 228 \end{aligned}$ | $\begin{aligned} & 210, \\ & 228 \end{aligned}$ |  |  | $\begin{aligned} & 210, \\ & 210 \end{aligned}$ | $\begin{aligned} & 226, \\ & 228 \end{aligned}$ | $\begin{aligned} & 210, \\ & 228 \end{aligned}$ | $\begin{aligned} & 210, \\ & 228 \end{aligned}$ | $\begin{aligned} & 210, \\ & 210 \end{aligned}$ | $\begin{aligned} & 210, \\ & 210 \end{aligned}$ | $\begin{aligned} & 224, \\ & 224 \end{aligned}$ | $\begin{aligned} & 224, \\ & 210 \end{aligned}$ |  |  |
| D8S272 | $\begin{gathered} 154 . \\ 02 \end{gathered}$ | $\begin{aligned} & 227, \\ & 227 \end{aligned}$ | $\begin{aligned} & 237, \\ & 227 \end{aligned}$ | $\begin{aligned} & 227, \\ & 237 \end{aligned}$ | $\begin{aligned} & 227, \\ & 237 \end{aligned}$ | $\begin{aligned} & 227, \\ & 237 \end{aligned}$ | $\begin{aligned} & 227, \\ & 237 \end{aligned}$ | $\begin{aligned} & 227, \\ & 237 \end{aligned}$ | $\begin{aligned} & 227, \\ & 237 \end{aligned}$ | $\begin{aligned} & 227, \\ & 237 \end{aligned}$ | $\begin{aligned} & 223, \\ & 229 \end{aligned}$ | $\begin{aligned} & 229, \\ & 237 \end{aligned}$ | $\begin{aligned} & 223, \\ & 227 \end{aligned}$ | $\begin{aligned} & 223, \\ & 227 \end{aligned}$ | $\begin{aligned} & 227, \\ & 237 \end{aligned}$ | $\begin{aligned} & 227, \\ & 235 \end{aligned}$ | $\begin{aligned} & 227, \\ & 227 \end{aligned}$ | $\begin{aligned} & 235, \\ & 237 \end{aligned}$ | $\begin{aligned} & 235 \\ & 237 \end{aligned}$ |
| D9S917 | 0.00 | $\begin{aligned} & 145 \\ & 145 \end{aligned}$ | $\begin{aligned} & 133, \\ & 142 \end{aligned}$ | $\begin{aligned} & 133, \\ & 145 \end{aligned}$ | $\begin{aligned} & 142, \\ & 145 \end{aligned}$ | $\begin{aligned} & 142, \\ & 145 \end{aligned}$ |  |  |  | $\begin{aligned} & 145 \\ & 145 \end{aligned}$ | $\begin{gathered} 133, \\ 133 \end{gathered}$ | $\begin{aligned} & 133, \\ & 145 \end{aligned}$ | $\begin{gathered} 145, \\ 133 \end{gathered}$ | $\begin{aligned} & 145 \\ & 145 \end{aligned}$ | $\begin{aligned} & 142, \\ & 145 \end{aligned}$ | $\begin{aligned} & 142, \\ & 148 \end{aligned}$ |  |  |  |
| D9S1813 | 9.83 | $\begin{aligned} & 226, \\ & 246 \end{aligned}$ | $\begin{aligned} & 226, \\ & 226 \end{aligned}$ | $\begin{aligned} & 226, \\ & 226 \end{aligned}$ | $\begin{gathered} 226, \\ 246 \end{gathered}$ | $\begin{aligned} & 226, \\ & 246 \end{aligned}$ | $\begin{aligned} & 226, \\ & 246 \end{aligned}$ |  |  | $\begin{aligned} & 226, \\ & 246 \end{aligned}$ | $\begin{aligned} & 226 \\ & 226 \end{aligned}$ | $\begin{aligned} & 226, \\ & 226 \end{aligned}$ | $\begin{aligned} & 226, \\ & 226 \end{aligned}$ | $\begin{aligned} & 226, \\ & 246 \end{aligned}$ | $\begin{aligned} & 226, \\ & 250 \end{aligned}$ | $\begin{aligned} & 252, \\ & 252 \end{aligned}$ | $\begin{aligned} & 250, \\ & 252 \end{aligned}$ |  |  |
| D9S288 | 9.83 |  | $\begin{aligned} & 130, \\ & 132 \end{aligned}$ | $\begin{gathered} 130, \\ 130 \end{gathered}$ | $\begin{gathered} 132, \\ 134 \end{gathered}$ | $\begin{gathered} 132, \\ 134 \end{gathered}$ | $\begin{aligned} & 130, \\ & 134 \end{aligned}$ | $\begin{gathered} 130, \\ 130 \end{gathered}$ | $\begin{aligned} & 130, \\ & 130 \end{aligned}$ | $\begin{aligned} & 130, \\ & 134 \end{aligned}$ |  | $\begin{aligned} & 130 \\ & 136 \end{aligned}$ |  | $\begin{aligned} & 130 \\ & 134 \end{aligned}$ | $\begin{aligned} & 128 \\ & 130 \end{aligned}$ | $\begin{gathered} 126, \\ 132 \end{gathered}$ | $\begin{aligned} & 128, \\ & 132 \end{aligned}$ | $\begin{aligned} & 128, \\ & 132 \end{aligned}$ | $\begin{gathered} 126 \\ 128 \end{gathered}$ |
| D9S1810 | $\begin{gathered} 12.7 \\ 8 \end{gathered}$ |  | $\begin{aligned} & 204, \\ & 212 \end{aligned}$ |  | $\begin{aligned} & \hline 204, \\ & 212 \end{aligned}$ | $\begin{aligned} & 204, \\ & 214 \end{aligned}$ |  |  |  | $\begin{aligned} & \hline 204, \\ & 212 \end{aligned}$ |  | $\begin{aligned} & 204, \\ & 212 \end{aligned}$ | $\begin{aligned} & 212, \\ & 212 \end{aligned}$ | $\begin{aligned} & 204, \\ & 212 \end{aligned}$ | $\begin{aligned} & 204, \\ & 204 \end{aligned}$ |  | $\begin{aligned} & 204, \\ & 204 \end{aligned}$ |  |  |
| D9S286 | $\begin{gathered} 18.0 \\ 6 \end{gathered}$ | $\begin{aligned} & 139, \\ & 159 \end{aligned}$ | $\begin{aligned} & \hline 153, \\ & 153 \end{aligned}$ | $\begin{aligned} & 153, \\ & 159 \end{aligned}$ | $\begin{aligned} & \hline 139, \\ & 153 \end{aligned}$ | $\begin{aligned} & 139 \\ & 153 \end{aligned}$ | $\begin{aligned} & \hline 139, \\ & 153 \end{aligned}$ | $\begin{aligned} & 153, \\ & 159 \end{aligned}$ | $\begin{aligned} & 153, \\ & 159 \end{aligned}$ | $\begin{aligned} & \hline 139, \\ & 159 \end{aligned}$ | $\begin{aligned} & 139, \\ & 145 \end{aligned}$ | $\begin{gathered} 145, \\ 159 \end{gathered}$ | $\begin{aligned} & 139, \\ & 145 \end{aligned}$ | $\begin{aligned} & 139, \\ & 159 \end{aligned}$ | $\begin{aligned} & 139, \\ & 159 \end{aligned}$ | $\begin{aligned} & 139 \\ & 155 \end{aligned}$ | $\begin{aligned} & 139, \\ & 139 \end{aligned}$ | $\begin{aligned} & 139, \\ & 139 \end{aligned}$ | $\begin{gathered} 155 \\ 159 \end{gathered}$ |
| D9S274 | $\begin{gathered} 28.4 \\ 2 \end{gathered}$ | $\begin{aligned} & 163, \\ & 171 \end{aligned}$ |  |  |  |  | $\begin{gathered} 163, \\ 171 \end{gathered}$ |  |  |  |  |  |  |  | $\begin{aligned} & 163, \\ & 165 \end{aligned}$ | $\begin{gathered} 163, \\ 171 \end{gathered}$ | $\begin{aligned} & 171, \\ & 165 \end{aligned}$ |  |  |


| D9S285 | $\begin{gathered} 29.5 \\ 2 \end{gathered}$ | $\begin{aligned} & 123, \\ & 125 \end{aligned}$ | $\begin{aligned} & \hline 123, \\ & 123 \end{aligned}$ | $\begin{aligned} & 123, \\ & 125 \end{aligned}$ | $\begin{aligned} & 123, \\ & 123 \end{aligned}$ | $\begin{aligned} & \hline 123, \\ & 123 \end{aligned}$ | $\begin{aligned} & \hline 123, \\ & 123 \end{aligned}$ | $\begin{aligned} & 123, \\ & 125 \end{aligned}$ | $\begin{aligned} & 123, \\ & 125 \end{aligned}$ | $\begin{aligned} & 123, \\ & 125 \end{aligned}$ | $\begin{aligned} & \hline 113, \\ & 113 \end{aligned}$ | $\begin{aligned} & 113, \\ & 125 \end{aligned}$ | $\begin{aligned} & \hline 113, \\ & 123 \end{aligned}$ | $\begin{aligned} & \hline 123, \\ & 123 \end{aligned}$ | $\begin{aligned} & \hline 107, \\ & 123 \end{aligned}$ | $\begin{aligned} & 125, \\ & 125 \end{aligned}$ | $\begin{aligned} & 123, \\ & 125 \end{aligned}$ | $\begin{aligned} & 123, \\ & 125 \end{aligned}$ | $\begin{aligned} & \hline 123, \\ & 125 \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D9S157 | $\begin{gathered} 32.2 \\ 4 \end{gathered}$ | $\begin{aligned} & \hline 133, \\ & 137 \end{aligned}$ | $\begin{aligned} & \hline 137, \\ & 145 \end{aligned}$ | $\begin{aligned} & 137, \\ & 137 \end{aligned}$ | $\begin{array}{\|c\|} \hline 133, \\ 137 \\ \hline \end{array}$ | $\begin{gathered} 133, \\ 137 \end{gathered}$ | $\begin{aligned} & \hline 133, \\ & 145 \end{aligned}$ | $\begin{aligned} & 137, \\ & 137 \end{aligned}$ | $\begin{aligned} & 137, \\ & 145 \end{aligned}$ | $\begin{aligned} & 133, \\ & 137 \end{aligned}$ | $\begin{gathered} 137, \\ 139 \end{gathered}$ | $\begin{gathered} 137, \\ 139 \end{gathered}$ | $\begin{aligned} & \hline 133, \\ & 139 \end{aligned}$ | $\begin{aligned} & 133, \\ & 133 \end{aligned}$ | $\begin{aligned} & 133, \\ & 145 \end{aligned}$ | $\begin{aligned} & 145, \\ & 145 \end{aligned}$ | $\begin{aligned} & 133, \\ & 145 \end{aligned}$ | $\begin{aligned} & 133, \\ & 145 \end{aligned}$ | $\begin{gathered} 133, \\ 145 \end{gathered}$ |
| D9S1679 | $\begin{gathered} 44.2 \\ 8 \end{gathered}$ | $\begin{aligned} & 135, \\ & 137 \end{aligned}$ | $\begin{aligned} & \hline 137, \\ & 141 \end{aligned}$ | $\begin{aligned} & 135, \\ & 141 \end{aligned}$ | $\begin{aligned} & \hline 135, \\ & 141 \end{aligned}$ | $\begin{aligned} & 137, \\ & 141 \end{aligned}$ | $\begin{array}{\|c\|} \hline 135, \\ 137 \\ \hline \end{array}$ | $\begin{aligned} & 137, \\ & 141 \end{aligned}$ | $\begin{array}{\|l\|} \hline 137, \\ 137 \\ \hline \end{array}$ | $\begin{array}{\|l\|} \hline 135, \\ 137 \end{array}$ | $\begin{aligned} & 139, \\ & 141 \end{aligned}$ | $\begin{aligned} & 137, \\ & 141 \end{aligned}$ | $\begin{aligned} & 135 \\ & 141 \end{aligned}$ | $\begin{aligned} & 135, \\ & 135 \end{aligned}$ | $\begin{aligned} & 141, \\ & 135 \end{aligned}$ | $\begin{array}{r} 139, \\ 141 \end{array}$ | $\begin{aligned} & \hline 135, \\ & 139 \end{aligned}$ |  |  |
| D9S1121 | $\begin{gathered} 44.2 \\ 8 \end{gathered}$ | $\begin{aligned} & \hline 184, \\ & 184 \end{aligned}$ | $\begin{array}{\|l\|} \hline 204, \\ 212 \\ \hline \end{array}$ | $\begin{aligned} & 184, \\ & 212 \end{aligned}$ | $\begin{array}{\|c\|} \hline 212, \\ 184 \\ \hline \end{array}$ | $\begin{aligned} & 184, \\ & 212 \end{aligned}$ | $\begin{aligned} & 184, \\ & 204 \end{aligned}$ |  |  | $\begin{aligned} & \hline 184, \\ & 184 \end{aligned}$ | $\begin{aligned} & 184, \\ & 184 \end{aligned}$ | $\begin{aligned} & 184, \\ & 184 \end{aligned}$ | $\begin{aligned} & 184, \\ & 184 \end{aligned}$ | $\begin{aligned} & 184, \\ & 212 \end{aligned}$ | $\begin{aligned} & 184, \\ & 212 \end{aligned}$ | $\begin{aligned} & 184, \\ & 212 \end{aligned}$ | $\begin{aligned} & 184, \\ & 212 \end{aligned}$ |  |  |
| D9S169 | $\begin{gathered} \hline 49.2 \\ 0 \end{gathered}$ | $\begin{aligned} & 269, \\ & 271 \end{aligned}$ | $\begin{aligned} & \hline 269, \\ & 277 \\ & \hline \end{aligned}$ | $\begin{aligned} & 271, \\ & 277 \end{aligned}$ | $\begin{aligned} & \hline 271, \\ & 277 \\ & \hline \end{aligned}$ | $\begin{aligned} & 269, \\ & 277 \end{aligned}$ | $\begin{array}{\|c\|} \hline 269, \\ 271 \\ \hline \end{array}$ | $\begin{aligned} & 269, \\ & 269 \end{aligned}$ | $\begin{aligned} & 269, \\ & 277 \end{aligned}$ | $\begin{aligned} & 279, \\ & 271 \end{aligned}$ | $\begin{aligned} & 271, \\ & 263 \end{aligned}$ | $\begin{aligned} & 263, \\ & 269 \end{aligned}$ | $\begin{aligned} & 263, \\ & 271 \end{aligned}$ | $\begin{aligned} & 271, \\ & 273 \end{aligned}$ | $\begin{aligned} & 269, \\ & 273 \end{aligned}$ | $\begin{aligned} & 265, \\ & 267 \end{aligned}$ | $\begin{aligned} & 267, \\ & 273 \end{aligned}$ |  |  |
| D9S161 | $\begin{gathered} 51.8 \\ 7 \end{gathered}$ | $\begin{aligned} & 121, \\ & 131 \end{aligned}$ | $\begin{aligned} & \hline 121, \\ & 133 \end{aligned}$ | $\begin{aligned} & 121, \\ & 131 \end{aligned}$ | $\begin{aligned} & 121, \\ & 131 \end{aligned}$ | $\begin{aligned} & 121, \\ & 121 \end{aligned}$ | $\begin{aligned} & 121, \\ & 131 \end{aligned}$ | $\begin{aligned} & 121, \\ & 121 \end{aligned}$ | $\begin{aligned} & \hline 121, \\ & 133 \end{aligned}$ | $\begin{aligned} & \hline 121, \\ & 131 \end{aligned}$ | $\begin{aligned} & 121, \\ & 131 \end{aligned}$ | $\begin{aligned} & 121, \\ & 131 \end{aligned}$ | $\begin{aligned} & 121, \\ & 131 \end{aligned}$ | $\begin{aligned} & \hline 121, \\ & 131 \end{aligned}$ | $\begin{aligned} & 121, \\ & 133 \end{aligned}$ | $\begin{aligned} & 131, \\ & 133 \end{aligned}$ | $\begin{aligned} & 121, \\ & 133 \end{aligned}$ | $\begin{aligned} & 121, \\ & 131 \end{aligned}$ | $\begin{aligned} & 121, \\ & 131 \end{aligned}$ |
| D9S1118 | $\begin{gathered} \hline 58.2 \\ 6 \end{gathered}$ | $\begin{aligned} & \hline 141, \\ & 169 \end{aligned}$ | $161,$ | $\begin{aligned} & \hline 161, \\ & 169 \end{aligned}$ | $\begin{aligned} & 141, \\ & 161 \end{aligned}$ | $\begin{aligned} & \hline 161, \\ & 169 \end{aligned}$ | $\begin{aligned} & \hline 161, \\ & 169 \end{aligned}$ |  |  | $\begin{array}{\|l\|} \hline 141, \\ 169 \\ \hline \end{array}$ | $\begin{aligned} & \hline 141, \\ & 141 \end{aligned}$ | $\begin{aligned} & \hline 141, \\ & 141 \end{aligned}$ | $\begin{aligned} & 141, \\ & 169 \end{aligned}$ | $\begin{aligned} & 157, \\ & 169 \end{aligned}$ | $157,$ | $\begin{aligned} & 157, \\ & 165 \end{aligned}$ | $\begin{aligned} & 157, \\ & 157 \end{aligned}$ |  |  |
| D9S273 | $\begin{gathered} 65.7 \\ 9 \end{gathered}$ | $\begin{aligned} & 211, \\ & 211 \end{aligned}$ | $\begin{array}{\|l\|} \hline 209, \\ 215 \\ \hline \end{array}$ | $\begin{aligned} & 209, \\ & 211 \end{aligned}$ | $\begin{aligned} & 209, \\ & 211 \end{aligned}$ | $\begin{aligned} & 209, \\ & 21, \end{aligned}$ | $\begin{aligned} & 209, \\ & 211 \end{aligned}$ | $\begin{aligned} & \hline 209, \\ & 211 \end{aligned}$ |  | $\begin{aligned} & 211, \\ & 211 \end{aligned}$ | $\begin{aligned} & 209, \\ & 211 \end{aligned}$ | $\begin{aligned} & 211, \\ & 211 \end{aligned}$ | $\begin{aligned} & 211, \\ & 211 \end{aligned}$ | $\begin{aligned} & 209, \\ & 211 \end{aligned}$ | $\begin{aligned} & 205, \\ & 209 \end{aligned}$ | $\begin{aligned} & \hline 205, \\ & 209 \end{aligned}$ | $\begin{aligned} & 209, \\ & 209 \end{aligned}$ | $\begin{aligned} & \hline 205, \\ & 209 \end{aligned}$ | $205,$ |
| D9S175 | $\begin{gathered} 70.3 \\ 3 \end{gathered}$ | $\begin{aligned} & 220, \\ & 226 \end{aligned}$ | $\begin{array}{\|l\|} \hline 208, \\ 218 \\ \hline \end{array}$ | $\begin{aligned} & 218, \\ & 220 \end{aligned}$ | $\begin{aligned} & \hline 218, \\ & 220 \\ & \hline \end{aligned}$ | $\begin{aligned} & 218, \\ & 226 \end{aligned}$ | $\begin{aligned} & 218, \\ & 220 \end{aligned}$ | $\begin{aligned} & 218, \\ & 220 \end{aligned}$ | $\begin{aligned} & 208, \\ & 226 \end{aligned}$ | $\begin{aligned} & 220, \\ & 226 \end{aligned}$ | $\begin{aligned} & 208, \\ & 210 \end{aligned}$ | $\begin{aligned} & 210, \\ & 226 \end{aligned}$ |  | $\begin{aligned} & 220, \\ & 226 \end{aligned}$ | $\begin{aligned} & 224, \\ & 226 \end{aligned}$ |  | $\begin{aligned} & 224, \\ & 226 \end{aligned}$ | $\begin{aligned} & 208, \\ & 226 \end{aligned}$ | $\begin{aligned} & 208, \\ & 226 \end{aligned}$ |
| D9S1122 | $\begin{gathered} 75.8 \\ 8 \end{gathered}$ | $\begin{aligned} & 203, \\ & 203 \end{aligned}$ | $\begin{aligned} & 191, \\ & 199 \end{aligned}$ | $199,$ | $\begin{gathered} \hline 203, \\ 199 \\ \hline \end{gathered}$ | $\begin{aligned} & 191, \\ & 203 \end{aligned}$ | $\begin{aligned} & \hline 199, \\ & 203 \end{aligned}$ |  |  | $\begin{aligned} & 203, \\ & 203 \end{aligned}$ | $\begin{gathered} 203, \\ 195 \end{gathered}$ | $\begin{aligned} & 203, \\ & 203 \\ & \hline \end{aligned}$ | $\begin{aligned} & 203, \\ & 203 \end{aligned}$ | $\begin{aligned} & 203, \\ & 203 \end{aligned}$ | $\begin{aligned} & 203, \\ & 203 \end{aligned}$ | $\begin{aligned} & 195, \\ & 199 \end{aligned}$ | $\begin{aligned} & 199, \\ & 203 \end{aligned}$ |  |  |
| D9S167 | $\begin{gathered} 83.4 \\ 1 \end{gathered}$ | $\begin{aligned} & 266, \\ & 270 \end{aligned}$ | $\begin{array}{\|l\|} \hline 276, \\ 278 \\ \hline \end{array}$ | $\begin{aligned} & 266, \\ & 276 \end{aligned}$ | $\begin{aligned} & 266, \\ & 276 \\ & \hline \end{aligned}$ | $\begin{gathered} 266 \\ 278 \end{gathered}$ | $\begin{array}{\|c\|} \hline 266, \\ 276 \\ \hline \end{array}$ | $\begin{aligned} & 270, \\ & 276 \end{aligned}$ | $\begin{aligned} & 266 \\ & 278 \end{aligned}$ | $\begin{gathered} 266, \\ 270 \end{gathered}$ | $\begin{gathered} 270, \\ 270 \end{gathered}$ | $\begin{aligned} & 270, \\ & 270 \end{aligned}$ | $\begin{aligned} & 266, \\ & 270 \end{aligned}$ | $\begin{aligned} & 266, \\ & 270 \end{aligned}$ | $\begin{aligned} & 270, \\ & 284 \end{aligned}$ | $\begin{gathered} 264, \\ 268 \end{gathered}$ | $\begin{aligned} & 264, \\ & 270 \end{aligned}$ | $\begin{aligned} & 264, \\ & 270 \end{aligned}$ | $\begin{gathered} 268, \\ 270 \end{gathered}$ |
| D9S257 | $\begin{gathered} 91.8 \\ 7 \end{gathered}$ | $\begin{aligned} & 261, \\ & 263 \end{aligned}$ | $\begin{array}{\|l\|} \hline 261, \\ 259 \\ \hline \end{array}$ | $\begin{aligned} & 259, \\ & 261 \end{aligned}$ | $\begin{aligned} & 259, \\ & 261 \end{aligned}$ | $\begin{aligned} & 261, \\ & 261 \end{aligned}$ | $\begin{array}{\|l\|} \hline 261, \\ 289 \\ \hline \end{array}$ |  |  | $\begin{aligned} & 261, \\ & 263 \end{aligned}$ | $\begin{aligned} & 261, \\ & 267 \end{aligned}$ | $\begin{aligned} & 267, \\ & 263 \end{aligned}$ | $\begin{aligned} & 267, \\ & 261 \end{aligned}$ | $\begin{aligned} & 263, \\ & 267 \end{aligned}$ | $\begin{aligned} & 267, \\ & 263 \end{aligned}$ | $\begin{aligned} & 263, \\ & 259 \end{aligned}$ | $\begin{aligned} & 259, \\ & 263 \end{aligned}$ |  |  |
| D9S287 | $\begin{gathered} 103 . \\ 42 \end{gathered}$ | $\begin{aligned} & \hline 168, \\ & 170 \end{aligned}$ | $\begin{aligned} & 160, \\ & 170 \end{aligned}$ | $\begin{aligned} & \hline 170, \\ & 170 \end{aligned}$ | $\begin{aligned} & \hline 170, \\ & 170 \end{aligned}$ | $\begin{gathered} 168, \\ 170 \end{gathered}$ | $\begin{array}{\|l\|} \hline 170, \\ 170 \\ \hline \end{array}$ | $\begin{aligned} & \hline 168, \\ & 170 \end{aligned}$ | $\begin{aligned} & 168, \\ & 170 \end{aligned}$ | $\begin{aligned} & 168, \\ & 172 \end{aligned}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ | $\begin{aligned} & 168, \\ & 170 \end{aligned}$ | $\begin{aligned} & \hline 170, \\ & 172 \end{aligned}$ | $\begin{aligned} & 168, \\ & 170 \end{aligned}$ | $\begin{aligned} & 168, \\ & 170 \end{aligned}$ | $\begin{aligned} & 170, \\ & 174 \end{aligned}$ | $\begin{aligned} & \hline 168, \\ & 170 \end{aligned}$ | $\begin{aligned} & \hline 168, \\ & 170 \end{aligned}$ | $\begin{aligned} & \hline 168, \\ & 174 \end{aligned}$ |
| D9S1690 | $\begin{gathered} 106 . \\ 63 \end{gathered}$ | $\begin{aligned} & 231, \\ & 233 \end{aligned}$ | $\begin{array}{\|l\|} \hline 225, \\ 227 \\ \hline \end{array}$ | $\begin{aligned} & \hline 227, \\ & 231 \end{aligned}$ | $\begin{gathered} 227, \end{gathered}$ | $\begin{aligned} & 225, \\ & 231 \end{aligned}$ | $\begin{aligned} & 227, \\ & 23, \end{aligned}$ | $\begin{aligned} & \hline 227, \\ & 233 \end{aligned}$ | $225,$ | $\begin{aligned} & 233, \\ & 237 \end{aligned}$ | $\begin{aligned} & 225, \\ & 233 \end{aligned}$ | $\begin{aligned} & 225, \\ & 237 \end{aligned}$ | $\begin{aligned} & 233, \\ & 237 \end{aligned}$ | $\begin{aligned} & 231, \\ & 233 \end{aligned}$ | $\begin{aligned} & 231, \\ & 233 \end{aligned}$ | $\begin{aligned} & 225, \\ & 237 \end{aligned}$ | $\begin{aligned} & 225, \\ & 233 \end{aligned}$ | $\begin{aligned} & \hline 233, \\ & 237 \end{aligned}$ | $233,$ |
| D9S1677 | $\begin{gathered} 117 . \\ 37 \end{gathered}$ | $\begin{aligned} & 251, \\ & 263 \end{aligned}$ | $\begin{aligned} & 251, \\ & 255 \end{aligned}$ | $\begin{aligned} & 251, \\ & 263 \end{aligned}$ | $\begin{aligned} & 251, \\ & 251 \end{aligned}$ | $\begin{aligned} & 255, \\ & 263 \end{aligned}$ | $\begin{aligned} & 251, \\ & 251 \end{aligned}$ | $\begin{aligned} & 251, \\ & 263 \end{aligned}$ | $\begin{aligned} & 251, \\ & 255 \end{aligned}$ | $\begin{aligned} & 263, \\ & 263 \end{aligned}$ | $\begin{aligned} & 251, \\ & 265 \end{aligned}$ | $\begin{aligned} & 251, \\ & 263 \end{aligned}$ | $\begin{aligned} & 263, \\ & 265 \end{aligned}$ | $\begin{aligned} & 251, \\ & 263 \end{aligned}$ | $\begin{aligned} & 251, \\ & 263 \end{aligned}$ | $\begin{aligned} & 259, \\ & 263 \end{aligned}$ | $\begin{aligned} & 259, \\ & 263 \end{aligned}$ | $\begin{aligned} & 259, \\ & 263 \end{aligned}$ | $\begin{aligned} & 263, \\ & 263 \end{aligned}$ |


| D9S1776 | $\begin{gathered} 123 . \\ 33 \end{gathered}$ |  | $\begin{aligned} & 113, \\ & 121 \end{aligned}$ | $\begin{aligned} & 113, \\ & 117 \end{aligned}$ | $\begin{aligned} & 117, \\ & 121 \end{aligned}$ | $\begin{aligned} & 113, \\ & 117 \end{aligned}$ | $\begin{aligned} & 117, \\ & 121 \end{aligned}$ | $\begin{aligned} & 117, \\ & 121 \end{aligned}$ | $\begin{aligned} & 113 \\ & 117 \end{aligned}$ | $\begin{gathered} 121, \\ 123 \end{gathered}$ | $\begin{aligned} & \hline 111, \\ & 123 \end{aligned}$ | $\begin{aligned} & 111, \\ & 123 \end{aligned}$ |  | $\begin{aligned} & 117, \\ & 121 \end{aligned}$ | $\begin{aligned} & 117, \\ & 121 \end{aligned}$ | $\begin{aligned} & 111, \\ & 113 \end{aligned}$ | $\begin{aligned} & 111, \\ & 121 \end{aligned}$ | $\begin{aligned} & 111, \\ & 121 \end{aligned}$ | $\begin{aligned} & 113, \\ & 121 \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D9S1682 | $\begin{gathered} 132 . \\ 09 \end{gathered}$ | $\begin{aligned} & 204, \\ & 204 \end{aligned}$ | $\begin{aligned} & 202, \\ & 204 \end{aligned}$ | $\begin{aligned} & 202, \\ & 204 \end{aligned}$ | $\begin{aligned} & 204, \\ & 204 \end{aligned}$ | $\begin{gathered} 202, \\ 204 \end{gathered}$ | $\begin{aligned} & 204, \\ & 204 \end{aligned}$ | $\begin{aligned} & 204, \\ & 204 \end{aligned}$ | $\begin{aligned} & 204, \\ & 204 \end{aligned}$ | $\begin{aligned} & 202, \\ & 204 \end{aligned}$ | $\begin{aligned} & 204, \\ & 204 \end{aligned}$ | $\begin{aligned} & 202, \\ & 204 \end{aligned}$ | $\begin{aligned} & 202, \\ & 204 \end{aligned}$ | $\begin{aligned} & 202, \\ & 204 \end{aligned}$ | $\begin{aligned} & 204, \\ & 204 \end{aligned}$ | $\begin{aligned} & 202, \\ & 204 \end{aligned}$ | $\begin{aligned} & 204, \\ & 204 \end{aligned}$ | $\begin{aligned} & 204, \\ & 204 \end{aligned}$ | $\begin{aligned} & 204, \\ & 204 \end{aligned}$ |
| D9S282 | $\begin{gathered} 136 . \\ 47 \end{gathered}$ | $\begin{aligned} & 233, \\ & 237 \end{aligned}$ | $\begin{aligned} & 233, \\ & 237 \end{aligned}$ | $\begin{aligned} & 233, \\ & 237 \end{aligned}$ | $\begin{aligned} & 233, \\ & 237 \end{aligned}$ | $\begin{aligned} & 233, \\ & 237 \end{aligned}$ | $\begin{aligned} & 233, \\ & 233 \end{aligned}$ |  |  | $\begin{aligned} & 235 \\ & 235 \end{aligned}$ | $\begin{aligned} & 233, \\ & 235 \end{aligned}$ | $\begin{aligned} & 233, \\ & 235 \end{aligned}$ | $\begin{aligned} & 233, \\ & 235 \end{aligned}$ | $\begin{aligned} & 235, \\ & 237 \end{aligned}$ | $\begin{aligned} & 235, \\ & 237 \end{aligned}$ | $\begin{aligned} & 235, \\ & 242 \end{aligned}$ | $\begin{aligned} & 235, \\ & 242 \end{aligned}$ |  |  |
| D9S290 | $\begin{gathered} 140 . \\ 86 \end{gathered}$ | $\begin{aligned} & 158, \\ & 160 \end{aligned}$ | $\begin{gathered} 152, \\ 158 \end{gathered}$ | $\begin{gathered} 152, \\ 158 \end{gathered}$ | $\begin{aligned} & \hline 158, \\ & 160 \end{aligned}$ | $\begin{gathered} 152, \\ 158 \end{gathered}$ | $\begin{aligned} & 158, \\ & 158 \end{aligned}$ | $\begin{aligned} & 158, \\ & 158 \end{aligned}$ | $\begin{aligned} & 152, \\ & 160 \end{aligned}$ |  | $\begin{gathered} 152, \\ 158 \end{gathered}$ | $\begin{gathered} \hline 158, \\ 160 \end{gathered}$ | $\begin{gathered} 158, \\ 158 \end{gathered}$ | $\begin{aligned} & 158, \\ & 160 \end{aligned}$ | $\begin{aligned} & 158, \\ & 160 \end{aligned}$ | $\begin{aligned} & 152, \\ & 156 \end{aligned}$ | $\begin{gathered} 152, \\ 158 \end{gathered}$ | $\begin{aligned} & 156, \\ & 160 \end{aligned}$ | $\begin{aligned} & 152, \\ & 158 \end{aligned}$ |
| D9S1826 | $\begin{gathered} 159 . \\ 61 \end{gathered}$ | $\begin{aligned} & 133, \\ & 135 \end{aligned}$ | $\begin{aligned} & 133, \\ & 147 \end{aligned}$ | $\begin{aligned} & 133, \\ & 147 \end{aligned}$ | $\begin{gathered} 133, \\ 135 \end{gathered}$ | $\begin{aligned} & 135 \\ & 147 \end{aligned}$ | $\begin{gathered} 135, \\ 147 \end{gathered}$ | $\begin{aligned} & 133, \\ & 147 \end{aligned}$ | $\begin{aligned} & 133, \\ & 147 \end{aligned}$ |  | $\begin{gathered} 135, \\ 139 \end{gathered}$ | $\begin{gathered} 135, \\ 135 \end{gathered}$ | $\begin{gathered} 135, \\ 139 \end{gathered}$ | $\begin{aligned} & 135, \\ & 135 \end{aligned}$ | $\begin{aligned} & 135 \\ & 135 \end{aligned}$ | $\begin{gathered} 135 \\ 145 \end{gathered}$ | $\begin{aligned} & 135 \\ & 135 \end{aligned}$ | $\begin{aligned} & 135 \\ & 145 \end{aligned}$ |  |
| D10S249 | 2.13 | $\begin{aligned} & 120, \\ & 126 \end{aligned}$ | $\begin{aligned} & \hline 118, \\ & 118 \end{aligned}$ | $\begin{aligned} & 118 \\ & 126 \end{aligned}$ | $\begin{aligned} & 118, \\ & 120 \end{aligned}$ | $\begin{aligned} & 118, \\ & 120 \end{aligned}$ | $\begin{aligned} & 118, \\ & 126 \end{aligned}$ | $\begin{aligned} & 118, \\ & 120 \end{aligned}$ |  | $\begin{gathered} 126, \\ 130 \end{gathered}$ | $\begin{aligned} & 118, \\ & 122 \end{aligned}$ | $\begin{aligned} & 118, \\ & 126 \end{aligned}$ | $\begin{aligned} & 122, \\ & 126 \end{aligned}$ | $\begin{aligned} & 120, \\ & 126 \end{aligned}$ | $\begin{aligned} & 120, \\ & 126 \end{aligned}$ | $\begin{aligned} & 122, \\ & 130 \end{aligned}$ | $\begin{aligned} & 120, \\ & 122 \end{aligned}$ | $\begin{aligned} & 122, \\ & 126 \end{aligned}$ | $\begin{aligned} & 120, \\ & 130 \end{aligned}$ |
| D10S189 | $\begin{gathered} 19.0 \\ 0 \end{gathered}$ | $\begin{aligned} & 186, \\ & 188 \end{aligned}$ | $\begin{aligned} & 186, \\ & 182 \end{aligned}$ | $\begin{aligned} & 186, \\ & 186 \end{aligned}$ | $\begin{gathered} 182, \\ 186 \end{gathered}$ | $\begin{gathered} 186, \\ 188 \end{gathered}$ | $\begin{aligned} & 182, \\ & 186 \end{aligned}$ |  |  | $\begin{gathered} 182, \\ 188 \end{gathered}$ | $\begin{gathered} 186, \\ 186 \end{gathered}$ | $\begin{gathered} 186, \\ 188 \end{gathered}$ | $\begin{gathered} 186, \\ 188 \end{gathered}$ | $\begin{aligned} & 186, \\ & 188 \end{aligned}$ | $\begin{aligned} & 182, \\ & 186 \end{aligned}$ | $\begin{gathered} 180, \\ 186 \end{gathered}$ | $\begin{aligned} & 180, \\ & 182 \end{aligned}$ |  |  |
| D10S547 | $\begin{gathered} 29.1 \\ 5 \end{gathered}$ | $\begin{aligned} & 236 \\ & 238 \end{aligned}$ | $\begin{aligned} & 236, \\ & 242 \end{aligned}$ | $\begin{aligned} & 236 \\ & 238 \end{aligned}$ | $\begin{aligned} & 236, \\ & 242 \end{aligned}$ |  | $\begin{aligned} & 236, \\ & 242 \end{aligned}$ | $\begin{aligned} & 236, \\ & 236 \end{aligned}$ | $\begin{aligned} & 236, \\ & 238 \end{aligned}$ | $\begin{aligned} & 236 \\ & 236 \end{aligned}$ | $\begin{aligned} & 236, \\ & 246 \end{aligned}$ | $\begin{aligned} & 236 \\ & 236 \end{aligned}$ | $\begin{aligned} & 236, \\ & 246 \end{aligned}$ | $\begin{aligned} & 238, \\ & 246 \end{aligned}$ | $\begin{aligned} & 236, \\ & 236 \end{aligned}$ | $\begin{aligned} & 236 \\ & 238 \end{aligned}$ | $\begin{aligned} & 236 \\ & 238 \end{aligned}$ | $\begin{aligned} & 236 \\ & 236 \end{aligned}$ | $\begin{aligned} & 236, \\ & 236 \end{aligned}$ |
| D10S1653 | $\begin{gathered} 40.3 \\ 6 \end{gathered}$ | $\begin{aligned} & 203, \\ & 205 \end{aligned}$ | $\begin{aligned} & 203, \\ & 207 \end{aligned}$ | $\begin{aligned} & 203, \\ & 205 \end{aligned}$ | $\begin{aligned} & \hline 203, \\ & 203 \end{aligned}$ | $\begin{gathered} 203, \\ 203 \end{gathered}$ | $\begin{aligned} & 203, \\ & 207 \end{aligned}$ | $\begin{aligned} & 203, \\ & 205 \end{aligned}$ | $\begin{aligned} & 203, \\ & 205 \end{aligned}$ | $\begin{aligned} & 203, \\ & 203 \end{aligned}$ | $\begin{aligned} & 203, \\ & 203 \end{aligned}$ | $\begin{aligned} & 203, \\ & 203 \end{aligned}$ | $\begin{aligned} & 203, \\ & 203 \end{aligned}$ | $\begin{aligned} & 203, \\ & 205 \end{aligned}$ | $\begin{aligned} & 203, \\ & 203 \end{aligned}$ | $\begin{aligned} & 203, \\ & 203 \end{aligned}$ | $\begin{aligned} & 203, \\ & 203 \end{aligned}$ | $\begin{aligned} & 203, \\ & 203 \end{aligned}$ | $\begin{aligned} & 203, \\ & 203 \end{aligned}$ |
| D10S548 | $\begin{gathered} 45.7 \\ 0 \end{gathered}$ | $\begin{aligned} & 140 \\ & 144 \end{aligned}$ | $\begin{aligned} & 140 \\ & 142 \end{aligned}$ | $\begin{aligned} & 140, \\ & 142 \end{aligned}$ | $\begin{aligned} & 142, \\ & 144 \end{aligned}$ | $\begin{gathered} 140 \\ 140 \end{gathered}$ | $\begin{gathered} 140, \\ 140 \end{gathered}$ | $\begin{aligned} & 142, \\ & 144 \end{aligned}$ | $\begin{aligned} & 142, \\ & 144 \end{aligned}$ | $\begin{gathered} 140 \\ 140 \end{gathered}$ | $\begin{aligned} & 142, \\ & 146 \end{aligned}$ | $\begin{aligned} & 140, \\ & 142 \end{aligned}$ | $\begin{aligned} & 140, \\ & 142 \end{aligned}$ | $\begin{aligned} & 140, \\ & 144 \end{aligned}$ | $\begin{aligned} & 140 \\ & 140 \end{aligned}$ | $\begin{aligned} & 140 \\ & 146 \end{aligned}$ | $\begin{aligned} & 140 \\ & 146 \end{aligned}$ | $\begin{aligned} & 140 \\ & 140 \end{aligned}$ | $\begin{aligned} & 140, \\ & 140 \end{aligned}$ |
| D10S1423 | $\begin{gathered} 46.2 \\ 3 \end{gathered}$ | $\begin{aligned} & 234, \\ & 238 \end{aligned}$ | $\begin{aligned} & 230, \\ & 234 \end{aligned}$ | $\begin{aligned} & 230, \\ & 234 \end{aligned}$ | $\begin{aligned} & 230, \\ & 238 \end{aligned}$ | $\begin{aligned} & 234, \\ & 238 \end{aligned}$ | $\begin{aligned} & 234, \\ & 238 \end{aligned}$ |  |  | $\begin{aligned} & 230 \\ & 238 \end{aligned}$ | $\begin{aligned} & 226 \\ & 226 \end{aligned}$ | $\begin{aligned} & 226, \\ & 238 \end{aligned}$ | $\begin{aligned} & 226, \\ & 238 \end{aligned}$ | $\begin{aligned} & 230 \\ & 234 \end{aligned}$ | $\begin{aligned} & 230 \\ & 238 \end{aligned}$ | $\begin{aligned} & 234, \\ & 226 \end{aligned}$ | $\begin{aligned} & 230 \\ & 234 \end{aligned}$ |  |  |
| D10S197 | $\begin{gathered} 52.1 \\ 0 \end{gathered}$ | $\begin{aligned} & 107, \\ & 171 \end{aligned}$ | $\begin{aligned} & 171, \\ & 171 \end{aligned}$ | $\begin{gathered} 171, \\ 171 \end{gathered}$ | $\begin{array}{r} 167, \\ 171 \end{array}$ | $\begin{gathered} 167, \\ 171 \end{gathered}$ | $\begin{gathered} 167, \\ 171 \end{gathered}$ | $\begin{aligned} & 171, \\ & 171 \end{aligned}$ | $\begin{aligned} & 171, \\ & 171 \end{aligned}$ | $\begin{gathered} 167, \\ 173 \end{gathered}$ | $\begin{gathered} 171, \\ 171 \end{gathered}$ | $\begin{gathered} 171, \\ 173 \end{gathered}$ | $\begin{gathered} 171, \\ 173 \end{gathered}$ | $\begin{aligned} & 167, \\ & 171 \end{aligned}$ | $\begin{gathered} 167, \\ 173 \end{gathered}$ | $\begin{gathered} 171, \\ 171 \end{gathered}$ | $\begin{aligned} & 167, \\ & 171 \end{aligned}$ | $\begin{aligned} & 167 \\ & 171 \end{aligned}$ | $\begin{aligned} & 171, \\ & 173 \end{aligned}$ |
| D10S1208 | $\begin{gathered} 63.3 \\ 0 \end{gathered}$ | $\begin{aligned} & 179, \\ & 179 \end{aligned}$ | $\begin{aligned} & 179, \\ & 194 \end{aligned}$ | $\begin{aligned} & 179, \\ & 179 \end{aligned}$ | $\begin{gathered} 179, \\ 179 \end{gathered}$ | $\begin{gathered} 179, \\ 194 \end{gathered}$ | $\begin{gathered} 179, \\ 194 \end{gathered}$ |  |  | $\begin{aligned} & 179, \\ & 179 \end{aligned}$ | $\begin{gathered} 191, \\ 197 \end{gathered}$ | $\begin{aligned} & 179, \\ & 197 \end{aligned}$ | $\begin{aligned} & 179, \\ & 191 \end{aligned}$ | $\begin{aligned} & 179, \\ & 179 \end{aligned}$ | $\begin{aligned} & 179, \\ & 179 \end{aligned}$ | $\begin{aligned} & 194, \\ & 194 \end{aligned}$ | $\begin{aligned} & 194, \\ & 179 \end{aligned}$ |  |  |
| D10S196 | $\begin{gathered} 70.2 \\ 3 \end{gathered}$ | $\begin{aligned} & 105 \\ & 109 \end{aligned}$ | $\begin{aligned} & 103 \\ & 105 \end{aligned}$ | $\begin{aligned} & 105 \\ & 105 \end{aligned}$ | $\begin{gathered} 105, \\ 109 \end{gathered}$ | $\begin{gathered} 103, \\ 109 \end{gathered}$ | $\begin{gathered} 103, \\ 105 \end{gathered}$ | $\begin{aligned} & 105 \\ & 105 \end{aligned}$ | $\begin{aligned} & 105 \\ & 109 \end{aligned}$ | $\begin{aligned} & 103 \\ & 105 \end{aligned}$ | $\begin{aligned} & 103 \\ & 105 \end{aligned}$ | $\begin{aligned} & 105 \\ & 105 \end{aligned}$ | $\begin{aligned} & 105 \\ & 105 \end{aligned}$ | $\begin{aligned} & 105 \\ & 109 \end{aligned}$ | $\begin{aligned} & 103, \\ & 105 \end{aligned}$ | $\begin{gathered} 103, \\ 103 \end{gathered}$ | $\begin{aligned} & 103, \\ & 103 \end{aligned}$ | $\begin{aligned} & 103, \\ & 103 \end{aligned}$ | $\begin{aligned} & 103, \\ & 105 \end{aligned}$ |
| $\begin{gathered} \text { Gata121A } \\ 08 \end{gathered}$ | $\begin{gathered} 88.4 \\ 1 \end{gathered}$ | $\begin{aligned} & 188, \\ & 192 \end{aligned}$ | $\begin{aligned} & 188, \\ & 196 \end{aligned}$ | $\begin{aligned} & 188, \\ & 188 \end{aligned}$ | $\begin{gathered} 188, \\ 192 \end{gathered}$ | $\begin{gathered} 192, \\ 196 \end{gathered}$ | $\begin{gathered} 196, \\ 192 \end{gathered}$ |  |  | $\begin{gathered} 188 \\ 192 \end{gathered}$ | $\begin{gathered} 188 \\ 184 \end{gathered}$ | $\begin{gathered} 188 \\ 188 \end{gathered}$ | $\begin{gathered} 188 \\ 184 \end{gathered}$ | $\begin{aligned} & 188, \\ & 188 \end{aligned}$ | $\begin{aligned} & 188, \\ & 192 \end{aligned}$ | $\begin{gathered} 184 \\ 188 \end{gathered}$ | $\begin{aligned} & 184, \\ & 192 \end{aligned}$ |  |  |


| D10S537 | $\begin{gathered} 91.1 \\ 3 \end{gathered}$ | $\begin{aligned} & 146, \\ & 160 \end{aligned}$ | $\begin{aligned} & 154, \\ & 160 \end{aligned}$ | $\begin{aligned} & 146, \\ & 160 \end{aligned}$ | $\begin{gathered} 154, \\ 160 \end{gathered}$ | $\begin{gathered} 160, \\ 160 \end{gathered}$ | $\begin{gathered} 160, \\ 160 \end{gathered}$ | $\begin{aligned} & 146, \\ & 160 \end{aligned}$ |  | $\begin{aligned} & 154, \\ & 160 \end{aligned}$ | $\begin{gathered} 154, \\ 160 \end{gathered}$ |  |  | $\begin{aligned} & 146, \\ & 160 \end{aligned}$ | $\begin{aligned} & 154, \\ & 160 \end{aligned}$ | $\begin{aligned} & 144, \\ & 144 \end{aligned}$ | $\begin{aligned} & 144, \\ & 154 \end{aligned}$ | $\begin{aligned} & 144, \\ & 154 \end{aligned}$ | $\begin{aligned} & 144, \\ & 160 \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D10S2327 | $\begin{gathered} 100 . \\ 92 \end{gathered}$ | $\begin{aligned} & 204, \\ & 220 \end{aligned}$ | $\begin{aligned} & 204, \\ & 220 \end{aligned}$ | $\begin{aligned} & 204, \\ & 220 \end{aligned}$ | $\begin{aligned} & 204, \\ & 220 \end{aligned}$ | $\begin{aligned} & 204, \\ & 204 \end{aligned}$ | $\begin{aligned} & 204, \\ & 204 \end{aligned}$ |  |  | $\begin{aligned} & 204, \\ & 220 \end{aligned}$ | $\begin{aligned} & 224, \\ & 224 \end{aligned}$ | $\begin{aligned} & 204, \\ & 224 \end{aligned}$ | $\begin{aligned} & 220, \\ & 224 \end{aligned}$ | $\begin{aligned} & 204, \\ & 220 \end{aligned}$ | $\begin{aligned} & 204, \\ & 220 \end{aligned}$ | $\begin{aligned} & 204, \\ & 204 \end{aligned}$ | $\begin{aligned} & 220, \\ & 204 \end{aligned}$ |  |  |
| D10S1686 | $\begin{gathered} 105 . \\ 04 \end{gathered}$ | $\begin{aligned} & 186 \\ & 202 \end{aligned}$ | $\begin{aligned} & 184, \\ & 200 \end{aligned}$ | $\begin{aligned} & 184, \\ & 202 \end{aligned}$ | $\begin{aligned} & 200, \\ & 202 \end{aligned}$ | $\begin{aligned} & 184, \\ & 202 \end{aligned}$ | $\begin{aligned} & 200, \\ & 202 \end{aligned}$ | $\begin{aligned} & 186, \\ & 200 \end{aligned}$ | $\begin{aligned} & 200, \\ & 202 \end{aligned}$ | $\begin{aligned} & 186, \\ & 200 \end{aligned}$ | $\begin{gathered} 184, \\ 196 \end{gathered}$ | $\begin{aligned} & 196 \\ & 200 \end{aligned}$ | $\begin{aligned} & 184, \\ & 186 \end{aligned}$ | $\begin{aligned} & \hline 186, \\ & 200 \\ & \hline \end{aligned}$ | $\begin{aligned} & 186, \\ & 200 \end{aligned}$ | $\begin{aligned} & 186, \\ & 204 \end{aligned}$ | $\begin{aligned} & 186, \\ & 204 \end{aligned}$ | $\begin{aligned} & 200, \\ & 204 \end{aligned}$ | $\begin{aligned} & 186, \\ & 204 \end{aligned}$ |
| D10S185 | $\begin{gathered} 116 . \\ 34 \end{gathered}$ |  | $\begin{aligned} & 149, \\ & 159 \end{aligned}$ | $\begin{aligned} & 149, \\ & 159 \end{aligned}$ | $\begin{aligned} & 149, \\ & 149 \end{aligned}$ | $\begin{gathered} 149, \\ 159 \end{gathered}$ | $\begin{aligned} & 149, \\ & 149 \end{aligned}$ | $\begin{aligned} & 149, \\ & 151 \end{aligned}$ | $\begin{aligned} & 149, \\ & 149 \end{aligned}$ | $\begin{aligned} & 151, \\ & 165 \end{aligned}$ | $\begin{aligned} & 147, \\ & 159 \end{aligned}$ | $\begin{aligned} & 159 \\ & 165 \end{aligned}$ |  | $\begin{aligned} & 157, \\ & 165 \end{aligned}$ | $\begin{aligned} & 151, \\ & 165 \end{aligned}$ | $\begin{aligned} & 147, \\ & 147 \end{aligned}$ | $\begin{aligned} & 147, \\ & 151 \end{aligned}$ | $\begin{aligned} & 147, \\ & 165 \end{aligned}$ | $\begin{aligned} & 147, \\ & 151 \end{aligned}$ |
| D10S192 | $\begin{gathered} 124 . \\ 27 \end{gathered}$ |  | $\begin{aligned} & \hline 192, \\ & 198 \end{aligned}$ | $\begin{gathered} 196, \\ 198 \end{gathered}$ | $\begin{aligned} & 192, \\ & 196 \end{aligned}$ | $\begin{gathered} 196, \\ 198 \end{gathered}$ | $\begin{aligned} & 192, \\ & 196 \end{aligned}$ | $\begin{aligned} & 192, \\ & 196 \end{aligned}$ | $\begin{aligned} & 192, \\ & 196 \end{aligned}$ |  | $\begin{aligned} & 196, \\ & 200 \end{aligned}$ | $\begin{gathered} 196, \\ 196 \end{gathered}$ | $\begin{aligned} & 196, \\ & 200 \end{aligned}$ | $\begin{aligned} & 186, \\ & 196 \end{aligned}$ | $\begin{aligned} & 196, \\ & 196 \end{aligned}$ | $\begin{aligned} & 196 \\ & 196 \end{aligned}$ | $\begin{aligned} & 196, \\ & 196 \end{aligned}$ | $\begin{aligned} & 196 \\ & 196 \end{aligned}$ | $\begin{aligned} & 196 \\ & 196 \end{aligned}$ |
| D10S1239 | $\begin{gathered} 125 . \\ 41 \end{gathered}$ | $\begin{aligned} & 160, \\ & 180 \end{aligned}$ | $\begin{aligned} & 176, \\ & 180 \end{aligned}$ | $\begin{aligned} & 176, \\ & 180 \end{aligned}$ | $\begin{aligned} & 180 \\ & 180 \end{aligned}$ | $\begin{gathered} 176, \\ 180 \end{gathered}$ | $\begin{aligned} & \hline 180, \\ & 180 \end{aligned}$ |  |  | $\begin{aligned} & 160, \\ & 180 \end{aligned}$ | $\begin{aligned} & 160 \\ & 176 \end{aligned}$ | $\begin{gathered} 176, \\ 180 \end{gathered}$ | $\begin{aligned} & \hline 160, \\ & 160 \end{aligned}$ | $\begin{aligned} & 180, \\ & 180 \end{aligned}$ | $\begin{aligned} & 160, \\ & 176 \end{aligned}$ | $\begin{aligned} & 176 \\ & 176 \end{aligned}$ | $\begin{aligned} & \hline 176, \\ & 160 \end{aligned}$ |  |  |
| D10S597 | $\begin{gathered} 128 . \\ 73 \end{gathered}$ | $\begin{aligned} & \hline 216, \\ & 216 \end{aligned}$ | $\begin{aligned} & \hline 216, \\ & 218 \end{aligned}$ | $\begin{aligned} & 216, \\ & 218 \end{aligned}$ | $\begin{aligned} & 216 \\ & 216 \end{aligned}$ | $\begin{aligned} & 216, \\ & 216 \end{aligned}$ | $\begin{aligned} & 216, \\ & 216 \end{aligned}$ | $\begin{aligned} & \hline 216, \\ & 216 \end{aligned}$ | $\begin{aligned} & 216, \\ & 216 \end{aligned}$ | $\begin{aligned} & 216, \\ & 216 \end{aligned}$ | $\begin{aligned} & \hline 216, \\ & 218 \end{aligned}$ | $\begin{gathered} 216, \\ 216 \end{gathered}$ | $\begin{aligned} & 216, \\ & 216 \end{aligned}$ | $\begin{aligned} & 216, \\ & 220 \end{aligned}$ | $\begin{aligned} & 216, \\ & 218 \end{aligned}$ | $\begin{aligned} & \hline 218, \\ & 218 \end{aligned}$ | $\begin{aligned} & 216, \\ & 218 \end{aligned}$ | $\begin{aligned} & 218, \\ & 218 \end{aligned}$ | $\begin{aligned} & 216, \\ & 218 \end{aligned}$ |
| D10S1693 | $\begin{gathered} 137 . \\ 39 \end{gathered}$ | $\begin{aligned} & 257, \\ & 259 \end{aligned}$ | $\begin{aligned} & 255, \\ & 259 \end{aligned}$ | $\begin{aligned} & 257, \\ & 259 \end{aligned}$ | $\begin{aligned} & 255, \\ & 257 \end{aligned}$ | $\begin{aligned} & 257, \\ & 259 \end{aligned}$ | $\begin{aligned} & 255, \\ & 259 \end{aligned}$ |  | $\begin{aligned} & 255, \\ & 259 \end{aligned}$ | $\begin{array}{\|l\|} \hline 257, \\ 259 \end{array}$ | $\begin{aligned} & 255, \\ & 255 \end{aligned}$ | $\begin{aligned} & 255, \\ & 257 \end{aligned}$ | $\begin{aligned} & 255, \\ & 259 \end{aligned}$ | $\begin{aligned} & 255, \\ & 257 \end{aligned}$ | $\begin{aligned} & 257, \\ & 259 \end{aligned}$ | $\begin{aligned} & 253, \\ & 255 \end{aligned}$ | $\begin{aligned} & 255, \\ & 259 \end{aligned}$ | $\begin{aligned} & 253, \\ & 257 \end{aligned}$ | $\begin{aligned} & 255, \\ & 259 \end{aligned}$ |
| D10S1230 | $\begin{gathered} 142 . \\ 78 \end{gathered}$ | $\begin{aligned} & 126, \\ & 132 \end{aligned}$ | $\begin{aligned} & 126, \\ & 132 \end{aligned}$ | $\begin{aligned} & 126, \\ & 126 \end{aligned}$ | $\begin{gathered} 132, \\ 132 \end{gathered}$ | $\begin{gathered} 132, \\ 126 \end{gathered}$ | $\begin{gathered} 132, \\ 132 \end{gathered}$ |  |  | $\begin{aligned} & 123, \\ & 126 \end{aligned}$ | $\begin{gathered} 126, \\ 129 \end{gathered}$ | $\begin{gathered} 129, \\ 126 \end{gathered}$ | $\begin{aligned} & 123, \\ & 126 \end{aligned}$ | $\begin{aligned} & 123, \\ & 126 \end{aligned}$ | $\begin{aligned} & 126, \\ & 132 \end{aligned}$ | $\begin{gathered} 132, \\ 132 \end{gathered}$ | $\begin{aligned} & 132, \\ & 132 \end{aligned}$ |  |  |
| D10S587 | $\begin{gathered} 147 . \\ 57 \end{gathered}$ | $\begin{aligned} & 174, \\ & 176 \end{aligned}$ | $\begin{aligned} & 176, \\ & 184 \end{aligned}$ | $\begin{aligned} & 176, \\ & 176 \end{aligned}$ | $\begin{gathered} 174, \\ 184 \end{gathered}$ | $\begin{gathered} 174, \\ 176 \end{gathered}$ | $\begin{aligned} & 174 \\ & 184 \end{aligned}$ | $\begin{aligned} & 176 \\ & 184 \end{aligned}$ | $\begin{aligned} & 174, \\ & 184 \end{aligned}$ | $\begin{gathered} 174, \\ 176 \end{gathered}$ | $\begin{gathered} 176, \\ 180 \end{gathered}$ | $\begin{gathered} 176, \\ 176 \end{gathered}$ | $\begin{gathered} 174, \\ 180 \end{gathered}$ | $\begin{aligned} & 174, \\ & 176 \end{aligned}$ | $\begin{aligned} & 174, \\ & 176 \end{aligned}$ | $\begin{gathered} 174, \\ 180 \end{gathered}$ | $\begin{aligned} & 174, \\ & 174 \end{aligned}$ | $\begin{aligned} & 176 \\ & 180 \end{aligned}$ | $\begin{aligned} & 174 \\ & 180 \end{aligned}$ |
| D10S217 | $\begin{gathered} 157 . \\ 89 \end{gathered}$ | $\begin{aligned} & 200, \\ & 202 \end{aligned}$ | $\begin{aligned} & 200, \\ & 214 \end{aligned}$ | $\begin{aligned} & 200, \\ & 214 \end{aligned}$ | $\begin{aligned} & 200 \\ & 214 \end{aligned}$ | $\begin{aligned} & 200, \\ & 202 \end{aligned}$ | $\begin{aligned} & 202, \\ & 214 \end{aligned}$ | $\begin{aligned} & 200, \\ & 214 \end{aligned}$ | $\begin{aligned} & 202, \\ & 214 \end{aligned}$ | $\begin{aligned} & 202, \\ & 202 \end{aligned}$ | $\begin{aligned} & 206 \\ & 206 \end{aligned}$ | $\begin{aligned} & 202, \\ & 206 \end{aligned}$ | $\begin{aligned} & 202, \\ & 206 \end{aligned}$ |  | $\begin{aligned} & 202, \\ & 202 \end{aligned}$ | $\begin{aligned} & 200, \\ & 200 \end{aligned}$ | $\begin{aligned} & 200, \\ & 202 \end{aligned}$ | $\begin{aligned} & 200, \\ & 202 \end{aligned}$ | $\begin{aligned} & 200, \\ & 202 \end{aligned}$ |
| D10S1651 | $\begin{gathered} 168 . \\ 77 \end{gathered}$ | $\begin{aligned} & 190, \\ & 190 \end{aligned}$ | $\begin{aligned} & 182, \\ & 196 \end{aligned}$ | $\begin{aligned} & 190, \\ & 196 \end{aligned}$ | $\begin{gathered} 182, \\ 190 \end{gathered}$ | $\begin{gathered} 190, \\ 196 \end{gathered}$ |  | $\begin{aligned} & 190, \\ & 196 \end{aligned}$ | $\begin{aligned} & 182, \\ & 190 \end{aligned}$ | $\begin{gathered} 180, \\ 180 \end{gathered}$ | $\begin{gathered} 182, \\ 190 \end{gathered}$ | $\begin{gathered} 180, \\ 190 \end{gathered}$ | $\begin{aligned} & 180, \\ & 182 \end{aligned}$ |  | $\begin{aligned} & 180, \\ & 190 \end{aligned}$ | $\begin{aligned} & 182, \\ & 196 \end{aligned}$ | $\begin{aligned} & 190, \\ & 196 \end{aligned}$ | $\begin{aligned} & 180, \\ & 182 \end{aligned}$ | $\begin{aligned} & 180 \\ & 182 \end{aligned}$ |
| D10S212 | $\begin{gathered} 170 . \\ 94 \end{gathered}$ | $\begin{gathered} 195 \\ 199 \end{gathered}$ | $\begin{aligned} & 195, \\ & 189 \end{aligned}$ | $\begin{aligned} & 189, \\ & 195 \end{aligned}$ | $\begin{gathered} 195 \\ 195 \end{gathered}$ | $\begin{array}{r} 189 \\ 195 \end{array}$ | $\begin{gathered} 195, \\ 195 \end{gathered}$ |  |  | $\begin{gathered} 189, \\ 191 \end{gathered}$ | $\begin{aligned} & 191, \\ & 195 \end{aligned}$ | $\begin{gathered} 191, \\ 191 \end{gathered}$ | $\begin{gathered} 195, \\ 189 \end{gathered}$ | $\begin{aligned} & 189, \\ & 199 \end{aligned}$ | $\begin{aligned} & 191, \\ & 195 \end{aligned}$ | $\begin{gathered} 195, \\ 195 \end{gathered}$ | $\begin{aligned} & 195, \\ & 195 \end{aligned}$ |  |  |
| D11S4046 | 2.79 | $\begin{aligned} & 187, \\ & 187 \end{aligned}$ | $\begin{aligned} & 195, \\ & 197 \end{aligned}$ | $\begin{aligned} & 187, \\ & 197 \end{aligned}$ | $\begin{gathered} 187, \\ 197 \end{gathered}$ | $\begin{gathered} 187, \\ 197 \end{gathered}$ | $\begin{gathered} 187, \\ 197 \end{gathered}$ | $\begin{aligned} & 187, \\ & 197 \end{aligned}$ | $\begin{aligned} & 187, \\ & 197 \end{aligned}$ | $\begin{gathered} 187, \\ 195 \end{gathered}$ | $\begin{gathered} 187, \\ 197 \end{gathered}$ | $\begin{aligned} & 195 \\ & 197 \end{aligned}$ | $\begin{gathered} 187, \\ 197 \end{gathered}$ | $\begin{aligned} & 183, \\ & 187 \end{aligned}$ | $\begin{aligned} & 183, \\ & 195 \end{aligned}$ | $\begin{aligned} & 183, \\ & 183 \end{aligned}$ | $\begin{aligned} & 183, \\ & 195 \end{aligned}$ | $\begin{aligned} & 183, \\ & 183 \end{aligned}$ | $\begin{aligned} & 183, \\ & 183 \end{aligned}$ |
| D11S1338 | $\begin{gathered} 12.9 \\ 2 \end{gathered}$ | $\begin{aligned} & 259 \\ & 265 \end{aligned}$ | $\begin{aligned} & 257, \\ & 259 \end{aligned}$ |  | $\begin{aligned} & 257, \\ & 265 \end{aligned}$ | $\begin{aligned} & 257, \\ & 259 \end{aligned}$ | $\begin{aligned} & 257, \\ & 259 \end{aligned}$ |  | $\begin{aligned} & 257, \\ & 265 \end{aligned}$ | $\begin{aligned} & 263, \\ & 265 \end{aligned}$ |  | $\begin{aligned} & 259, \\ & 265 \end{aligned}$ | $\begin{aligned} & 257, \\ & 263 \end{aligned}$ | $\begin{aligned} & 259, \\ & 263 \end{aligned}$ | $\begin{aligned} & 263, \\ & 265 \end{aligned}$ | $\begin{aligned} & 257, \\ & 259 \end{aligned}$ | $\begin{aligned} & 257, \\ & 265 \end{aligned}$ | $\begin{aligned} & 257, \\ & 263 \end{aligned}$ | $\begin{aligned} & 257, \\ & 263 \end{aligned}$ |


| D11S902 | $\begin{gathered} 21.4 \\ 7 \end{gathered}$ | $\begin{aligned} & 149, \\ & 161 \end{aligned}$ | $\begin{aligned} & 151, \\ & 155 \end{aligned}$ | $\begin{aligned} & 149 \\ & 155 \end{aligned}$ | $\begin{gathered} 149, \\ 151 \end{gathered}$ | $\begin{aligned} & 151, \\ & 161 \end{aligned}$ | $\begin{aligned} & \hline 151, \\ & 161 \end{aligned}$ | $\begin{aligned} & 149 \\ & 151 \end{aligned}$ |  | $\begin{aligned} & 147, \\ & 157 \end{aligned}$ | $\begin{aligned} & 155 \\ & 157 \end{aligned}$ | $\begin{aligned} & 155, \\ & 157 \end{aligned}$ | $\begin{aligned} & 155, \\ & 157 \end{aligned}$ | $\begin{aligned} & 149, \\ & 157 \end{aligned}$ | $\begin{aligned} & 147, \\ & 157 \end{aligned}$ | $\begin{aligned} & 151, \\ & 153 \end{aligned}$ | $\begin{aligned} & 153, \\ & 157 \end{aligned}$ | $\begin{aligned} & 153, \\ & 157 \end{aligned}$ |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D11S1981 | $\begin{gathered} 21.4 \\ 7 \end{gathered}$ | $\begin{aligned} & 162, \\ & 162 \end{aligned}$ | $\begin{aligned} & 154, \\ & 162 \end{aligned}$ | $\begin{aligned} & 162, \\ & 162 \end{aligned}$ | $\begin{aligned} & 154, \\ & 162 \end{aligned}$ | $\begin{aligned} & \hline 154, \\ & 162 \end{aligned}$ | $\begin{aligned} & \hline 154, \\ & 162 \\ & \hline \end{aligned}$ |  |  | $\begin{aligned} & 174, \\ & 158 \end{aligned}$ | $\begin{aligned} & 162, \\ & 162 \end{aligned}$ | $\begin{aligned} & 162, \\ & 174 \end{aligned}$ | $\begin{aligned} & 162, \\ & 174 \end{aligned}$ | $\begin{aligned} & 162, \\ & 174 \end{aligned}$ | $\begin{aligned} & 174, \\ & 158 \end{aligned}$ | $\begin{aligned} & 158, \\ & 166 \end{aligned}$ | $\begin{aligned} & 158, \\ & 174 \end{aligned}$ |  |  |
| D11S904 | $\begin{gathered} 33.5 \\ 7 \end{gathered}$ | $\begin{aligned} & 193, \\ & 201 \end{aligned}$ | $\begin{aligned} & 185, \\ & 201 \end{aligned}$ | $\begin{aligned} & 201, \\ & 201 \end{aligned}$ | $\begin{aligned} & 185, \\ & 201 \end{aligned}$ | $\begin{aligned} & \hline 185, \\ & 193 \end{aligned}$ | $\begin{aligned} & 185, \\ & 201 \end{aligned}$ | $\begin{aligned} & 185 \\ & 201 \end{aligned}$ |  | $\begin{gathered} 191, \\ 199 \end{gathered}$ | $\begin{aligned} & 191, \\ & 201 \end{aligned}$ | $\begin{gathered} \hline 191, \\ 191 \end{gathered}$ | $\begin{aligned} & 191, \\ & 201 \end{aligned}$ | $\begin{aligned} & 191, \\ & 201 \end{aligned}$ | $\begin{aligned} & 193, \\ & 199 \end{aligned}$ |  | $\begin{aligned} & 185, \\ & 193 \end{aligned}$ | $\begin{aligned} & 187, \\ & 193 \end{aligned}$ | $\begin{gathered} 187, \\ 199 \end{gathered}$ |
| D11S935 | $\begin{gathered} 45.9 \\ 4 \end{gathered}$ |  | $\begin{aligned} & 204, \\ & 206 \end{aligned}$ | $\begin{aligned} & 206, \\ & 206 \end{aligned}$ | $\begin{aligned} & 196, \\ & 204 \end{aligned}$ | $\begin{aligned} & 196, \\ & 204 \end{aligned}$ | $\begin{aligned} & 196, \\ & 204 \end{aligned}$ | $\begin{aligned} & 204, \\ & 206 \end{aligned}$ |  | $\begin{aligned} & 206, \\ & 206 \end{aligned}$ | $\begin{aligned} & 206, \\ & 206 \end{aligned}$ | $\begin{aligned} & 206, \\ & 206 \end{aligned}$ | $\begin{aligned} & 206 \\ & 206 \end{aligned}$ | $\begin{aligned} & 196, \\ & 206 \end{aligned}$ |  | $\begin{aligned} & 196, \\ & 204 \end{aligned}$ | $\begin{aligned} & 204, \\ & 206 \end{aligned}$ | $\begin{aligned} & 196, \\ & 206 \end{aligned}$ | $\begin{aligned} & 196, \\ & 206 \end{aligned}$ |
| D11S905 | $\begin{gathered} 51.9 \\ 5 \end{gathered}$ | $\begin{aligned} & 210, \\ & 210 \end{aligned}$ | $\begin{aligned} & 208, \\ & 224 \end{aligned}$ | $\begin{aligned} & 210, \\ & 224 \end{aligned}$ | $\begin{aligned} & 210, \\ & 224 \end{aligned}$ | $\begin{aligned} & 208, \\ & 210 \end{aligned}$ |  | $\begin{aligned} & 208, \\ & 210 \end{aligned}$ | $\begin{aligned} & 208, \\ & 210 \end{aligned}$ | $\begin{aligned} & 208, \\ & 210 \end{aligned}$ | $\begin{aligned} & 212, \\ & 224 \end{aligned}$ | $\begin{aligned} & 208, \\ & 212 \end{aligned}$ | $\begin{aligned} & 208, \\ & 224 \end{aligned}$ | $\begin{aligned} & 208, \\ & 210 \end{aligned}$ | $\begin{aligned} & 210, \\ & 210 \end{aligned}$ | $\begin{aligned} & 208, \\ & 224 \end{aligned}$ | $\begin{aligned} & 210, \\ & 224 \end{aligned}$ | $\begin{aligned} & 208, \\ & 210 \end{aligned}$ | $\begin{aligned} & 210, \\ & 224 \end{aligned}$ |
| D11S4191 | $\begin{gathered} 60.0 \\ 9 \end{gathered}$ |  | $\begin{aligned} & 111, \\ & 131 \end{aligned}$ | $\begin{aligned} & 111, \\ & 115 \end{aligned}$ | $\begin{aligned} & 113, \\ & 131 \end{aligned}$ | $\begin{aligned} & 113, \\ & 131 \end{aligned}$ | $\begin{aligned} & 113, \\ & 131 \end{aligned}$ | $\begin{aligned} & 115, \\ & 131 \end{aligned}$ | $\begin{aligned} & 113, \\ & 131 \end{aligned}$ |  | $\begin{aligned} & \hline 111, \\ & 113 \end{aligned}$ |  | $\begin{aligned} & 111, \\ & 119 \end{aligned}$ | $\begin{aligned} & 115, \\ & 119 \end{aligned}$ | $\begin{aligned} & 113, \\ & 115 \end{aligned}$ | $\begin{aligned} & \hline 111, \\ & 113 \end{aligned}$ | $\begin{aligned} & 113, \\ & 115 \end{aligned}$ | $\begin{aligned} & 113, \\ & 115 \end{aligned}$ | $\begin{aligned} & \hline 111, \\ & 113 \end{aligned}$ |
| D11S987 | $\begin{gathered} 67.4 \\ 8 \end{gathered}$ | $\begin{aligned} & 110, \\ & 116 \end{aligned}$ | $\begin{aligned} & 110, \\ & 122 \end{aligned}$ | $\begin{aligned} & 110, \\ & 122 \end{aligned}$ | $\begin{aligned} & 110 \\ & 116 \end{aligned}$ | $\begin{aligned} & 110, \\ & 116 \end{aligned}$ | $\begin{aligned} & \hline 110 \\ & 110 \\ & \hline \end{aligned}$ | $\begin{aligned} & 110, \\ & 110 \end{aligned}$ |  | $\begin{aligned} & 110, \\ & 116 \end{aligned}$ | $\begin{aligned} & 112, \\ & 116 \end{aligned}$ | $\begin{aligned} & \hline 110, \\ & 116 \end{aligned}$ | $\begin{aligned} & 110, \\ & 112 \end{aligned}$ | $\begin{aligned} & 108, \\ & 112 \end{aligned}$ | $\begin{aligned} & 110, \\ & 116 \end{aligned}$ | $\begin{aligned} & 116, \\ & 120 \end{aligned}$ | $\begin{aligned} & 110, \\ & 116 \end{aligned}$ | $\begin{aligned} & 110, \\ & 116 \end{aligned}$ |  |
| D11S1314 | $\begin{gathered} 73.6 \\ 4 \end{gathered}$ | $\begin{aligned} & 225, \\ & 225 \end{aligned}$ | $\begin{aligned} & 209 \\ & 215 \end{aligned}$ | $\begin{aligned} & 209, \\ & 225 \end{aligned}$ | $\begin{aligned} & 215, \\ & 225 \end{aligned}$ | $\begin{aligned} & 215, \\ & 225 \end{aligned}$ | $\begin{aligned} & 215, \\ & 225 \end{aligned}$ | $\begin{aligned} & 215 \\ & 225 \end{aligned}$ |  | $\begin{aligned} & 225, \\ & 225 \end{aligned}$ | $\begin{aligned} & 209, \\ & 223 \end{aligned}$ | $\begin{aligned} & 209 \\ & 225 \end{aligned}$ | $\begin{aligned} & 223, \\ & 225 \end{aligned}$ | $\begin{aligned} & 215, \\ & 221 \end{aligned}$ | $\begin{aligned} & 225, \\ & 225 \end{aligned}$ | $\begin{aligned} & 209, \\ & 223 \end{aligned}$ | $\begin{aligned} & 223, \\ & 225 \end{aligned}$ | $\begin{aligned} & 223, \\ & 225 \end{aligned}$ | $\begin{aligned} & 223, \\ & 225 \end{aligned}$ |
| D11S2371 | $\begin{gathered} 76.1 \\ 3 \end{gathered}$ | $\begin{aligned} & 201, \\ & 209 \end{aligned}$ | $\begin{aligned} & 201, \\ & 201 \end{aligned}$ | $\begin{aligned} & 201, \\ & 201 \end{aligned}$ | $\begin{gathered} 201, \\ 209 \end{gathered}$ | $\begin{aligned} & 201, \\ & 209 \end{aligned}$ | $\begin{aligned} & 201, \\ & 201 \end{aligned}$ |  |  | $\begin{aligned} & 201, \\ & 209 \end{aligned}$ | $\begin{aligned} & 201, \\ & 201 \end{aligned}$ | $\begin{aligned} & 201, \\ & 201 \end{aligned}$ | $\begin{aligned} & 201, \\ & 201 \end{aligned}$ | $\begin{aligned} & 201, \\ & 201 \end{aligned}$ | $\begin{aligned} & 201, \\ & 209 \end{aligned}$ | $\begin{aligned} & 197, \\ & 213 \end{aligned}$ | $\begin{aligned} & \hline 201, \\ & 213 \end{aligned}$ |  |  |
| D11S937 | $\begin{gathered} 79.9 \\ 8 \end{gathered}$ | $\begin{aligned} & 256, \\ & 260 \end{aligned}$ | $\begin{aligned} & 248 \\ & 264 \end{aligned}$ | $\begin{aligned} & 260, \\ & 264 \end{aligned}$ | $\begin{aligned} & 248 \\ & 256 \end{aligned}$ | $\begin{aligned} & 248 \\ & 256 \end{aligned}$ | $\begin{aligned} & 248, \\ & 260 \end{aligned}$ | $\begin{aligned} & 248, \\ & 260 \end{aligned}$ |  | $\begin{aligned} & 256 \\ & 260 \end{aligned}$ | $\begin{aligned} & 250 \\ & 254 \end{aligned}$ | $\begin{aligned} & 254, \\ & 260 \end{aligned}$ | $\begin{aligned} & 250, \\ & 260 \end{aligned}$ | $\begin{aligned} & 246, \\ & 256 \end{aligned}$ | $\begin{aligned} & 256, \\ & 260 \end{aligned}$ |  | $\begin{aligned} & 258 \\ & 260 \end{aligned}$ | $\begin{aligned} & 258, \\ & 260 \end{aligned}$ | $\begin{aligned} & 256 \\ & 258 \end{aligned}$ |
| D11S901 | $\begin{gathered} 85.4 \\ 8 \end{gathered}$ | $\begin{aligned} & 176, \\ & 176 \end{aligned}$ | $\begin{gathered} 168, \\ 178 \end{gathered}$ | $\begin{aligned} & 168, \\ & 176 \end{aligned}$ | $\begin{gathered} 176 \\ 178 \end{gathered}$ | $\begin{gathered} 176 \\ 178 \end{gathered}$ | $\begin{gathered} 176 \\ 178 \end{gathered}$ | $\begin{aligned} & 176 \\ & 178 \end{aligned}$ | $\begin{aligned} & 176, \\ & 178 \end{aligned}$ | $\begin{aligned} & 176 \\ & 176 \end{aligned}$ | $\begin{aligned} & 168 \\ & 180 \end{aligned}$ | $\begin{aligned} & 176 \\ & 180 \end{aligned}$ | $\begin{aligned} & 168, \\ & 176 \end{aligned}$ | $\begin{aligned} & 180, \\ & 188 \end{aligned}$ | $\begin{aligned} & 176 \\ & 176 \end{aligned}$ |  | $\begin{aligned} & 176 \\ & 176 \end{aligned}$ | $\begin{aligned} & 176, \\ & 180 \end{aligned}$ | $\begin{aligned} & 176 \\ & 176 \end{aligned}$ |
| D11S4175 | $\begin{gathered} 91.4 \\ 7 \end{gathered}$ | $\begin{aligned} & 151, \\ & 193 \end{aligned}$ | $\begin{aligned} & 151, \\ & 187 \end{aligned}$ | $\begin{aligned} & 151, \\ & 151 \end{aligned}$ | $\begin{gathered} 187, \\ 193 \end{gathered}$ | $\begin{array}{r} 187, \\ 193 \end{array}$ | $\begin{aligned} & 151, \\ & 187 \end{aligned}$ | $\begin{aligned} & 151, \\ & 187 \end{aligned}$ |  | $\begin{aligned} & 151, \\ & 193 \end{aligned}$ | $\begin{aligned} & 171, \\ & 189 \end{aligned}$ | $\begin{aligned} & 151, \\ & 189 \end{aligned}$ | $\begin{aligned} & 151, \\ & 189 \end{aligned}$ | $\begin{aligned} & 175 \\ & 189 \end{aligned}$ | $\begin{aligned} & 151, \\ & 193 \end{aligned}$ | $\begin{aligned} & 151, \\ & 151 \end{aligned}$ | $\begin{aligned} & 151, \\ & 151 \end{aligned}$ | $\begin{aligned} & 151, \\ & 151 \end{aligned}$ | $\begin{aligned} & 151, \\ & 193 \end{aligned}$ |
| D11S898 | $\begin{gathered} 98.9 \\ 8 \end{gathered}$ | $\begin{aligned} & 146, \\ & 152 \end{aligned}$ | $\begin{gathered} 146, \\ 150 \end{gathered}$ | $\begin{aligned} & 146, \\ & 146 \end{aligned}$ | $\begin{gathered} 146, \\ 152 \end{gathered}$ | $\begin{aligned} & 146, \\ & 152 \end{aligned}$ | $\begin{aligned} & 146, \\ & 146 \end{aligned}$ | $\begin{aligned} & 146, \\ & 146 \end{aligned}$ | $\begin{aligned} & 146, \\ & 152 \end{aligned}$ | $\begin{aligned} & 146, \\ & 152 \end{aligned}$ | $\begin{aligned} & 146, \\ & 146 \end{aligned}$ | $\begin{aligned} & 146 \\ & 146 \end{aligned}$ | $\begin{aligned} & 146, \\ & 146 \end{aligned}$ | $\begin{aligned} & 152, \\ & 152 \end{aligned}$ | $\begin{aligned} & 146, \\ & 152 \end{aligned}$ | $\begin{aligned} & 142, \\ & 150 \end{aligned}$ | $\begin{aligned} & 142, \\ & 146 \end{aligned}$ | $\begin{aligned} & 142, \\ & 152 \end{aligned}$ | $\begin{aligned} & 142, \\ & 152 \end{aligned}$ |
| D11S2000 | $\begin{gathered} 100 . \\ 62 \end{gathered}$ | $\begin{aligned} & 219, \\ & 221 \end{aligned}$ | $\begin{aligned} & 215, \\ & 235 \end{aligned}$ | $\begin{aligned} & 219, \\ & 215 \end{aligned}$ | $\begin{aligned} & 215 \\ & 219 \end{aligned}$ | $\begin{aligned} & 215, \\ & 219 \end{aligned}$ | $\begin{aligned} & 215, \\ & 221 \end{aligned}$ |  |  | $\begin{aligned} & 219, \\ & 219 \end{aligned}$ | $\begin{aligned} & 215, \\ & 219 \end{aligned}$ | $\begin{aligned} & 215, \\ & 219 \end{aligned}$ | $\begin{aligned} & 215, \\ & 219 \end{aligned}$ | $\begin{aligned} & 215, \\ & 221 \end{aligned}$ | $\begin{aligned} & 219, \\ & 219 \end{aligned}$ | $\begin{aligned} & 219, \\ & 225 \end{aligned}$ | $\begin{aligned} & 219, \\ & 219 \end{aligned}$ |  |  |
| D11S4090 | $\begin{gathered} 105 . \\ 74 \end{gathered}$ | $\begin{aligned} & 173 \\ & 197 \end{aligned}$ | $\begin{gathered} 183, \\ 189 \end{gathered}$ | $\begin{gathered} 183, \\ 197 \end{gathered}$ | $\begin{aligned} & 183, \\ & 197 \end{aligned}$ | $\begin{gathered} 197, \\ 183 \end{gathered}$ | $\begin{gathered} 173, \\ 183 \end{gathered}$ |  |  | $\begin{gathered} 173 \\ 197 \end{gathered}$ | $\begin{gathered} 179 \\ 189 \end{gathered}$ | $\begin{aligned} & 179, \\ & 173 \end{aligned}$ | $\begin{aligned} & 173 \\ & 179 \end{aligned}$ | $\begin{aligned} & 173, \\ & 191 \end{aligned}$ | $\begin{aligned} & 177, \\ & 197 \end{aligned}$ | $\begin{gathered} 183, \\ 191 \end{gathered}$ | $\begin{aligned} & 177 \\ & 191 \end{aligned}$ |  |  |


| D11S908 | $\begin{gathered} 108 . \\ 59 \end{gathered}$ | $\begin{aligned} & 145, \\ & 147 \end{aligned}$ | $\begin{aligned} & 147, \\ & 149 \end{aligned}$ | $\begin{gathered} 145, \\ 149 \end{gathered}$ | $\begin{gathered} 145, \\ 149 \end{gathered}$ | $\begin{aligned} & 145 \\ & 149 \end{aligned}$ | $\begin{gathered} 147, \\ 149 \end{gathered}$ | $\begin{aligned} & 147, \\ & 149 \end{aligned}$ | $\begin{aligned} & 145, \\ & 149 \end{aligned}$ | $\begin{aligned} & 145 \\ & 147 \end{aligned}$ | $\begin{gathered} 147, \\ 151 \end{gathered}$ | $\begin{aligned} & 147, \\ & 147 \end{aligned}$ | $\begin{gathered} 147, \\ 147 \end{gathered}$ | $\begin{aligned} & 145 \\ & 147 \end{aligned}$ | $\begin{aligned} & 145 \\ & 145 \end{aligned}$ | $\begin{aligned} & 145, \\ & 147 \end{aligned}$ | $\begin{aligned} & 145, \\ & 147 \end{aligned}$ | $\begin{aligned} & 145, \\ & 147 \end{aligned}$ | $\begin{aligned} & 145 \\ & 147 \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D11S1998 | $\begin{gathered} 113 . \\ 13 \end{gathered}$ | $\begin{aligned} & 153, \\ & 153 \end{aligned}$ | $\begin{aligned} & 149, \\ & 153 \end{aligned}$ | $\begin{aligned} & 149, \\ & 153 \end{aligned}$ | $\begin{aligned} & \hline 149 \\ & 153 \\ & \hline \end{aligned}$ | $\begin{aligned} & \hline 149, \\ & 153 \\ & \hline \end{aligned}$ | $\begin{aligned} & \hline 149, \\ & 153 \\ & \hline \end{aligned}$ |  |  | $\begin{aligned} & 153, \\ & 153 \end{aligned}$ | $\begin{aligned} & \hline 149 \\ & 153 \\ & \hline \end{aligned}$ | $\begin{aligned} & \hline 149 \\ & 153 \\ & \hline \end{aligned}$ | $\begin{aligned} & \hline 149, \\ & 153 \\ & \hline \end{aligned}$ | $\begin{aligned} & \hline 153, \\ & 153 \\ & \hline \end{aligned}$ | $\begin{aligned} & 149, \\ & 153 \end{aligned}$ | $\begin{aligned} & 149, \\ & 157 \end{aligned}$ | $\begin{aligned} & 149, \\ & 149 \end{aligned}$ |  |  |
| D11S925 | $\begin{gathered} 118 . \\ 47 \end{gathered}$ |  | $\begin{aligned} & 191, \\ & 195 \end{aligned}$ | $\begin{aligned} & 195, \\ & 195 \end{aligned}$ | $\begin{gathered} 195 \\ 195 \end{gathered}$ | $\begin{gathered} 195, \\ 195 \end{gathered}$ | $\begin{gathered} 195, \\ 195 \end{gathered}$ | $\begin{aligned} & 187, \\ & 195 \end{aligned}$ | $\begin{gathered} 195, \\ 195 \end{gathered}$ | $\begin{gathered} 187, \\ 195 \end{gathered}$ | $\begin{gathered} 173, \\ 189 \end{gathered}$ | $\begin{gathered} 187, \\ 189 \end{gathered}$ | $\begin{gathered} 187, \\ 189 \end{gathered}$ | $\begin{aligned} & 187, \\ & 195 \end{aligned}$ | $\begin{aligned} & 173, \\ & 195 \end{aligned}$ | $\begin{gathered} 173, \\ 195 \end{gathered}$ | $\begin{aligned} & 173, \\ & 195 \end{aligned}$ |  | $\begin{aligned} & 173 \\ & 195 \end{aligned}$ |
| D11S912 | $\begin{gathered} 131 . \\ 26 \end{gathered}$ | $\begin{gathered} 109, \\ 105 \end{gathered}$ | $\begin{aligned} & 103, \\ & 109 \end{aligned}$ | $\begin{gathered} 109, \\ 103 \end{gathered}$ | $\begin{gathered} 103, \\ 109 \end{gathered}$ | $\begin{gathered} 103, \\ 109 \end{gathered}$ | $\begin{gathered} 103, \\ 105 \end{gathered}$ |  |  | $\begin{gathered} 101, \\ 105 \end{gathered}$ | $\begin{gathered} 107, \\ 111 \end{gathered}$ | $\begin{gathered} 101, \\ 111 \end{gathered}$ | $\begin{gathered} 105, \\ 107 \end{gathered}$ | $\begin{gathered} 105, \\ 109 \end{gathered}$ | $\begin{aligned} & 101, \\ & 105 \end{aligned}$ | $\begin{gathered} 107, \\ 107 \end{gathered}$ | $\begin{aligned} & 101, \\ & 107 \end{aligned}$ |  |  |
| D11S1320 | $\begin{gathered} 141 . \\ 91 \end{gathered}$ | $\begin{aligned} & 227, \\ & 233 \end{aligned}$ | $\begin{aligned} & 227, \\ & 227 \end{aligned}$ | $\begin{aligned} & 227, \\ & 227 \end{aligned}$ | $\begin{aligned} & 227, \\ & 227 \end{aligned}$ | $\begin{aligned} & 227, \\ & 227 \end{aligned}$ | $\begin{aligned} & 227, \\ & 233 \end{aligned}$ | $\begin{aligned} & 227, \\ & 227 \end{aligned}$ | $\begin{aligned} & 227, \\ & 233 \end{aligned}$ | $\begin{aligned} & 233, \\ & 233 \end{aligned}$ | $\begin{aligned} & 231, \\ & 231 \end{aligned}$ | $\begin{aligned} & 231, \\ & 233 \end{aligned}$ | $\begin{aligned} & 231, \\ & 233 \end{aligned}$ | $\begin{aligned} & 227, \\ & 233 \end{aligned}$ | $\begin{aligned} & 233, \\ & 233 \end{aligned}$ | $\begin{aligned} & 227, \\ & 227 \end{aligned}$ | $\begin{aligned} & 227, \\ & 233 \end{aligned}$ | $\begin{aligned} & 227, \\ & 233 \end{aligned}$ | $\begin{aligned} & 227, \\ & 233 \end{aligned}$ |
| D11S968 | $\begin{gathered} 147 . \\ 77 \end{gathered}$ |  | $\begin{aligned} & 147, \\ & 147 \end{aligned}$ | $\begin{aligned} & 147, \\ & 147 \end{aligned}$ | $\begin{aligned} & 147, \\ & 147 \end{aligned}$ | $\begin{gathered} 147, \\ 147 \end{gathered}$ | $\begin{gathered} 147, \\ 147 \end{gathered}$ | $\begin{aligned} & 147, \\ & 147 \end{aligned}$ | $\begin{aligned} & 147, \\ & 147 \end{aligned}$ |  | $\begin{aligned} & 145, \\ & 149 \end{aligned}$ | $\begin{gathered} 147, \\ 149 \end{gathered}$ | $\begin{aligned} & 145 \\ & 147 \end{aligned}$ | $\begin{aligned} & 147, \\ & 147 \end{aligned}$ | $\begin{aligned} & 147, \\ & 147 \end{aligned}$ | $\begin{gathered} 143, \\ 149 \end{gathered}$ |  | $\begin{aligned} & 147, \\ & 149 \end{aligned}$ | $\begin{aligned} & 147, \\ & 149 \end{aligned}$ |
| D12S99 | $\begin{gathered} 12.6 \\ 0 \end{gathered}$ | $\begin{aligned} & 210, \\ & 210 \end{aligned}$ | $\begin{aligned} & 208, \\ & 212 \end{aligned}$ | $\begin{aligned} & 208, \\ & 210 \end{aligned}$ | $\begin{aligned} & 208, \\ & 210 \end{aligned}$ | $\begin{aligned} & \hline 208, \\ & 210 \end{aligned}$ | $\begin{aligned} & 210, \\ & 212 \end{aligned}$ | $\begin{aligned} & 210, \\ & 212 \end{aligned}$ | $\begin{aligned} & 210, \\ & 212 \end{aligned}$ | $\begin{aligned} & \hline 210, \\ & 212 \end{aligned}$ | $\begin{aligned} & \hline 212, \\ & 212 \end{aligned}$ | $\begin{aligned} & 212, \\ & 212 \end{aligned}$ | $\begin{aligned} & 212, \\ & 212 \end{aligned}$ | $\begin{aligned} & 212, \\ & 212 \end{aligned}$ | $\begin{aligned} & 212, \\ & 212 \end{aligned}$ |  | $\begin{aligned} & 208, \\ & 212 \end{aligned}$ | $\begin{aligned} & 208, \\ & 212 \end{aligned}$ | $\begin{aligned} & 210, \\ & 212 \end{aligned}$ |
| D12S1617 | $\begin{gathered} 44.0 \\ 3 \end{gathered}$ |  | $\begin{aligned} & 257, \\ & 257 \end{aligned}$ | $\begin{aligned} & 257, \\ & 259 \end{aligned}$ | $\begin{aligned} & 257, \\ & 259 \end{aligned}$ | $\begin{aligned} & 257, \\ & 261 \end{aligned}$ | $\begin{aligned} & 257, \\ & 259 \end{aligned}$ | $\begin{aligned} & 257, \\ & 261 \end{aligned}$ | $\begin{aligned} & 257, \\ & 259 \end{aligned}$ | $\begin{aligned} & 255, \\ & 259 \end{aligned}$ | $\begin{aligned} & 247, \\ & 257 \end{aligned}$ | $\begin{aligned} & 247, \\ & 255 \end{aligned}$ | $\begin{aligned} & 247, \\ & 255 \end{aligned}$ | $\begin{aligned} & 259, \\ & 261 \end{aligned}$ | $\begin{aligned} & 259, \\ & 261 \end{aligned}$ | $\begin{aligned} & 247, \\ & 261 \end{aligned}$ | $\begin{aligned} & 247, \\ & 261 \end{aligned}$ | $\begin{aligned} & 261, \\ & 261 \end{aligned}$ | $\begin{aligned} & 247, \\ & 259 \end{aligned}$ |
| D12S345 | $\begin{gathered} 53.0 \\ 9 \end{gathered}$ | $\begin{aligned} & 212, \\ & 226 \end{aligned}$ | $\begin{aligned} & 224, \\ & 228 \end{aligned}$ | $\begin{aligned} & 212, \\ & 224 \end{aligned}$ | $\begin{aligned} & 226, \\ & 228 \end{aligned}$ | $\begin{aligned} & 226, \\ & 228 \end{aligned}$ | $\begin{aligned} & 224, \\ & 226 \end{aligned}$ | $\begin{aligned} & 224, \\ & 226 \end{aligned}$ | $\begin{aligned} & 212, \\ & 228 \end{aligned}$ | $\begin{aligned} & 210, \\ & 216 \end{aligned}$ | $\begin{aligned} & 224, \\ & 224 \end{aligned}$ | $\begin{aligned} & 210, \\ & 224 \end{aligned}$ | $\begin{aligned} & 210, \\ & 224 \end{aligned}$ | $\begin{aligned} & 212, \\ & 226 \end{aligned}$ | $\begin{aligned} & 212, \\ & 226 \end{aligned}$ | $\begin{aligned} & 210, \\ & 230 \end{aligned}$ | $\begin{aligned} & 210, \\ & 226 \end{aligned}$ | $\begin{aligned} & 210, \\ & 226 \end{aligned}$ | $\begin{aligned} & 212, \\ & 230 \end{aligned}$ |
| D12S398 | $\begin{gathered} 68.1 \\ 6 \end{gathered}$ | $\begin{gathered} 132, \\ 140 \end{gathered}$ | $\begin{aligned} & 132, \\ & 128 \end{aligned}$ | $\begin{aligned} & 128, \\ & 140 \end{aligned}$ | $\begin{aligned} & 140, \\ & 132 \end{aligned}$ | $\begin{aligned} & 132, \\ & 132 \end{aligned}$ | $\begin{gathered} 128, \\ 140 \end{gathered}$ |  |  | $\begin{aligned} & 128, \\ & 132 \end{aligned}$ | $\begin{gathered} 128, \\ 132 \end{gathered}$ | $\begin{aligned} & 132, \\ & 132 \end{aligned}$ | $\begin{aligned} & 132, \\ & 132 \end{aligned}$ | $\begin{aligned} & 132, \\ & 140 \end{aligned}$ | $\begin{aligned} & 128, \\ & 132 \end{aligned}$ | $\begin{aligned} & 128, \\ & 140 \end{aligned}$ | $\begin{aligned} & 128, \\ & 140 \end{aligned}$ |  |  |
| D12S83 | $\begin{gathered} \hline 75.1 \\ 7 \\ \hline \end{gathered}$ | $\begin{gathered} 87,9 \\ 3 \end{gathered}$ | $\begin{gathered} 85,9 \\ 3 \end{gathered}$ | $\begin{gathered} 85,9 \\ 3 \end{gathered}$ | $\begin{gathered} 93,9 \\ 3 \end{gathered}$ | $\begin{gathered} 87,9 \\ 3 \end{gathered}$ |  | $\begin{gathered} 85,9 \\ 3 \end{gathered}$ | $\begin{gathered} 87,9 \\ 3 \end{gathered}$ | $\begin{gathered} 85,8 \\ 7 \end{gathered}$ | $\begin{gathered} 85,9 \\ 1 \end{gathered}$ | $\begin{gathered} 87,9 \\ 1 \end{gathered}$ | $\begin{gathered} 87,9 \\ 1 \end{gathered}$ | $\begin{gathered} 93,1 \\ 02 \end{gathered}$ | $\begin{gathered} 85,1 \\ 01 \end{gathered}$ | $\begin{gathered} 87,8 \\ 9 \end{gathered}$ | $\begin{gathered} 85,8 \\ 7 \end{gathered}$ | $\begin{gathered} 85,8 \\ 7 \end{gathered}$ | $\begin{gathered} 89,1 \\ 01 \end{gathered}$ |
| D12S1300 | $\begin{gathered} 104 . \\ 12 \end{gathered}$ | $\begin{aligned} & 123, \\ & 123 \end{aligned}$ | $\begin{aligned} & 119, \\ & 123 \end{aligned}$ | $\begin{aligned} & 123, \\ & 123 \end{aligned}$ | $\begin{aligned} & 119, \\ & 123 \end{aligned}$ | $\begin{aligned} & 119, \\ & 123 \end{aligned}$ | $\begin{gathered} 119, \\ 123 \end{gathered}$ |  |  | $\begin{gathered} 107 \\ 123 \end{gathered}$ | $\begin{gathered} 119 \\ 127 \end{gathered}$ | $\begin{gathered} 107, \\ 119 \end{gathered}$ | $\begin{gathered} 107, \\ 127 \end{gathered}$ | $\begin{aligned} & 123, \\ & 123 \end{aligned}$ | $\begin{gathered} 107, \\ 123 \end{gathered}$ | $\begin{gathered} 119, \\ 119 \end{gathered}$ | $\begin{aligned} & 119, \\ & 123 \end{aligned}$ |  |  |
| D12S346 | $\begin{gathered} 104 . \\ 65 \end{gathered}$ |  | $\begin{aligned} & 172, \\ & 174 \end{aligned}$ | $\begin{aligned} & 154, \\ & 174 \end{aligned}$ | $\begin{gathered} 154, \\ 172 \end{gathered}$ | $\begin{gathered} 162, \\ 172 \end{gathered}$ | $\begin{gathered} 162, \\ 172 \end{gathered}$ | $\begin{aligned} & 162, \\ & 174 \end{aligned}$ | $\begin{aligned} & 154, \\ & 174 \end{aligned}$ | $\begin{aligned} & \hline 160, \\ & 170 \end{aligned}$ | $\begin{gathered} 164, \\ 172 \end{gathered}$ | $\begin{aligned} & 160, \\ & 172 \end{aligned}$ | $\begin{aligned} & 160, \\ & 164 \end{aligned}$ | $\begin{aligned} & 154, \\ & 162 \end{aligned}$ | $\begin{aligned} & 160, \\ & 170 \end{aligned}$ | $\begin{aligned} & 172, \\ & 176 \end{aligned}$ | $\begin{aligned} & 170, \\ & 172 \end{aligned}$ | $\begin{aligned} & 170 \\ & 176 \end{aligned}$ | $\begin{aligned} & 170, \\ & 176 \end{aligned}$ |
| D12S2070 | $\begin{gathered} 125 . \\ 30 \end{gathered}$ | $\begin{gathered} 89,9 \\ 8 \end{gathered}$ | $\begin{gathered} \hline 89,1 \\ 01 \end{gathered}$ | $\begin{gathered} 101, \\ 89 \end{gathered}$ | $\begin{gathered} 89,8 \\ 9 \end{gathered}$ | $\begin{gathered} 89,8 \\ 9 \end{gathered}$ | $\begin{gathered} 89,8 \\ 9 \end{gathered}$ |  |  | $\begin{gathered} 89,8 \\ 9 \end{gathered}$ | $\begin{gathered} 98,9 \\ 8 \end{gathered}$ | $\begin{gathered} 89,9 \\ 8 \end{gathered}$ | $\begin{gathered} 89,9 \\ 8 \end{gathered}$ | $\begin{gathered} 89,9 \\ 8 \end{gathered}$ | $\begin{gathered} \hline 89,9 \\ 8 \end{gathered}$ | $\begin{gathered} 89,9 \\ 8 \end{gathered}$ | $\begin{gathered} 89,8 \\ 9 \end{gathered}$ |  |  |
| D12S79 | $\begin{gathered} 125 . \\ 31 \end{gathered}$ | $\begin{aligned} & 159 \\ & 179 \end{aligned}$ | $\begin{aligned} & 161, \\ & 171 \end{aligned}$ | $\begin{aligned} & 159 \\ & 171 \end{aligned}$ | $\begin{gathered} 159, \\ 161 \end{gathered}$ | $\begin{aligned} & 159, \\ & 161 \end{aligned}$ | $\begin{gathered} 159 \\ 161 \end{gathered}$ | $\begin{aligned} & 159 \\ & 171 \end{aligned}$ | $\begin{aligned} & 159 \\ & 171 \end{aligned}$ | $\begin{aligned} & 159, \\ & 165 \end{aligned}$ | $\begin{gathered} 181, \\ 183 \end{gathered}$ |  | $\begin{gathered} 165 \\ 181 \end{gathered}$ | $\begin{aligned} & 165, \\ & 179 \end{aligned}$ | $\begin{gathered} 165 \\ 179 \end{gathered}$ |  | $\begin{aligned} & 159 \\ & 165 \end{aligned}$ | $\begin{aligned} & 165 \\ & 179 \end{aligned}$ |  |


| D12S2082 | $\begin{gathered} 130 . \\ 94 \end{gathered}$ | $\begin{aligned} & 286, \\ & 290 \end{aligned}$ | $\begin{aligned} & 290, \\ & 294 \end{aligned}$ | $\begin{aligned} & 286, \\ & 290 \end{aligned}$ | $\begin{aligned} & 286, \\ & 294 \end{aligned}$ |  | $\begin{aligned} & 290, \\ & 294 \end{aligned}$ | $\begin{aligned} & 286, \\ & 290 \end{aligned}$ | $\begin{aligned} & 286, \\ & 290 \end{aligned}$ | $\begin{gathered} 286 \\ 294 \end{gathered}$ | $\begin{aligned} & \hline 290, \\ & 298 \end{aligned}$ | $\begin{aligned} & 286 \\ & 298 \end{aligned}$ | $\begin{aligned} & \hline 294, \\ & 298 \end{aligned}$ | $\begin{aligned} & 290, \\ & 294 \end{aligned}$ | $\begin{aligned} & 290, \\ & 294 \end{aligned}$ | $\begin{aligned} & 294, \\ & 294 \end{aligned}$ | $\begin{aligned} & 294, \\ & 294 \end{aligned}$ | $\begin{aligned} & 294, \\ & 294 \end{aligned}$ |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D12S366 | $\begin{gathered} 133 . \\ 33 \end{gathered}$ | $\begin{gathered} 193, \\ 195 \end{gathered}$ | $\begin{gathered} 195, \\ 195 \end{gathered}$ | $\begin{gathered} 195, \\ 195 \end{gathered}$ | $\begin{gathered} 195 \\ 195 \end{gathered}$ |  | $\begin{gathered} 193, \\ 195 \end{gathered}$ | $\begin{gathered} 195, \\ 195 \end{gathered}$ | $\begin{gathered} 195, \\ 195 \end{gathered}$ | $\begin{gathered} 189, \\ 193 \end{gathered}$ | $\begin{gathered} 191, \\ 195 \end{gathered}$ | $\begin{gathered} 189 \\ 189 \end{gathered}$ |  | $\begin{gathered} 189, \\ 193 \end{gathered}$ | $\begin{gathered} 189, \\ 193 \end{gathered}$ |  | $\begin{gathered} 189, \\ 189 \end{gathered}$ | $\begin{aligned} & 189, \\ & 189 \end{aligned}$ | $\begin{gathered} 189, \\ 189 \end{gathered}$ |
| D12S86 | $\begin{gathered} 134 . \\ 54 \end{gathered}$ | $\begin{aligned} & 140, \\ & 146 \end{aligned}$ | $\begin{aligned} & 128, \\ & 144 \end{aligned}$ | $\begin{gathered} 138, \\ 140 \end{gathered}$ | $\begin{aligned} & 138 \\ & 140 \end{aligned}$ | $\begin{aligned} & 140, \\ & 144 \end{aligned}$ | $\begin{aligned} & 138, \\ & 146 \end{aligned}$ | $\begin{aligned} & 140, \\ & 144 \end{aligned}$ | $\begin{aligned} & 140, \\ & 144 \end{aligned}$ | $\begin{aligned} & 142, \\ & 152 \end{aligned}$ | $\begin{aligned} & 128, \\ & 132 \end{aligned}$ | $\begin{aligned} & 132, \\ & 142 \end{aligned}$ | $\begin{gathered} 132, \\ 142 \end{gathered}$ | $\begin{aligned} & 146, \\ & 152 \end{aligned}$ | $\begin{aligned} & 146, \\ & 152 \end{aligned}$ | $\begin{aligned} & 144, \\ & 146 \end{aligned}$ | $\begin{aligned} & 144, \\ & 152 \end{aligned}$ |  |  |
| D12S2078 | $\begin{gathered} 149 . \\ 60 \end{gathered}$ | $\begin{aligned} & 258, \\ & 270 \end{aligned}$ | $\begin{aligned} & 274, \\ & 274 \end{aligned}$ | $\begin{aligned} & 274, \\ & 258 \end{aligned}$ | $\begin{aligned} & 270, \\ & 274 \end{aligned}$ | $\begin{aligned} & 274, \\ & 258 \end{aligned}$ | $\begin{aligned} & 274, \\ & 270 \end{aligned}$ |  |  |  |  |  |  | $\begin{aligned} & 258, \\ & 270 \end{aligned}$ | $\begin{aligned} & 258, \\ & 270 \end{aligned}$ | $\begin{aligned} & 258, \\ & 262 \end{aligned}$ | $\begin{aligned} & 258, \\ & 270 \end{aligned}$ |  |  |
| D12S1659 | $\begin{gathered} 155 . \\ 94 \end{gathered}$ | $\begin{aligned} & 105, \\ & 109 \end{aligned}$ | $\begin{gathered} 109, \\ 109 \end{gathered}$ |  | $\begin{gathered} 109, \\ 109 \end{gathered}$ | $\begin{gathered} 105, \\ 109 \end{gathered}$ | $\begin{gathered} 109, \\ 109 \end{gathered}$ | $\begin{aligned} & 105, \\ & 109 \end{aligned}$ | $\begin{aligned} & 105 \\ & 109 \end{aligned}$ | $\begin{gathered} 101, \\ 109 \end{gathered}$ | $\begin{aligned} & 109, \\ & 109 \end{aligned}$ |  | $\begin{aligned} & 101, \\ & 109 \end{aligned}$ | $\begin{aligned} & 105, \\ & 109 \end{aligned}$ | $\begin{aligned} & 105, \\ & 109 \end{aligned}$ | $\begin{gathered} 109, \\ 109 \end{gathered}$ | $\begin{aligned} & 109, \\ & 109 \end{aligned}$ |  | $\begin{aligned} & 105, \\ & 109 \end{aligned}$ |
| D12S1723 | $\begin{gathered} 164 . \\ 63 \end{gathered}$ | $\begin{gathered} 167, \\ 169 \end{gathered}$ | $\begin{aligned} & 167, \\ & 167 \end{aligned}$ | $\begin{aligned} & 167, \\ & 169 \end{aligned}$ | $\begin{gathered} 167, \\ 169 \end{gathered}$ | $\begin{gathered} 167, \\ 169 \end{gathered}$ | $\begin{gathered} 167, \\ 169 \end{gathered}$ | $\begin{aligned} & 167, \\ & 169 \end{aligned}$ | $\begin{gathered} 167, \\ 167 \end{gathered}$ | $\begin{gathered} 167, \\ 169 \end{gathered}$ | $\begin{aligned} & 173, \\ & 175 \end{aligned}$ | $\begin{gathered} 167, \\ 175 \end{gathered}$ | $\begin{gathered} 167, \\ 175 \end{gathered}$ | $\begin{aligned} & 167, \\ & 169 \end{aligned}$ | $\begin{aligned} & 167, \\ & 169 \end{aligned}$ | $\begin{gathered} 167, \\ 175 \end{gathered}$ | $\begin{gathered} 169, \\ 175 \end{gathered}$ | $\begin{aligned} & 167, \\ & 175 \end{aligned}$ | $\begin{aligned} & 167, \\ & 175 \end{aligned}$ |
| D13S175 | 6.03 | $\begin{gathered} 103, \\ 105 \end{gathered}$ | $\begin{aligned} & \hline 101, \\ & 103 \end{aligned}$ | $\begin{gathered} 103, \\ 103 \end{gathered}$ | $\begin{aligned} & 101, \\ & 105 \end{aligned}$ |  | $\begin{gathered} 103, \\ 105 \end{gathered}$ | $\begin{gathered} 101, \\ 103 \end{gathered}$ | $\begin{gathered} 103, \\ 103 \end{gathered}$ | $\begin{gathered} 105, \\ 109 \end{gathered}$ | $\begin{gathered} 107, \\ 107 \end{gathered}$ | $\begin{gathered} 107, \\ 109 \end{gathered}$ | $\begin{gathered} 107, \\ 109 \end{gathered}$ | $\begin{gathered} 103, \\ 105 \end{gathered}$ | $\begin{gathered} 103, \\ 103 \end{gathered}$ | $\begin{gathered} 103, \\ 103 \end{gathered}$ | $\begin{gathered} 103, \\ 103 \end{gathered}$ | $\begin{aligned} & 103, \\ & 103 \end{aligned}$ | $\begin{gathered} 103, \\ 103 \end{gathered}$ |
| D13S1493 | $\begin{gathered} 25.8 \\ 0 \end{gathered}$ | $\begin{aligned} & 231, \\ & 243 \end{aligned}$ | $\begin{aligned} & 223, \\ & 235 \end{aligned}$ | $\begin{aligned} & 231, \\ & 235 \end{aligned}$ | $\begin{aligned} & 223, \\ & 231 \end{aligned}$ | $\begin{aligned} & 235, \\ & 243 \end{aligned}$ | $\begin{aligned} & 235, \\ & 243 \end{aligned}$ |  |  | $\begin{aligned} & \hline 231, \\ & 243 \end{aligned}$ | $\begin{aligned} & 223, \\ & 243 \end{aligned}$ | $\begin{aligned} & 243, \\ & 243 \end{aligned}$ | $\begin{aligned} & 243, \\ & 243 \end{aligned}$ | $\begin{aligned} & 231, \\ & 243 \end{aligned}$ | $\begin{aligned} & 231, \\ & 243 \end{aligned}$ | $\begin{aligned} & 231, \\ & 243 \end{aligned}$ | $\begin{aligned} & 231, \\ & 243 \end{aligned}$ |  |  |
| D13S218 | $\begin{gathered} 32.9 \\ 0 \end{gathered}$ | $\begin{aligned} & \hline 191, \\ & 191 \end{aligned}$ | $\begin{aligned} & 191, \\ & 197 \end{aligned}$ | $\begin{gathered} 191, \\ 197 \end{gathered}$ |  | $\begin{gathered} 191, \\ 197 \end{gathered}$ | $\begin{gathered} 191, \\ 197 \end{gathered}$ | $\begin{gathered} 191, \\ 191 \end{gathered}$ | $\begin{aligned} & \hline 191, \\ & 191 \end{aligned}$ | $\begin{gathered} 191, \\ 191 \end{gathered}$ | $\begin{gathered} 189, \\ 189 \end{gathered}$ | $\begin{gathered} 189, \\ 191 \end{gathered}$ | $\begin{gathered} 189, \\ 191 \end{gathered}$ | $\begin{gathered} 189, \\ 191 \end{gathered}$ | $\begin{gathered} 191, \\ 191 \end{gathered}$ | $\begin{gathered} 191, \\ 195 \end{gathered}$ |  | $\begin{aligned} & 191, \\ & 195 \end{aligned}$ | $\begin{gathered} 191, \\ 195 \end{gathered}$ |
| D13S800 | $\begin{gathered} 55.3 \\ 1 \end{gathered}$ | $\begin{aligned} & 299, \\ & 303 \end{aligned}$ | $\begin{aligned} & 307, \\ & 311 \end{aligned}$ | $\begin{aligned} & 299 \\ & 307 \end{aligned}$ | $\begin{gathered} 303, \\ 311 \end{gathered}$ | $\begin{aligned} & 303, \\ & 311 \end{aligned}$ | $\begin{gathered} 299 \\ 311 \end{gathered}$ |  |  | $\begin{aligned} & 303, \\ & 311 \end{aligned}$ | $\begin{gathered} 307, \\ 307 \end{gathered}$ | $\begin{gathered} 303, \\ 307 \end{gathered}$ | $\begin{aligned} & 307, \\ & 311 \end{aligned}$ | $\begin{aligned} & 307, \\ & 311 \end{aligned}$ | $\begin{aligned} & 303, \\ & 303 \end{aligned}$ | $\begin{aligned} & 311, \\ & 323 \end{aligned}$ | $\begin{aligned} & 303, \\ & 323 \end{aligned}$ |  |  |
| D13S156 | $\begin{gathered} 55.8 \\ 5 \end{gathered}$ | $\begin{aligned} & 280, \\ & 280 \end{aligned}$ | $\begin{aligned} & 280, \\ & 282 \end{aligned}$ | $\begin{aligned} & 280, \\ & 280 \end{aligned}$ | $\begin{aligned} & 280, \\ & 282 \end{aligned}$ | $\begin{aligned} & 280, \\ & 282 \end{aligned}$ | $\begin{aligned} & 280, \\ & 282 \end{aligned}$ | $\begin{aligned} & 280, \\ & 280 \end{aligned}$ |  | $\begin{aligned} & 276 \\ & 278 \end{aligned}$ | $\begin{aligned} & 278, \\ & 280 \end{aligned}$ | $\begin{aligned} & 278, \\ & 280 \end{aligned}$ | $\begin{aligned} & 278, \\ & 280 \end{aligned}$ | $\begin{aligned} & 276 \\ & 278 \end{aligned}$ | $\begin{aligned} & 278, \\ & 280 \end{aligned}$ |  | $\begin{aligned} & 280, \\ & 282 \end{aligned}$ | $\begin{aligned} & 280, \\ & 282 \end{aligned}$ | $\begin{aligned} & 278, \\ & 280 \end{aligned}$ |
| D13S170 | $\begin{gathered} 63.9 \\ 0 \end{gathered}$ | $\begin{gathered} 125, \\ 127 \end{gathered}$ | $\begin{aligned} & 127, \\ & 127 \end{aligned}$ | $\begin{aligned} & 127 \\ & 127 \end{aligned}$ | $\begin{gathered} 125, \\ 127 \end{gathered}$ | $\begin{gathered} 125, \\ 127 \end{gathered}$ | $\begin{aligned} & 127, \\ & 127 \end{aligned}$ | $\begin{aligned} & 127 \\ & 127 \end{aligned}$ | $\begin{gathered} 125, \\ 127 \end{gathered}$ | $\begin{gathered} 117, \\ 121 \end{gathered}$ | $\begin{aligned} & 123, \\ & 125 \end{aligned}$ |  | $\begin{gathered} 121, \\ 123 \end{gathered}$ | $\begin{aligned} & 121, \\ & 125 \end{aligned}$ | $\begin{aligned} & 117, \\ & 121 \end{aligned}$ | $\begin{gathered} 125, \\ 131 \end{gathered}$ | $\begin{aligned} & 117, \\ & 125 \end{aligned}$ | $\begin{aligned} & 117, \\ & 125 \end{aligned}$ |  |
| D13S793 | $\begin{gathered} 76.2 \\ 6 \end{gathered}$ | $\begin{aligned} & 257, \\ & 261 \end{aligned}$ | $\begin{aligned} & 257, \\ & 265 \end{aligned}$ | $\begin{aligned} & 257, \\ & 257 \end{aligned}$ | $\begin{aligned} & 261, \\ & 265 \end{aligned}$ | $\begin{aligned} & 257, \\ & 261 \end{aligned}$ | $\begin{aligned} & 257, \\ & 265 \end{aligned}$ |  |  | $\begin{aligned} & 257, \\ & 265 \end{aligned}$ | $\begin{aligned} & 265, \\ & 269 \end{aligned}$ | $\begin{aligned} & 265, \\ & 265 \end{aligned}$ | $\begin{aligned} & 265, \\ & 265 \end{aligned}$ | $\begin{aligned} & 265, \\ & 257 \end{aligned}$ | $\begin{aligned} & 261, \\ & 265 \end{aligned}$ | $\begin{aligned} & 257, \\ & 261 \end{aligned}$ | $\begin{aligned} & 261, \\ & 261 \end{aligned}$ |  |  |
| D13S779 | $\begin{gathered} 82.9 \\ 3 \end{gathered}$ | $\begin{gathered} 189, \\ 195 \end{gathered}$ | $\begin{aligned} & 195, \\ & 180 \end{aligned}$ | $\begin{aligned} & \hline 180, \\ & 189 \end{aligned}$ | $\begin{gathered} 195 \\ 195 \end{gathered}$ | $\begin{gathered} 180, \\ 195 \end{gathered}$ | $\begin{gathered} 189, \\ 195 \end{gathered}$ |  |  | $\begin{gathered} 189, \\ 189 \end{gathered}$ | $\begin{array}{r} 189, \\ 195 \end{array}$ | $\begin{gathered} 189, \\ 195 \end{gathered}$ | $\begin{gathered} 189, \\ 195 \end{gathered}$ | $\begin{gathered} 180, \\ 195 \end{gathered}$ | $\begin{aligned} & 189, \\ & 195 \end{aligned}$ | $\begin{aligned} & 192, \\ & 192 \end{aligned}$ | $\begin{aligned} & 192, \\ & 195 \end{aligned}$ |  |  |
| D14S283 | $\begin{gathered} 13.8 \\ 9 \end{gathered}$ | $\begin{aligned} & 137 \\ & 145 \end{aligned}$ | $\begin{aligned} & 131, \\ & 147 \end{aligned}$ | $\begin{aligned} & 131, \\ & 145 \end{aligned}$ | 131 | $\begin{gathered} 145 \\ 147 \end{gathered}$ | $\begin{aligned} & 145 \\ & 147 \end{aligned}$ | $\begin{aligned} & 145 \\ & 147 \end{aligned}$ | $\begin{aligned} & 145 \\ & 147 \end{aligned}$ | $\begin{gathered} 137 \\ 145 \end{gathered}$ | $\begin{gathered} 133 \\ 133 \end{gathered}$ | $\begin{aligned} & 133, \\ & 145 \end{aligned}$ | $\begin{aligned} & 133, \\ & 137 \end{aligned}$ | $\begin{aligned} & 125 \\ & 137 \end{aligned}$ | $\begin{aligned} & 145 \\ & 145 \end{aligned}$ | $\begin{aligned} & 143 \\ & 145 \end{aligned}$ | $\begin{aligned} & 143 \\ & 145 \end{aligned}$ | $\begin{aligned} & 143 \\ & 145 \end{aligned}$ | $\begin{aligned} & 143 \\ & 145 \end{aligned}$ |


| D14S70 | $\begin{gathered} 14.1 \\ 1 \end{gathered}$ | $\begin{array}{\|l} \hline 210, \\ 210 \end{array}$ | $\begin{aligned} & 214, \\ & 214 \end{aligned}$ | $\begin{aligned} & 210, \\ & 214 \end{aligned}$ | $\begin{aligned} & 210, \\ & 214 \end{aligned}$ | $\begin{aligned} & 210, \\ & 214 \end{aligned}$ | $\begin{aligned} & 210, \\ & 214 \end{aligned}$ |  |  | $\begin{aligned} & 210, \\ & 214 \end{aligned}$ | $\begin{aligned} & 210, \\ & 214 \end{aligned}$ | $\begin{aligned} & 210, \\ & 210 \end{aligned}$ | $\begin{aligned} & 210, \\ & 214 \end{aligned}$ | $\begin{aligned} & 210, \\ & 212 \end{aligned}$ | $\begin{aligned} & 210, \\ & 212 \end{aligned}$ | $\begin{aligned} & 212, \\ & 216 \end{aligned}$ |  | $\begin{aligned} & 210, \\ & 216 \end{aligned}$ | $\begin{gathered} 210, \\ 216 \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D14S608 | $\begin{gathered} 28.0 \\ 1 \end{gathered}$ | $\begin{aligned} & 200, \\ & 200 \end{aligned}$ | $\begin{aligned} & 200, \\ & 220 \end{aligned}$ | $\begin{aligned} & 200, \\ & 220 \end{aligned}$ | $\begin{aligned} & 200, \\ & 220 \end{aligned}$ | $\begin{aligned} & 200, \\ & 200 \end{aligned}$ | $\begin{aligned} & 200, \\ & 220 \end{aligned}$ |  |  | $\begin{aligned} & 200, \\ & 200 \end{aligned}$ | $\begin{aligned} & 200, \\ & 224 \end{aligned}$ | $\begin{aligned} & 200, \\ & 224 \end{aligned}$ | $\begin{aligned} & 200, \\ & 200 \end{aligned}$ | $\begin{aligned} & 200, \\ & 224 \end{aligned}$ | $\begin{aligned} & \hline 200, \\ & 200 \end{aligned}$ | $\begin{aligned} & 200, \\ & 216 \end{aligned}$ | $\begin{aligned} & 200, \\ & 200 \end{aligned}$ |  |  |
| D14S599 | $\begin{gathered} 40.6 \\ 8 \end{gathered}$ | $\begin{array}{\|c\|} \hline 90,9 \\ 0 \end{array}$ | $\begin{gathered} 90,8 \\ 4 \end{gathered}$ | $\begin{gathered} 90,9 \\ 0 \end{gathered}$ | $\begin{gathered} 90,9 \\ 0 \end{gathered}$ | $\begin{gathered} 90,8 \\ 4 \end{gathered}$ | $\begin{gathered} 90,9 \\ 0 \end{gathered}$ |  |  | $\begin{array}{\|c\|} \hline 90,9 \\ 0 \end{array}$ | $\begin{gathered} 84,9 \\ 0 \end{gathered}$ | $\begin{gathered} 90,8 \\ 4 \end{gathered}$ | $\begin{gathered} 90,9 \\ 0 \end{gathered}$ | $\begin{gathered} 90,9 \\ 0 \end{gathered}$ | $\begin{gathered} 90,9 \\ 0 \end{gathered}$ | $\begin{gathered} 96,9 \\ 6 \end{gathered}$ | $\begin{gathered} 90,9 \\ 6 \end{gathered}$ |  |  |
| D14S587 | $\begin{gathered} 55.8 \\ 2 \end{gathered}$ | $\begin{aligned} & 274, \\ & 274 \end{aligned}$ | $\begin{aligned} & 250, \\ & 254 \end{aligned}$ | $\begin{aligned} & 254, \\ & 274 \end{aligned}$ | $\begin{aligned} & 250 \\ & 274 \end{aligned}$ | $\begin{aligned} & 250, \\ & 274 \end{aligned}$ | $\begin{aligned} & 254, \\ & 274 \end{aligned}$ |  |  | $\begin{aligned} & 254, \\ & 274 \end{aligned}$ | $\begin{aligned} & 250, \\ & 254 \end{aligned}$ | $\begin{aligned} & 254, \\ & 250 \end{aligned}$ | $\begin{aligned} & 254, \\ & 254 \end{aligned}$ | $\begin{aligned} & 254, \\ & 274 \end{aligned}$ | $\begin{aligned} & 254, \\ & 266 \end{aligned}$ | $\begin{aligned} & 254, \\ & 266 \end{aligned}$ | $\begin{aligned} & \hline 266, \\ & 254 \end{aligned}$ |  |  |
| D14S592 | $\begin{gathered} 66.8 \\ 0 \end{gathered}$ | $\begin{aligned} & 228, \\ & 237 \end{aligned}$ | $\begin{aligned} & 237, \\ & 240 \end{aligned}$ | $\begin{aligned} & 237, \\ & 237 \end{aligned}$ | $\begin{gathered} 237, \\ 240 \end{gathered}$ | $\begin{gathered} 237, \\ 240 \end{gathered}$ | $\begin{aligned} & 237, \\ & 237 \end{aligned}$ |  |  | $\begin{aligned} & 228, \\ & 237 \end{aligned}$ | $\begin{aligned} & 231, \\ & 234 \end{aligned}$ | $\begin{aligned} & 234, \\ & 237 \end{aligned}$ | $\begin{aligned} & 228, \\ & 231 \end{aligned}$ | $\begin{aligned} & 228, \\ & 237 \end{aligned}$ | $\begin{aligned} & \hline 228, \\ & 234 \end{aligned}$ | $\begin{aligned} & 234, \\ & 237 \end{aligned}$ | $\begin{aligned} & 234, \\ & 237 \end{aligned}$ |  |  |
| D14S63 | $\begin{gathered} 69.1 \\ 8 \end{gathered}$ | $\begin{aligned} & 205, \\ & 209 \end{aligned}$ | $\begin{aligned} & 205 \\ & 215 \end{aligned}$ | $\begin{aligned} & 205 \\ & 215 \end{aligned}$ | $\begin{aligned} & 205, \\ & 205 \end{aligned}$ | $\begin{aligned} & 205, \\ & 215 \end{aligned}$ | $\begin{gathered} 205 \\ 205 \end{gathered}$ | $\begin{aligned} & 205 \\ & 205 \end{aligned}$ | $\begin{aligned} & 205 \\ & 205 \end{aligned}$ | $\begin{aligned} & 209, \\ & 211 \end{aligned}$ |  | $\begin{aligned} & 211, \\ & 215 \end{aligned}$ | $\begin{aligned} & 209 \\ & 215 \end{aligned}$ | $\begin{aligned} & 205, \\ & 209 \end{aligned}$ |  | $\begin{aligned} & 205 \\ & 207 \end{aligned}$ | $\begin{aligned} & 207, \\ & 207 \end{aligned}$ | $\begin{aligned} & 205, \\ & 211 \end{aligned}$ | $\begin{aligned} & 205, \\ & 211 \end{aligned}$ |
| D14S588 | $\begin{gathered} 75.6 \\ 1 \end{gathered}$ | $\begin{aligned} & 125, \\ & 133 \end{aligned}$ | $\begin{aligned} & 121, \\ & 125 \end{aligned}$ | $\begin{aligned} & 121, \\ & 125 \end{aligned}$ | $\begin{gathered} 125, \\ 125 \end{gathered}$ | $\begin{gathered} 125 \\ 125 \end{gathered}$ | $\begin{gathered} 121, \\ 133 \end{gathered}$ |  |  | $\begin{aligned} & 129, \\ & 1333 \end{aligned}$ | $\begin{aligned} & 121, \\ & 129 \end{aligned}$ | $\begin{gathered} 129, \\ 129 \end{gathered}$ | $\begin{gathered} 121, \\ 133 \end{gathered}$ | $\begin{gathered} 133, \\ 125 \end{gathered}$ | $\begin{aligned} & 129, \\ & 137 \end{aligned}$ | $\begin{aligned} & 121 \\ & 125 \end{aligned}$ | $\begin{aligned} & 121, \\ & 137 \end{aligned}$ |  |  |
| D14S258 | $\begin{gathered} 76.2 \\ 8 \end{gathered}$ | $\begin{aligned} & 170, \\ & 178 \end{aligned}$ | $\begin{aligned} & 172, \\ & 174 \end{aligned}$ | $\begin{aligned} & 170, \\ & 174 \end{aligned}$ | $\begin{gathered} 170, \\ 172 \end{gathered}$ | $\begin{aligned} & 170, \\ & 172 \end{aligned}$ | $\begin{gathered} 174, \\ 178 \end{gathered}$ | $\begin{aligned} & 170, \\ & 172 \end{aligned}$ | $\begin{aligned} & 172, \\ & 178 \end{aligned}$ | $\begin{aligned} & 174, \\ & 178 \end{aligned}$ | $\begin{gathered} 172, \\ 178 \end{gathered}$ | $\begin{aligned} & 174, \\ & 178 \end{aligned}$ | $\begin{aligned} & 172, \\ & 178 \end{aligned}$ | $\begin{aligned} & 170, \\ & 178 \end{aligned}$ | $\begin{aligned} & 174, \\ & 174 \end{aligned}$ | $\begin{aligned} & 170, \\ & 178 \end{aligned}$ | $\begin{aligned} & 170, \\ & 174 \end{aligned}$ |  | $\begin{aligned} & 174, \\ & 178 \end{aligned}$ |
| D14S53 | $\begin{gathered} 86.2 \\ 9 \end{gathered}$ | $\begin{gathered} 149, \\ 151 \end{gathered}$ | $\begin{gathered} 149, \\ 151 \end{gathered}$ | $\begin{aligned} & 149, \\ & 151 \end{aligned}$ | $\begin{gathered} 151, \\ 151 \end{gathered}$ | $\begin{gathered} 151, \\ 151 \end{gathered}$ | $\begin{gathered} 149, \\ 149 \end{gathered}$ |  |  | $\begin{aligned} & \hline 149, \\ & 151 \end{aligned}$ | $\begin{gathered} 149 \\ 151 \end{gathered}$ | $\begin{gathered} 149, \\ 151 \end{gathered}$ | $\begin{aligned} & \hline 149, \\ & 149 \end{aligned}$ | $\begin{aligned} & 149, \\ & 153 \end{aligned}$ | $\begin{aligned} & \hline 143, \\ & 151 \end{aligned}$ | $\begin{gathered} 143, \\ 151 \end{gathered}$ | $\begin{aligned} & 143, \\ & 151 \end{aligned}$ |  |  |
| D14S1279 | $\begin{gathered} 95.8 \\ 9 \end{gathered}$ | $\begin{aligned} & 131, \\ & 135 \end{aligned}$ | $\begin{aligned} & 131, \\ & 135 \end{aligned}$ | $\begin{aligned} & 131, \\ & 135 \end{aligned}$ | $\begin{gathered} 131, \\ 135 \end{gathered}$ | $\begin{gathered} 135, \\ 135 \end{gathered}$ | $\begin{gathered} 131, \\ 131 \end{gathered}$ |  |  | $\begin{gathered} 131, \\ 135 \end{gathered}$ | $\begin{aligned} & 131, \\ & 135 \end{aligned}$ | $\begin{gathered} 135, \\ 135 \end{gathered}$ | $\begin{aligned} & 131, \\ & 135 \end{aligned}$ | $\begin{aligned} & 131, \\ & 135 \end{aligned}$ | $\begin{aligned} & 135, \\ & 127 \end{aligned}$ | $\begin{aligned} & 131, \\ & 131 \end{aligned}$ | $\begin{aligned} & 127, \\ & 131 \end{aligned}$ |  |  |
| D14S65 | $\begin{gathered} 117 . \\ 30 \end{gathered}$ | $\begin{aligned} & 135 \\ & 145 \end{aligned}$ | $\begin{aligned} & 147, \\ & 149 \end{aligned}$ | $\begin{aligned} & 145 \\ & 147 \end{aligned}$ | $\begin{gathered} 135 \\ 147 \end{gathered}$ |  | $\begin{gathered} 145 \\ 149 \end{gathered}$ | $\begin{gathered} 135 \\ 149 \end{gathered}$ |  | $\begin{aligned} & 145 \\ & 147 \end{aligned}$ | $\begin{aligned} & 141, \\ & 147 \end{aligned}$ | $\begin{aligned} & 145 \\ & 147 \end{aligned}$ | $\begin{aligned} & 141, \\ & 147 \end{aligned}$ | $\begin{aligned} & 143 \\ & 147 \end{aligned}$ | $\begin{gathered} 135 \\ 145 \end{gathered}$ | $\begin{gathered} 141, \\ 141 \end{gathered}$ | $\begin{aligned} & 135 \\ & 141 \end{aligned}$ | $\begin{aligned} & 141, \\ & 145 \end{aligned}$ | $\begin{aligned} & 141 \\ & 145 \end{aligned}$ |
| D14S985 | $\begin{gathered} 126 . \\ 61 \end{gathered}$ | $\begin{aligned} & 136 \\ & 142 \end{aligned}$ | $\begin{aligned} & 138, \\ & 142 \end{aligned}$ | $\begin{aligned} & 136, \\ & 138 \end{aligned}$ | $\begin{gathered} 136, \\ 142 \end{gathered}$ | $\begin{gathered} 136, \\ 138 \end{gathered}$ | $\begin{gathered} 136 \\ 142 \end{gathered}$ | $\begin{aligned} & 142, \\ & 142 \end{aligned}$ | $\begin{aligned} & 138 \\ & 142 \end{aligned}$ | $\begin{gathered} 136, \\ 142 \end{gathered}$ | $\begin{aligned} & 134, \\ & 136 \end{aligned}$ | $\begin{aligned} & 134, \\ & 136 \end{aligned}$ | $\begin{gathered} 136, \\ 142 \end{gathered}$ | $\begin{aligned} & 142, \\ & 142 \end{aligned}$ |  | $\begin{gathered} 138 \\ 138 \end{gathered}$ | $\begin{aligned} & 138, \\ & 142 \end{aligned}$ |  | $\begin{aligned} & 138, \\ & 142 \end{aligned}$ |
| D15S128 | 6.11 | $\begin{aligned} & 201, \\ & 203 \end{aligned}$ | $\begin{aligned} & 199, \\ & 201 \end{aligned}$ | $\begin{aligned} & 201, \\ & 203 \end{aligned}$ | $\begin{aligned} & 199, \\ & 203 \end{aligned}$ | $\begin{aligned} & 199, \\ & 201 \end{aligned}$ | $\begin{aligned} & 199, \\ & 203 \end{aligned}$ | $\begin{aligned} & 199 \\ & 201 \end{aligned}$ | $\begin{aligned} & 201, \\ & 201 \end{aligned}$ | $\begin{aligned} & 199, \\ & 203 \end{aligned}$ | $\begin{gathered} 201, \\ 201 \end{gathered}$ | $\begin{aligned} & 201, \\ & 203 \end{aligned}$ | $\begin{aligned} & \hline 201, \\ & 203 \end{aligned}$ | $\begin{aligned} & 201, \\ & 203 \end{aligned}$ | $\begin{aligned} & 201, \\ & 201 \end{aligned}$ | $\begin{aligned} & 201, \\ & 207 \end{aligned}$ |  | $\begin{aligned} & 201, \\ & 201 \end{aligned}$ | $\begin{gathered} 201, \\ 207 \end{gathered}$ |
| D15S1002 | $\begin{gathered} 14.5 \\ 8 \end{gathered}$ | $\begin{gathered} 105, \\ 107 \end{gathered}$ | $\begin{aligned} & \hline 119, \\ & 119 \end{aligned}$ | $\begin{aligned} & 105, \\ & 119 \end{aligned}$ | $\begin{aligned} & \hline 107, \\ & 119 \end{aligned}$ | $\begin{gathered} 107, \\ 119 \end{gathered}$ | $\begin{gathered} 105 \\ 119 \end{gathered}$ | $\begin{aligned} & 107 \\ & 119 \end{aligned}$ | $\begin{aligned} & \hline 107, \\ & 119 \end{aligned}$ | $\begin{aligned} & 107, \\ & 111 \end{aligned}$ | $\begin{aligned} & 119, \\ & 121 \end{aligned}$ | $\begin{aligned} & \hline 107, \\ & 119 \end{aligned}$ | $\begin{gathered} 107, \\ 121 \end{gathered}$ | $\begin{aligned} & 107, \\ & 111 \end{aligned}$ | $\begin{aligned} & 107, \\ & 107 \end{aligned}$ | $\begin{gathered} 107 \\ 119 \end{gathered}$ | $\begin{gathered} 107, \\ 119 \end{gathered}$ | $\begin{aligned} & \hline 107, \\ & 119 \end{aligned}$ | $\begin{gathered} 107, \\ 119 \end{gathered}$ |
| D15S1007 | $\begin{gathered} 25.8 \\ 6 \end{gathered}$ | $\begin{gathered} 175, \\ 179 \end{gathered}$ | $\begin{aligned} & 177, \\ & 185 \end{aligned}$ | $\begin{aligned} & 175, \\ & 185 \end{aligned}$ | $\begin{aligned} & 179, \\ & 185 \end{aligned}$ | $\begin{aligned} & 179, \\ & 185 \end{aligned}$ |  | $\begin{aligned} & 179 \\ & 185 \end{aligned}$ | $\begin{aligned} & 177, \\ & 179 \end{aligned}$ | $\begin{gathered} 165, \\ 177 \end{gathered}$ | $\begin{aligned} & 173, \\ & 177 \end{aligned}$ | $\begin{gathered} 165, \\ 173 \end{gathered}$ | $\begin{aligned} & 173, \\ & 177 \end{aligned}$ | $\begin{aligned} & 165, \\ & 177 \end{aligned}$ |  | $\begin{aligned} & 179 \\ & 187 \end{aligned}$ | $\begin{aligned} & 179, \\ & 179 \end{aligned}$ | $\begin{aligned} & 179, \\ & 187 \end{aligned}$ | $\begin{gathered} 177, \\ 179 \end{gathered}$ |


| D15S994 | $\begin{gathered} 40.2 \\ 5 \end{gathered}$ | $\begin{aligned} & 206, \\ & 218 \end{aligned}$ | $\begin{aligned} & 210, \\ & 212 \end{aligned}$ | $\begin{aligned} & 206, \\ & 210 \end{aligned}$ | $\begin{aligned} & 212, \\ & 218 \end{aligned}$ | $\begin{aligned} & 212, \\ & 218 \end{aligned}$ | $\begin{aligned} & 212, \\ & 218 \end{aligned}$ |  |  | $\begin{aligned} & 204, \\ & 218 \end{aligned}$ | $\begin{aligned} & 204, \\ & 206 \end{aligned}$ | $\begin{aligned} & \hline 206 \\ & 218 \end{aligned}$ | $\begin{aligned} & 204, \\ & 206 \end{aligned}$ | $\begin{aligned} & 204, \\ & 218 \end{aligned}$ | $\begin{aligned} & 204, \\ & 210 \end{aligned}$ |  | $\begin{aligned} & 204, \\ & 210 \end{aligned}$ | $\begin{aligned} & 206, \\ & 210 \end{aligned}$ | $\begin{aligned} & 204, \\ & 210 \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D15S978 | $\begin{gathered} 45.6 \\ 2 \end{gathered}$ | $\begin{aligned} & 221, \\ & 237 \end{aligned}$ | $\begin{aligned} & 221, \\ & 225 \end{aligned}$ | $\begin{aligned} & 221, \\ & 221 \end{aligned}$ | $\begin{aligned} & 225, \\ & 237 \end{aligned}$ | $\begin{aligned} & 225, \\ & 237 \end{aligned}$ | $\begin{aligned} & 225, \\ & 237 \end{aligned}$ | $\begin{aligned} & 225, \\ & 237 \end{aligned}$ | $\begin{aligned} & 221, \\ & 221 \end{aligned}$ | $\begin{aligned} & 225, \\ & 237 \end{aligned}$ | $\begin{aligned} & 225, \\ & 237 \end{aligned}$ |  | $\begin{aligned} & 225, \\ & 225 \end{aligned}$ | $\begin{aligned} & 225, \\ & 237 \end{aligned}$ | $\begin{aligned} & 225, \\ & 231 \end{aligned}$ | $\begin{aligned} & 225, \\ & 241 \end{aligned}$ | $\begin{aligned} & 225, \\ & 241 \end{aligned}$ | $\begin{aligned} & 225, \\ & 231 \end{aligned}$ |  |
| D15S117 | $\begin{gathered} 51.2 \\ 1 \end{gathered}$ | $\begin{aligned} & 146, \\ & 148 \end{aligned}$ | $\begin{aligned} & 140, \\ & 148 \end{aligned}$ | $\begin{aligned} & 146, \\ & 148 \end{aligned}$ | $\begin{gathered} 140, \\ 148 \end{gathered}$ |  | $\begin{aligned} & 140 \\ & 148 \end{aligned}$ | $\begin{aligned} & 140, \\ & 148 \end{aligned}$ | $\begin{aligned} & 140, \\ & 146 \end{aligned}$ | $\begin{gathered} 134, \\ 148 \end{gathered}$ | $\begin{gathered} 134, \\ 146 \end{gathered}$ | $\begin{gathered} 146, \\ 148 \end{gathered}$ | $\begin{gathered} 134, \\ 134 \end{gathered}$ | $\begin{gathered} 134, \\ 138 \end{gathered}$ | $\begin{gathered} 134, \\ 138 \end{gathered}$ | $\begin{gathered} 138, \\ 138 \end{gathered}$ | $\begin{gathered} 134, \\ 138 \end{gathered}$ | $\begin{aligned} & 138 \\ & 138 \end{aligned}$ | $\begin{gathered} 134, \\ 138 \end{gathered}$ |
| D15S1507 | $\begin{gathered} 60.1 \\ 7 \end{gathered}$ | $\begin{aligned} & 208, \\ & 212 \end{aligned}$ | $\begin{aligned} & 204, \\ & 208 \end{aligned}$ | $\begin{aligned} & 204, \\ & 212 \end{aligned}$ | $\begin{gathered} 208, \\ 208 \end{gathered}$ | $\begin{aligned} & \hline 208, \\ & 208 \end{aligned}$ | $\begin{gathered} 208, \\ 204 \end{gathered}$ |  |  | $\begin{aligned} & 204, \\ & 208 \end{aligned}$ | $\begin{aligned} & 208, \\ & 212 \end{aligned}$ | $\begin{aligned} & 204, \\ & 212 \end{aligned}$ | $\begin{aligned} & 204, \\ & 208 \end{aligned}$ | $\begin{aligned} & 204, \\ & 220 \end{aligned}$ | $\begin{aligned} & 204, \\ & 220 \end{aligned}$ | $\begin{aligned} & 204, \\ & 208 \end{aligned}$ | $\begin{aligned} & 204, \\ & 208 \end{aligned}$ |  |  |
| D15S1015 | $\begin{gathered} 66.9 \\ 0 \end{gathered}$ | $\begin{gathered} 103, \\ 105 \end{gathered}$ | $\begin{aligned} & 103, \\ & 103 \end{aligned}$ | $\begin{aligned} & 103, \\ & 105 \end{aligned}$ | $\begin{aligned} & 103 \\ & 103 \end{aligned}$ | $\begin{aligned} & 103, \\ & 103 \end{aligned}$ | $\begin{gathered} 103 \\ 103 \end{gathered}$ |  |  | $\begin{gathered} 103, \\ 103 \end{gathered}$ | $\begin{aligned} & 103 \\ & 105 \end{aligned}$ | $\begin{aligned} & 103 \\ & 105 \end{aligned}$ | $\begin{gathered} 103, \\ 103 \end{gathered}$ | $\begin{aligned} & 103 \\ & 103 \end{aligned}$ | $\begin{gathered} 103, \\ 103 \end{gathered}$ | $\begin{gathered} 103, \\ 103 \end{gathered}$ | $\begin{gathered} 103, \\ 103 \end{gathered}$ |  |  |
| D15S153 | $\begin{gathered} 68.1 \\ 0 \end{gathered}$ | $\begin{aligned} & 216, \\ & 222 \end{aligned}$ | $\begin{aligned} & \hline 212, \\ & 214 \end{aligned}$ | $\begin{aligned} & 212, \\ & 222 \end{aligned}$ | $\begin{aligned} & 214, \\ & 216 \end{aligned}$ | $\begin{aligned} & \hline 214, \\ & 216 \end{aligned}$ | $\begin{gathered} 214 \\ 216 \end{gathered}$ |  |  | $\begin{aligned} & \hline 216, \\ & 218 \end{aligned}$ | $\begin{aligned} & 218 \\ & 216 \end{aligned}$ | $\begin{aligned} & 216 \\ & 218 \end{aligned}$ | $\begin{aligned} & 218, \\ & 218 \end{aligned}$ | $\begin{aligned} & 212, \\ & 218 \end{aligned}$ | $\begin{aligned} & 212, \\ & 218 \end{aligned}$ | $\begin{aligned} & 212, \\ & 216 \end{aligned}$ | $\begin{aligned} & 212, \\ & 218 \end{aligned}$ |  |  |
| D15S131 | $\begin{gathered} 71.2 \\ 8 \end{gathered}$ | $\begin{aligned} & 260, \\ & 262 \end{aligned}$ | $\begin{aligned} & 260, \\ & 260 \end{aligned}$ | $\begin{aligned} & 260, \\ & 260 \end{aligned}$ | $\begin{aligned} & 260, \\ & 260 \end{aligned}$ | $\begin{aligned} & 260, \\ & 260 \end{aligned}$ | $\begin{aligned} & 260, \\ & 262 \end{aligned}$ |  |  | $\begin{aligned} & 256, \\ & 260 \end{aligned}$ | $\begin{gathered} 240, \\ 258 \end{gathered}$ | $\begin{aligned} & 240, \\ & 256 \end{aligned}$ | $\begin{aligned} & 256 \\ & 258 \end{aligned}$ | $\begin{aligned} & 240, \\ & 256 \end{aligned}$ | $\begin{aligned} & 240, \\ & 262 \end{aligned}$ | $\begin{aligned} & 254, \\ & 262 \end{aligned}$ | $\begin{aligned} & 262, \\ & 262 \end{aligned}$ |  |  |
| D15S211 | $\begin{gathered} 75.8 \\ 5 \end{gathered}$ | $\begin{aligned} & 225, \\ & 231 \end{aligned}$ | $\begin{aligned} & 229, \\ & 235 \end{aligned}$ | $\begin{aligned} & 231, \\ & 235 \end{aligned}$ | $\begin{aligned} & 229, \\ & 231 \end{aligned}$ | $\begin{aligned} & 229, \\ & 231 \end{aligned}$ | $\begin{aligned} & 225, \\ & 229 \end{aligned}$ |  |  | $\begin{aligned} & 227, \\ & 231 \end{aligned}$ | $\begin{aligned} & 229, \\ & 252 \end{aligned}$ | $\begin{aligned} & 227, \\ & 252 \end{aligned}$ | $\begin{aligned} & 227, \\ & 229 \end{aligned}$ | $\begin{aligned} & 225, \\ & 227 \end{aligned}$ | $\begin{aligned} & 225, \\ & 225 \end{aligned}$ | $\begin{aligned} & 240, \\ & 247 \end{aligned}$ | $\begin{aligned} & 225, \\ & 247 \end{aligned}$ |  |  |
| D15S205 | $\begin{gathered} 78.9 \\ 2 \end{gathered}$ | $\begin{aligned} & 156, \\ & 160 \end{aligned}$ |  | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ | $\begin{gathered} 156, \\ 160 \end{gathered}$ | $\begin{aligned} & \hline 156, \\ & 160 \end{aligned}$ | $\begin{aligned} & 160 \\ & 160 \end{aligned}$ | $\begin{aligned} & 156, \\ & 160 \end{aligned}$ | $\begin{aligned} & 160, \\ & 160 \end{aligned}$ | $\begin{aligned} & 156, \\ & 166 \end{aligned}$ | $\begin{aligned} & 150 \\ & 156 \end{aligned}$ | $\begin{aligned} & 156 \\ & 166 \end{aligned}$ | $\begin{aligned} & \hline 150, \\ & 166 \end{aligned}$ |  | $\begin{aligned} & \hline 160, \\ & 160 \end{aligned}$ | $\begin{aligned} & 158, \\ & 160 \end{aligned}$ |  | $\begin{aligned} & 160, \\ & 160 \end{aligned}$ | $\begin{aligned} & 158, \\ & 160 \end{aligned}$ |
| D15S127 | $\begin{gathered} 86.8 \\ 1 \\ \hline \end{gathered}$ | $\begin{aligned} & 130 \\ & 146 \end{aligned}$ | $\begin{aligned} & 135, \\ & 144 \end{aligned}$ | $\begin{aligned} & 144, \\ & 146 \end{aligned}$ | $\begin{aligned} & 135, \\ & 146 \end{aligned}$ |  | $\begin{gathered} 130, \\ 135 \end{gathered}$ | $\begin{aligned} & 135, \\ & 146 \end{aligned}$ | $\begin{aligned} & 130, \\ & 135 \end{aligned}$ | $\begin{aligned} & 130, \\ & 146 \end{aligned}$ | $\begin{gathered} 114, \\ 144 \end{gathered}$ | $\begin{gathered} 114, \\ 130 \end{gathered}$ | $\begin{aligned} & 130, \\ & 144 \end{aligned}$ | $\begin{aligned} & 128, \\ & 134 \end{aligned}$ | $\begin{aligned} & 130 \\ & 146 \end{aligned}$ | $\begin{aligned} & 130 \\ & 146 \end{aligned}$ |  | $\begin{aligned} & 140 \\ & 146 \end{aligned}$ | $\begin{gathered} 130, \\ 130 \end{gathered}$ |
| D15S816 | $\begin{gathered} 100 . \\ 59 \end{gathered}$ | $\begin{aligned} & 128, \\ & 136 \end{aligned}$ | $\begin{aligned} & 132, \\ & 136 \end{aligned}$ | $\begin{aligned} & 132, \\ & 136 \end{aligned}$ | $\begin{aligned} & 136, \\ & 136 \end{aligned}$ | $\begin{aligned} & 136, \\ & 136 \end{aligned}$ | $\begin{gathered} 136 \\ 128 \end{gathered}$ |  |  | $\begin{gathered} 128, \\ 136 \end{gathered}$ | $\begin{aligned} & 128, \\ & 128 \end{aligned}$ | $\begin{aligned} & 128, \\ & 136 \end{aligned}$ | $\begin{aligned} & 128, \\ & 136 \end{aligned}$ | $\begin{aligned} & 128, \\ & 136 \end{aligned}$ | $\begin{aligned} & 136, \\ & 136 \end{aligned}$ | $\begin{aligned} & 132, \\ & 132 \end{aligned}$ | $\begin{aligned} & 132, \\ & 136 \end{aligned}$ |  |  |
| D15S130 | $\begin{gathered} 100 . \\ 59 \end{gathered}$ | $\begin{aligned} & 222, \\ & 222 \end{aligned}$ | $\begin{aligned} & 222, \\ & 222 \end{aligned}$ | $\begin{aligned} & 222, \\ & 222 \end{aligned}$ | $\begin{aligned} & 222, \\ & 222 \end{aligned}$ | $\begin{aligned} & 222, \\ & 222 \end{aligned}$ | $\begin{aligned} & 222, \\ & 222 \end{aligned}$ | $\begin{aligned} & 222, \\ & 222 \end{aligned}$ | $\begin{aligned} & 222, \\ & 222 \end{aligned}$ | $\begin{aligned} & 222, \\ & 222 \end{aligned}$ | $\begin{aligned} & 222, \\ & 222 \end{aligned}$ | $\begin{aligned} & 222, \\ & 222 \end{aligned}$ | $\begin{aligned} & 222, \\ & 222 \end{aligned}$ | $\begin{aligned} & 220, \\ & 222 \end{aligned}$ | $\begin{aligned} & 222, \\ & 222 \end{aligned}$ | $\begin{aligned} & 220, \\ & 230 \end{aligned}$ | $\begin{aligned} & 222, \\ & 230 \end{aligned}$ |  |  |
| D15S120 | $\begin{gathered} 112 . \\ 58 \end{gathered}$ | $\begin{aligned} & 170, \\ & 172 \end{aligned}$ | $\begin{aligned} & \hline 160, \\ & 170 \end{aligned}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ | $\begin{aligned} & 160, \\ & 170 \end{aligned}$ | $\begin{gathered} 160, \\ 170 \end{gathered}$ | $\begin{gathered} 160, \\ 170 \end{gathered}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ |  | $\begin{gathered} 170, \\ 170 \end{gathered}$ |  | $\begin{gathered} 170, \\ 170 \end{gathered}$ | $\begin{gathered} 170, \\ 170 \end{gathered}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ | $\begin{gathered} 170, \\ 170 \end{gathered}$ | $\begin{gathered} 170, \\ 170 \end{gathered}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ |  |
| D16S404 | $\begin{gathered} 18.0 \\ 7 \end{gathered}$ | $\begin{aligned} & 131, \\ & 135 \end{aligned}$ | $\begin{aligned} & 131, \\ & 135 \end{aligned}$ | $\begin{aligned} & 135, \\ & 135 \end{aligned}$ | $\begin{aligned} & 135, \\ & 135 \end{aligned}$ | $\begin{aligned} & 131, \\ & 131 \end{aligned}$ | $\begin{gathered} 131 \\ 131 \end{gathered}$ | $\begin{aligned} & 135, \\ & 135 \end{aligned}$ | $\begin{aligned} & 131, \\ & 131 \end{aligned}$ | $\begin{aligned} & \hline 131, \\ & 133 \end{aligned}$ | $\begin{aligned} & \hline 129, \\ & 133 \\ & \hline \end{aligned}$ | $\begin{aligned} & 131, \\ & 133 \end{aligned}$ | $\begin{aligned} & \hline 133, \\ & 133 \end{aligned}$ | $\begin{aligned} & 135, \\ & 133 \end{aligned}$ | $\begin{aligned} & 131, \\ & 135 \end{aligned}$ | $\begin{gathered} 131, \\ 133 \end{gathered}$ | $\begin{aligned} & 131, \\ & 133 \end{aligned}$ | $\begin{aligned} & 131, \\ & 131 \end{aligned}$ | $\begin{gathered} 131, \\ 133 \end{gathered}$ |
| D16S3075 | $\begin{gathered} 23.2 \\ 8 \end{gathered}$ | $\begin{aligned} & 206 \\ & 208 \end{aligned}$ | $\begin{aligned} & 208, \\ & 212 \end{aligned}$ | $\begin{aligned} & 208, \\ & 212 \end{aligned}$ | $\begin{aligned} & 208 \\ & 212 \end{aligned}$ | $\begin{aligned} & 206 \\ & 208 \end{aligned}$ | $\begin{aligned} & 208 \\ & 208 \end{aligned}$ | $\begin{aligned} & 208 \\ & 208 \end{aligned}$ | $\begin{aligned} & 208 \\ & 208 \end{aligned}$ | $\begin{aligned} & 206 \\ & 212 \end{aligned}$ | $\begin{aligned} & 206 \\ & 212 \end{aligned}$ | $\begin{gathered} 206 \\ 206 \end{gathered}$ | $\begin{aligned} & 206, \\ & 212 \end{aligned}$ | $\begin{aligned} & 206, \\ & 212 \end{aligned}$ | $\begin{aligned} & 206 \\ & 208 \end{aligned}$ | $\begin{aligned} & 212, \\ & 214 \end{aligned}$ |  | $\begin{aligned} & 206 \\ & 214 \end{aligned}$ | $\begin{gathered} 206 \\ 214 \end{gathered}$ |


| D16S3046 | $\begin{gathered} 40.6 \\ 5 \end{gathered}$ | $\begin{gathered} 84,1 \\ 02 \end{gathered}$ | $\begin{aligned} & 102, \\ & 104 \end{aligned}$ | $\begin{aligned} & 102, \\ & 104 \end{aligned}$ | $\begin{gathered} 102, \\ 104 \end{gathered}$ | $\begin{gathered} 84,1 \\ 04 \end{gathered}$ | $\begin{gathered} 102, \\ 102 \end{gathered}$ | $\begin{aligned} & 102, \\ & 104 \end{aligned}$ | $\begin{aligned} & 102, \\ & 102 \end{aligned}$ | $\begin{aligned} & \hline 100, \\ & 102 \end{aligned}$ | $\begin{gathered} 100, \\ 104 \end{gathered}$ | $\begin{aligned} & 100 \\ & 100 \end{aligned}$ | $\begin{aligned} & \hline 100 \\ & 100 \end{aligned}$ | $\begin{aligned} & \hline 100, \\ & 102 \end{aligned}$ | $\begin{gathered} 84,1 \\ 02 \end{gathered}$ | $\begin{gathered} 102, \\ 104 \end{gathered}$ | $\begin{gathered} 84,1 \\ 02 \end{gathered}$ | $\begin{gathered} 84,1 \\ 04 \end{gathered}$ | $\begin{gathered} \hline 84,1 \\ 04 \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D16S403 | $\begin{gathered} 43.8 \\ 9 \end{gathered}$ | $\begin{aligned} & 140, \\ & 140 \end{aligned}$ | $\begin{aligned} & 138, \\ & 152 \end{aligned}$ | $\begin{aligned} & 140, \\ & 152 \end{aligned}$ | $\begin{aligned} & 140, \\ & 152 \end{aligned}$ | $\begin{aligned} & 140, \\ & 152 \end{aligned}$ | $\begin{aligned} & 140, \\ & 138 \end{aligned}$ |  |  | $\begin{aligned} & 140, \\ & 140 \end{aligned}$ | $\begin{aligned} & 144, \\ & 152 \end{aligned}$ | $\begin{aligned} & 140, \\ & 144 \end{aligned}$ | $\begin{aligned} & \hline 140, \\ & 144 \end{aligned}$ | $\begin{aligned} & \hline 140, \\ & 140 \end{aligned}$ | $\begin{aligned} & 142, \\ & 150 \end{aligned}$ | $\begin{aligned} & 138, \\ & 138 \end{aligned}$ | $\begin{aligned} & 138, \\ & 150 \end{aligned}$ |  |  |
| D16S3068 | $\begin{gathered} 48.5 \\ 3 \end{gathered}$ | $\begin{aligned} & 137, \\ & 147 \end{aligned}$ | $\begin{array}{\|l\|} \hline 139, \\ 143 \\ \hline \end{array}$ | $\begin{aligned} & 139, \\ & 147 \end{aligned}$ | $\begin{aligned} & 139, \\ & 147 \end{aligned}$ | $\begin{aligned} & 139, \\ & 147 \end{aligned}$ | $\begin{gathered} 143, \\ 147 \end{gathered}$ | $\begin{aligned} & 139, \\ & 147 \end{aligned}$ | $\begin{aligned} & 143, \\ & 147 \end{aligned}$ | $\begin{aligned} & 133, \\ & 147 \end{aligned}$ | $\begin{gathered} 141, \\ 143 \\ \hline \end{gathered}$ | $\begin{gathered} 133, \\ 143 \end{gathered}$ | $\begin{gathered} 133, \\ 143 \end{gathered}$ | $\begin{aligned} & 133, \\ & 147 \end{aligned}$ | $\begin{aligned} & 137, \\ & 141 \end{aligned}$ | $\begin{gathered} 139, \\ 139 \end{gathered}$ | $\begin{gathered} 137, \\ 139 \end{gathered}$ | $\begin{aligned} & 137, \\ & 139 \end{aligned}$ | $\begin{gathered} 137, \\ 139 \end{gathered}$ |
| D16S3136 | $\begin{gathered} 62.1 \\ 1 \end{gathered}$ | $\begin{aligned} & 203, \\ & 203 \end{aligned}$ | $\begin{aligned} & 201, \\ & 205 \end{aligned}$ | $\begin{aligned} & 201, \\ & 203 \end{aligned}$ | $\begin{aligned} & 201, \\ & 203 \end{aligned}$ | $\begin{aligned} & 201, \\ & 203 \end{aligned}$ | $\begin{aligned} & 203, \\ & 205 \end{aligned}$ | $\begin{aligned} & 201, \\ & 203 \end{aligned}$ | $\begin{aligned} & 203, \\ & 205 \end{aligned}$ | $\begin{aligned} & 203, \\ & 203 \end{aligned}$ |  |  | $\begin{gathered} 203, \\ 205 \end{gathered}$ | $\begin{aligned} & 203, \\ & 203 \end{aligned}$ | $\begin{aligned} & 201, \\ & 203 \end{aligned}$ | $\begin{aligned} & 201, \\ & 205 \end{aligned}$ | $\begin{aligned} & 201, \\ & 203 \end{aligned}$ | $\begin{aligned} & 201, \\ & 203 \end{aligned}$ | $\begin{aligned} & 201, \\ & 203 \end{aligned}$ |
| D16S415 | $\begin{gathered} 67.6 \\ 2 \end{gathered}$ | $\begin{aligned} & 220, \\ & 226 \end{aligned}$ | $\begin{aligned} & 222, \\ & 226 \end{aligned}$ | $\begin{aligned} & 220, \\ & 226 \end{aligned}$ | $\begin{aligned} & 220, \\ & 226 \end{aligned}$ | $\begin{aligned} & 226 \\ & 226 \end{aligned}$ | $\begin{aligned} & 222, \\ & 226 \end{aligned}$ | $\begin{aligned} & 226, \\ & 226 \end{aligned}$ | $\begin{aligned} & 220, \\ & 226 \end{aligned}$ | $\begin{aligned} & 220, \\ & 224 \end{aligned}$ | $\begin{aligned} & 220, \\ & 224 \end{aligned}$ | $\begin{aligned} & 224, \\ & 224 \end{aligned}$ | $\begin{aligned} & 224, \\ & 224 \end{aligned}$ | $\begin{aligned} & 220, \\ & 224 \end{aligned}$ | $\begin{aligned} & 224, \\ & 226 \end{aligned}$ | $\begin{aligned} & 224, \\ & 224 \end{aligned}$ | $\begin{aligned} & 224, \\ & 226 \end{aligned}$ | $\begin{aligned} & 224, \\ & 226 \end{aligned}$ |  |
| D16S3253 | $\begin{gathered} 71.7 \\ 7 \end{gathered}$ | $\begin{aligned} & 179, \\ & 187 \end{aligned}$ | $\begin{aligned} & \hline 179, \\ & 187 \end{aligned}$ | $\begin{aligned} & 179 \\ & 187 \end{aligned}$ | $\begin{gathered} 187, \\ 187 \end{gathered}$ | $\begin{aligned} & 179 \\ & 187 \end{aligned}$ | $\begin{aligned} & \hline 179, \\ & 179 \end{aligned}$ |  |  | $\begin{aligned} & 187, \\ & 175 \end{aligned}$ | $\begin{gathered} 187, \\ 187 \end{gathered}$ | $\begin{gathered} 187, \\ 175 \end{gathered}$ | $\begin{aligned} & 175, \\ & 187 \end{aligned}$ | $\begin{aligned} & 175, \\ & 187 \end{aligned}$ | $\begin{array}{\|l\|} \hline 179, \\ 187 \\ \hline \end{array}$ | $\begin{aligned} & 179 \\ & 183 \end{aligned}$ | $\begin{aligned} & 179, \\ & 183 \end{aligned}$ |  |  |
| D16S503 | $\begin{gathered} 83.5 \\ 5 \end{gathered}$ | $\begin{aligned} & 223, \\ & 223 \end{aligned}$ | $\begin{aligned} & \hline 219, \\ & 229 \end{aligned}$ | $\begin{aligned} & 223, \\ & 229 \end{aligned}$ | $\begin{aligned} & 223, \\ & 229 \end{aligned}$ | $\begin{aligned} & 223, \\ & 229 \end{aligned}$ | $\begin{aligned} & 219, \\ & 223 \end{aligned}$ | $\begin{aligned} & 223, \\ & 229 \end{aligned}$ | $\begin{aligned} & 219, \\ & 223 \end{aligned}$ | $\begin{aligned} & 223, \\ & 223 \end{aligned}$ | $\begin{aligned} & 229, \\ & 229 \end{aligned}$ | $\begin{aligned} & 223, \\ & 229 \end{aligned}$ |  | $\begin{aligned} & 223, \\ & 229 \end{aligned}$ | $\begin{aligned} & 223, \\ & 223 \end{aligned}$ | $\begin{aligned} & 223, \\ & 229 \end{aligned}$ | $\begin{aligned} & 223, \\ & 229 \end{aligned}$ | $\begin{aligned} & 223, \\ & 229 \end{aligned}$ | $\begin{aligned} & 223, \\ & 223 \end{aligned}$ |
| D16S515 | $\begin{gathered} 92.1 \\ 0 \end{gathered}$ | $\begin{aligned} & 226, \\ & 230 \end{aligned}$ | $\begin{aligned} & 230, \\ & 232 \end{aligned}$ | $\begin{aligned} & 226, \\ & 232 \end{aligned}$ | $\begin{aligned} & 230, \\ & 230 \end{aligned}$ | $\begin{aligned} & 226, \\ & 232 \end{aligned}$ | $\begin{aligned} & 226, \\ & 230 \end{aligned}$ |  | $\begin{aligned} & 230, \\ & 230 \end{aligned}$ | $\begin{aligned} & 230, \\ & 230 \end{aligned}$ | $\begin{aligned} & 230, \\ & 238 \end{aligned}$ | $\begin{aligned} & 230, \\ & 230 \end{aligned}$ | $\begin{aligned} & 230, \\ & 230 \end{aligned}$ | $\begin{aligned} & 230, \\ & 230 \end{aligned}$ | $\begin{aligned} & 226, \\ & 230 \end{aligned}$ | $\begin{aligned} & 234, \\ & 236 \end{aligned}$ | $\begin{aligned} & 226, \\ & 236 \end{aligned}$ | $\begin{aligned} & 226, \\ & 234 \end{aligned}$ | $\begin{aligned} & 226, \\ & 236 \end{aligned}$ |
| D16S516 | $\begin{gathered} 100 . \\ 39 \end{gathered}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ |  | $\begin{gathered} 170, \\ 170 \end{gathered}$ | $\begin{gathered} 170, \\ 170 \end{gathered}$ | $\begin{gathered} 170, \\ 170 \end{gathered}$ | $\begin{gathered} 170, \\ 170 \end{gathered}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ | $\begin{gathered} 170, \\ 170 \end{gathered}$ | $\begin{aligned} & 168, \\ & 170 \end{aligned}$ | $\begin{aligned} & 170, \\ & 174 \end{aligned}$ | $\begin{aligned} & 168 \\ & 178 \end{aligned}$ | $\begin{gathered} 168, \\ 170 \end{gathered}$ | $\begin{gathered} 170, \\ 170 \end{gathered}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ | $\begin{gathered} 170, \\ 170 \end{gathered}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ |  |
| D16S3091 | $\begin{gathered} 111 . \\ 12 \end{gathered}$ | $\begin{aligned} & 117, \\ & 119 \end{aligned}$ | $\begin{aligned} & 119, \\ & 119 \end{aligned}$ | $\begin{aligned} & 117, \\ & 119 \end{aligned}$ | $\begin{aligned} & 119, \\ & 119 \end{aligned}$ | $\begin{gathered} 117, \\ 119 \end{gathered}$ | $\begin{gathered} 117, \\ 119 \end{gathered}$ | $\begin{aligned} & 117, \\ & 119 \end{aligned}$ | $\begin{aligned} & 119 \\ & 119 \end{aligned}$ | $\begin{gathered} 119 \\ 119 \end{gathered}$ | $\begin{gathered} 125 \\ 125 \end{gathered}$ | $\begin{aligned} & 119 \\ & 125 \end{aligned}$ | $\begin{aligned} & 119, \\ & 125 \end{aligned}$ | $\begin{aligned} & 117, \\ & 123 \end{aligned}$ | $\begin{aligned} & 117, \\ & 119 \end{aligned}$ | $\begin{aligned} & 119, \\ & 127 \end{aligned}$ | $\begin{aligned} & 119, \\ & 119 \end{aligned}$ |  |  |
| D16S520 | $\begin{gathered} 125 . \\ 82 \end{gathered}$ | $\begin{aligned} & 185 \\ & 197 \end{aligned}$ | $\begin{aligned} & 193, \\ & 195 \end{aligned}$ | $\begin{aligned} & 185, \\ & 193 \end{aligned}$ | $\begin{aligned} & 195 \\ & 197 \end{aligned}$ |  |  | $\begin{aligned} & 185, \\ & 193 \end{aligned}$ | $\begin{aligned} & 185 \\ & 195 \end{aligned}$ | $\begin{gathered} 189 \\ 191 \end{gathered}$ | $\begin{gathered} 189, \\ 189 \end{gathered}$ | $\begin{array}{r} 189 \\ 191 \end{array}$ | $\begin{gathered} 189 \\ 191 \end{gathered}$ | $\begin{aligned} & 185 \\ & 197 \end{aligned}$ | $\begin{aligned} & 185, \\ & 189 \end{aligned}$ | $\begin{array}{r} 187 \\ 191 \end{array}$ |  |  | $\begin{aligned} & 189 \\ & 191 \end{aligned}$ |
| D17S849 | 0.63 | $\begin{aligned} & 255, \\ & 257 \end{aligned}$ | $\begin{aligned} & 253, \\ & 253 \end{aligned}$ | $\begin{aligned} & 253, \\ & 255 \end{aligned}$ | $\begin{aligned} & 253, \\ & 257 \end{aligned}$ | $\begin{aligned} & 253, \\ & 255 \end{aligned}$ | $\begin{aligned} & 253, \\ & 257 \end{aligned}$ | $\begin{aligned} & 253, \\ & 255 \end{aligned}$ | $\begin{aligned} & 253, \\ & 257 \end{aligned}$ | $\begin{aligned} & 255 \\ & 257 \end{aligned}$ | $\begin{aligned} & 253, \\ & 257 \end{aligned}$ |  | $\begin{aligned} & 253, \\ & 257 \end{aligned}$ | $\begin{aligned} & 255, \\ & 257 \end{aligned}$ | $\begin{aligned} & 253, \\ & 257 \end{aligned}$ | $\begin{aligned} & 253, \\ & 253 \end{aligned}$ | $\begin{aligned} & 253, \\ & 257 \end{aligned}$ | $\begin{aligned} & 253 \\ & 253 \end{aligned}$ |  |
| D17S831 | 6.60 | $\begin{aligned} & 224, \\ & 224 \end{aligned}$ | $\begin{aligned} & 230, \\ & 230 \end{aligned}$ | $\begin{aligned} & 224, \\ & 230 \end{aligned}$ | $\begin{aligned} & 224, \\ & 230 \end{aligned}$ | $\begin{aligned} & 224, \\ & 230 \end{aligned}$ | $\begin{aligned} & 224, \\ & 230 \end{aligned}$ | $\begin{aligned} & 224, \\ & 230 \end{aligned}$ | $\begin{aligned} & 224, \\ & 230 \end{aligned}$ | $\begin{aligned} & 224, \\ & 228 \end{aligned}$ | $\begin{aligned} & 224, \\ & 234 \end{aligned}$ |  | $\begin{aligned} & 224, \\ & 228 \end{aligned}$ | $\begin{aligned} & 224, \\ & 224 \end{aligned}$ | $\begin{aligned} & 228, \\ & 236 \end{aligned}$ | $\begin{aligned} & 230, \\ & 230 \end{aligned}$ | $\begin{aligned} & 236, \\ & 230 \end{aligned}$ | $\begin{aligned} & 228, \\ & 230 \end{aligned}$ | $\begin{aligned} & 228, \\ & 230 \end{aligned}$ |
| D17S938 | $\begin{gathered} 14.6 \\ 9 \end{gathered}$ | $\begin{aligned} & 180 \\ & 182 \end{aligned}$ | $\begin{aligned} & \hline 180, \\ & 180 \end{aligned}$ | $\begin{aligned} & 180 \\ & 180 \end{aligned}$ | $\begin{aligned} & 180 \\ & 182 \end{aligned}$ | $\begin{gathered} 180, \\ 180 \end{gathered}$ | $\begin{aligned} & \hline 180, \\ & 182 \end{aligned}$ | $\begin{aligned} & 180, \\ & 180 \end{aligned}$ | $\begin{aligned} & 180 \\ & 182 \end{aligned}$ | $\begin{aligned} & 180, \\ & 182 \end{aligned}$ | $\begin{gathered} 166, \\ 178 \end{gathered}$ | $\begin{aligned} & 178, \\ & 182 \end{aligned}$ | $\begin{aligned} & \hline 178, \\ & 180 \end{aligned}$ | $\begin{aligned} & 166, \\ & 180 \end{aligned}$ |  |  | $\begin{aligned} & 166, \\ & 178 \end{aligned}$ |  |  |
| D17S974 | $\begin{gathered} 22.2 \\ 4 \end{gathered}$ | $\begin{aligned} & 213, \\ & 217 \end{aligned}$ | $\begin{gathered} 209, \\ 123 \end{gathered}$ | $\begin{aligned} & 213, \\ & 217 \end{aligned}$ | $\begin{aligned} & 213, \\ & 217 \end{aligned}$ | $\begin{gathered} 109, \\ 123 \end{gathered}$ | $\begin{aligned} & 109 \\ & 217 \end{aligned}$ |  |  | $\begin{aligned} & 205 \\ & 217 \end{aligned}$ | $\begin{aligned} & 205 \\ & 213 \end{aligned}$ | $\begin{aligned} & 205, \\ & 213 \end{aligned}$ | $\begin{aligned} & 205, \\ & 213 \end{aligned}$ | $\begin{aligned} & 205 \\ & 205 \end{aligned}$ | $\begin{aligned} & 295, \\ & 213 \end{aligned}$ | $\begin{aligned} & 209 \\ & 205 \end{aligned}$ | $\begin{aligned} & 295, \\ & 213 \end{aligned}$ |  |  |


| D17S1852 | $\begin{gathered} 22.2 \\ 4 \end{gathered}$ | $\begin{aligned} & 210, \\ & 210 \end{aligned}$ | $\begin{aligned} & 200, \\ & 202 \end{aligned}$ | $\begin{aligned} & 202, \\ & 210 \end{aligned}$ | $\begin{aligned} & 202, \\ & 210 \end{aligned}$ | $\begin{aligned} & 200, \\ & 210 \end{aligned}$ | $\begin{aligned} & 200, \\ & 210 \end{aligned}$ | $\begin{aligned} & 202, \\ & 210 \end{aligned}$ | $\begin{aligned} & 200, \\ & 210 \end{aligned}$ | $\begin{aligned} & 200, \\ & 210 \end{aligned}$ | $\begin{aligned} & 202, \\ & 212 \end{aligned}$ |  | $\begin{aligned} & 200, \\ & 212 \end{aligned}$ | $\begin{aligned} & 200, \\ & 204 \end{aligned}$ | $\begin{aligned} & 204, \\ & 210 \end{aligned}$ |  | $\begin{aligned} & 202, \\ & 210 \end{aligned}$ | $\begin{aligned} & 204, \\ & 208 \end{aligned}$ |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D17S799 | $\begin{gathered} 31.9 \\ 6 \end{gathered}$ | $\begin{aligned} & 192, \\ & 196 \end{aligned}$ | $\begin{aligned} & 192, \\ & 200 \end{aligned}$ | $\begin{aligned} & 192, \\ & 196 \end{aligned}$ | $\begin{aligned} & 192, \\ & 196 \end{aligned}$ | $\begin{gathered} 192, \\ 192 \end{gathered}$ | $\begin{gathered} 192, \\ 196 \end{gathered}$ | $\begin{aligned} & 192, \\ & 192 \end{aligned}$ | $\begin{aligned} & 192, \\ & 196 \end{aligned}$ | $\begin{gathered} 192, \\ 196 \end{gathered}$ | $\begin{aligned} & 186, \\ & 200 \end{aligned}$ |  | $\begin{gathered} 186, \\ 192 \end{gathered}$ | $\begin{aligned} & 192, \\ & 196 \end{aligned}$ | $\begin{aligned} & 192, \\ & 196 \end{aligned}$ | $\begin{gathered} 192, \\ 196 \end{gathered}$ | $\begin{aligned} & 192, \\ & 196 \end{aligned}$ |  | $\begin{gathered} 192, \\ 196 \end{gathered}$ |
| D17S921 | $\begin{gathered} 36.1 \\ 4 \end{gathered}$ | $\begin{gathered} 175, \\ 183 \end{gathered}$ | $\begin{aligned} & 175, \\ & 183 \end{aligned}$ | $\begin{aligned} & 175 \\ & 175 \end{aligned}$ | $\begin{aligned} & 175, \\ & 175 \end{aligned}$ | $\begin{gathered} 183, \\ 183 \end{gathered}$ | $\begin{gathered} 175 \\ 175 \end{gathered}$ | $\begin{gathered} 175, \\ 183 \end{gathered}$ | $\begin{gathered} 175, \\ 183 \end{gathered}$ | $\begin{gathered} 175, \\ 183 \end{gathered}$ | $\begin{gathered} 181, \\ 183 \end{gathered}$ |  | $\begin{gathered} 181, \\ 183 \end{gathered}$ | $\begin{gathered} 183, \\ 183 \end{gathered}$ | $\begin{gathered} 183, \\ 183 \end{gathered}$ |  | $\begin{gathered} 183, \\ 183 \end{gathered}$ | $\begin{gathered} 183, \\ 183 \end{gathered}$ | $\begin{gathered} 183, \\ 183 \end{gathered}$ |
| D17S1857 | $\begin{gathered} 43.0 \\ 1 \end{gathered}$ | $\begin{aligned} & 181, \\ & 181 \end{aligned}$ | $\begin{aligned} & 177, \\ & 181 \end{aligned}$ | $\begin{aligned} & 181, \\ & 181 \end{aligned}$ | $\begin{gathered} 181, \\ 181 \end{gathered}$ | $\begin{aligned} & 177, \\ & 181 \end{aligned}$ | $\begin{gathered} 181, \\ 181 \end{gathered}$ | $\begin{aligned} & 181, \\ & 181 \end{aligned}$ | $\begin{aligned} & 177, \\ & 181 \end{aligned}$ | $\begin{aligned} & 181, \\ & 181 \end{aligned}$ | $\begin{aligned} & 177, \\ & 181 \end{aligned}$ |  | $\begin{gathered} 181, \\ 181 \end{gathered}$ | $\begin{aligned} & 181, \\ & 185 \end{aligned}$ | $\begin{aligned} & 181, \\ & 185 \end{aligned}$ | $\begin{aligned} & \hline 179, \\ & 181 \end{aligned}$ | $\begin{aligned} & 179, \\ & 185 \end{aligned}$ | $\begin{aligned} & 181, \\ & 185 \end{aligned}$ | $\begin{gathered} 181, \\ 181 \end{gathered}$ |
| D17S2196 | $\begin{gathered} 44.6 \\ 2 \end{gathered}$ | $\begin{aligned} & 139, \\ & 151 \end{aligned}$ | $\begin{aligned} & 155, \\ & 147 \end{aligned}$ | $\begin{aligned} & 139, \\ & 155 \end{aligned}$ | $\begin{aligned} & 139 \\ & 155 \end{aligned}$ | $\begin{aligned} & 139 \\ & 155 \end{aligned}$ | $\begin{aligned} & \hline 139, \\ & 155 \\ & \hline \end{aligned}$ |  |  | $\begin{aligned} & 139, \\ & 155 \end{aligned}$ | $\begin{aligned} & 151, \\ & 155 \end{aligned}$ | $\begin{aligned} & 159, \\ & 159 \end{aligned}$ | $\begin{aligned} & 159, \\ & 159 \end{aligned}$ | $\begin{aligned} & 147, \\ & 151 \end{aligned}$ | $\begin{aligned} & 147, \\ & 151 \end{aligned}$ | $\begin{aligned} & 147, \\ & 151 \end{aligned}$ | $\begin{aligned} & 147, \\ & 147 \end{aligned}$ |  |  |
| D17S1294 | $\begin{gathered} 50.7 \\ 4 \end{gathered}$ | $\begin{aligned} & 252, \\ & 256 \end{aligned}$ |  | $\begin{aligned} & 252, \\ & 252 \end{aligned}$ | $\begin{aligned} & 252, \\ & 252 \end{aligned}$ | $\begin{aligned} & 256 \\ & 256 \end{aligned}$ | $\begin{aligned} & 252, \\ & 252 \end{aligned}$ |  | $\begin{aligned} & 252, \\ & 256 \end{aligned}$ | $\begin{aligned} & 252, \\ & 252 \end{aligned}$ | $\begin{aligned} & 260, \\ & 264 \end{aligned}$ | $\begin{aligned} & 252, \\ & 260 \end{aligned}$ | $\begin{aligned} & 252, \\ & 260 \end{aligned}$ | $\begin{aligned} & 252, \\ & 256 \end{aligned}$ | $\begin{aligned} & 252, \\ & 260 \end{aligned}$ |  | $\begin{aligned} & 252, \\ & 256 \end{aligned}$ | $\begin{aligned} & 252, \\ & 256 \end{aligned}$ | $\begin{aligned} & 256, \\ & 260 \end{aligned}$ |
| D17S798 | $\begin{gathered} 53.4 \\ 1 \end{gathered}$ | $\begin{aligned} & 223, \\ & 229 \end{aligned}$ | $\begin{aligned} & 223, \\ & 223 \end{aligned}$ | $\begin{aligned} & 223, \\ & 229 \end{aligned}$ | $\begin{aligned} & 223, \\ & 229 \end{aligned}$ | $\begin{aligned} & 223, \\ & 223 \end{aligned}$ | $\begin{aligned} & 223, \\ & 229 \end{aligned}$ | $\begin{aligned} & 223, \\ & 223 \end{aligned}$ | $\begin{aligned} & 223, \\ & 229 \end{aligned}$ | $\begin{aligned} & 209, \\ & 229 \end{aligned}$ | $\begin{aligned} & 209 \\ & 225 \end{aligned}$ | $\begin{aligned} & \hline 209 \\ & 209 \end{aligned}$ | $\begin{aligned} & 209, \\ & 209 \end{aligned}$ | $\begin{aligned} & 223, \\ & 223 \end{aligned}$ | $\begin{aligned} & 223, \\ & 223 \end{aligned}$ | $\begin{aligned} & \hline 209, \\ & 217 \end{aligned}$ | $\begin{aligned} & 209, \\ & 223 \end{aligned}$ | $\begin{aligned} & 209, \\ & 223 \end{aligned}$ | $\begin{aligned} & 217, \\ & 223 \end{aligned}$ |
| D17S1868 | $\begin{gathered} 64.1 \\ 6 \end{gathered}$ | $\begin{gathered} 190, \\ 196 \end{gathered}$ | $\begin{gathered} 188, \\ 198 \end{gathered}$ | $\begin{gathered} 188, \\ 196 \end{gathered}$ | $\begin{gathered} 188, \\ 190 \end{gathered}$ | $\begin{gathered} 190, \\ 198 \end{gathered}$ | $\begin{gathered} 188, \\ 196 \end{gathered}$ | $\begin{aligned} & 188, \\ & 190 \end{aligned}$ | $\begin{gathered} 196, \\ 198 \end{gathered}$ | $\begin{gathered} 190, \\ 196 \end{gathered}$ | $\begin{aligned} & 196, \\ & 202 \end{aligned}$ | $\begin{gathered} 190, \\ 196 \end{gathered}$ | $\begin{aligned} & 190, \\ & 196 \end{aligned}$ | $\begin{aligned} & 192, \\ & 196 \end{aligned}$ | $\begin{aligned} & 190, \\ & 196 \end{aligned}$ | $\begin{aligned} & 192, \\ & 192 \end{aligned}$ | $\begin{aligned} & 190, \\ & 192 \end{aligned}$ | $\begin{aligned} & 192, \\ & 196 \end{aligned}$ | $\begin{gathered} 190, \\ 192 \end{gathered}$ |
| D17S2180 | $\begin{gathered} 66.8 \\ 5 \end{gathered}$ | $\begin{aligned} & 122, \\ & 122 \end{aligned}$ | $\begin{aligned} & 119, \\ & 119 \end{aligned}$ | $\begin{aligned} & 119, \\ & 122 \end{aligned}$ | $\begin{aligned} & 119, \\ & 122 \end{aligned}$ | $\begin{aligned} & 119, \\ & 122 \end{aligned}$ | $\begin{aligned} & 119, \\ & 122 \end{aligned}$ |  |  | $\begin{aligned} & 122, \\ & 122 \end{aligned}$ | $\begin{gathered} 119, \\ 119 \end{gathered}$ | $\begin{aligned} & 119, \\ & 122 \end{aligned}$ | $\begin{aligned} & 119, \\ & 122 \end{aligned}$ | $\begin{aligned} & 122, \\ & 122 \end{aligned}$ | $\begin{aligned} & 119, \\ & 122 \end{aligned}$ | $\begin{aligned} & 116, \\ & 122 \end{aligned}$ | $\begin{aligned} & 116, \\ & 122 \end{aligned}$ |  |  |
| D17S787 | $\begin{gathered} 74.9 \\ 9 \end{gathered}$ | $\begin{aligned} & 142, \\ & 152 \end{aligned}$ | $\begin{aligned} & 138 \\ & 138 \end{aligned}$ | $\begin{aligned} & 138, \\ & 152 \end{aligned}$ | $\begin{aligned} & 138, \\ & 142 \end{aligned}$ | $\begin{aligned} & 138, \\ & 142 \end{aligned}$ | $\begin{aligned} & 138, \\ & 152 \end{aligned}$ | $\begin{aligned} & 138, \\ & 142 \end{aligned}$ | $\begin{aligned} & 138 \\ & 142 \end{aligned}$ | $\begin{aligned} & 138, \\ & 152 \end{aligned}$ | $\begin{gathered} 140, \\ 142 \end{gathered}$ |  | $\begin{aligned} & 138, \\ & 142 \end{aligned}$ | $\begin{aligned} & 142, \\ & 152 \end{aligned}$ | $\begin{aligned} & 138, \\ & 144 \end{aligned}$ | $\begin{aligned} & 142, \\ & 152 \end{aligned}$ | $\begin{aligned} & 138, \\ & 142 \end{aligned}$ | $\begin{aligned} & 144, \\ & 152 \end{aligned}$ | $\begin{aligned} & 138, \\ & 152 \end{aligned}$ |
| D17S944 | $\begin{gathered} 82.5 \\ 6 \end{gathered}$ | $\begin{aligned} & 212, \\ & 216 \end{aligned}$ | $\begin{aligned} & 212, \\ & 216 \end{aligned}$ | $\begin{aligned} & 212, \\ & 216 \end{aligned}$ | $\begin{gathered} 216 \\ 216 \end{gathered}$ | $\begin{aligned} & 212, \\ & 216 \end{aligned}$ | $\begin{aligned} & 212, \\ & 212 \end{aligned}$ | $\begin{aligned} & 216, \\ & 216 \end{aligned}$ | $\begin{aligned} & 216 \\ & 216 \end{aligned}$ | $\begin{aligned} & 212, \\ & 212 \end{aligned}$ | $\begin{aligned} & 216, \\ & 218 \end{aligned}$ |  | $\begin{aligned} & 212, \\ & 216 \end{aligned}$ | $\begin{aligned} & 212, \\ & 216 \end{aligned}$ | $\begin{aligned} & 212, \\ & 212 \end{aligned}$ | $\begin{aligned} & 216, \\ & 222 \end{aligned}$ | $\begin{aligned} & 212, \\ & 216 \end{aligned}$ | $\begin{aligned} & 212, \\ & 222 \end{aligned}$ | $\begin{aligned} & 212, \\ & 222 \end{aligned}$ |
| D17S2193 | $\begin{gathered} 89.3 \\ 2 \end{gathered}$ | $\begin{aligned} & 141, \\ & 153 \end{aligned}$ | $\begin{aligned} & 141, \\ & 147 \end{aligned}$ | $\begin{aligned} & 141, \\ & 141 \end{aligned}$ | $\begin{gathered} 141, \\ 153 \end{gathered}$ | $\begin{gathered} 141, \\ 153 \end{gathered}$ | $\begin{aligned} & 141, \\ & 147 \end{aligned}$ |  |  | $\begin{gathered} 141 \\ 141 \end{gathered}$ | $\begin{aligned} & 141, \\ & 147 \end{aligned}$ | $\begin{gathered} 141, \\ 141 \end{gathered}$ | $\begin{aligned} & 141, \\ & 147 \end{aligned}$ | $\begin{aligned} & 141, \\ & 153 \end{aligned}$ | $\begin{aligned} & 141, \\ & 141 \end{aligned}$ | $\begin{aligned} & 147, \\ & 156 \end{aligned}$ | $\begin{aligned} & 141, \\ & 147 \end{aligned}$ |  |  |
| D17S949 | $\begin{gathered} 93.2 \\ 7 \end{gathered}$ | $\begin{aligned} & 207, \\ & 221 \end{aligned}$ | $\begin{aligned} & 215, \\ & 223 \end{aligned}$ | $\begin{aligned} & 215, \\ & 221 \end{aligned}$ | $\begin{gathered} 207, \\ 215 \end{gathered}$ | $\begin{aligned} & 207, \\ & 223 \end{aligned}$ | $\begin{aligned} & 221, \\ & 223 \end{aligned}$ | $\begin{aligned} & 207, \\ & 215 \end{aligned}$ | $\begin{aligned} & 207, \\ & 215 \end{aligned}$ | $\begin{aligned} & 211, \\ & 221 \end{aligned}$ | $\begin{aligned} & 217, \\ & 221 \end{aligned}$ |  | $\begin{aligned} & 217, \\ & 221 \end{aligned}$ | $\begin{aligned} & 207, \\ & 221 \end{aligned}$ | $\begin{aligned} & 211, \\ & 221 \end{aligned}$ | $\begin{aligned} & 219, \\ & 221 \end{aligned}$ | $\begin{aligned} & 211, \\ & 221 \end{aligned}$ | $\begin{aligned} & 219 . \\ & 221 \end{aligned}$ |  |
| D17S785 | $\begin{gathered} 103 . \\ 53 \end{gathered}$ | $\begin{aligned} & 185, \\ & 189 \end{aligned}$ | $\begin{aligned} & 189, \\ & 189 \end{aligned}$ | $\begin{aligned} & 189, \\ & 189 \end{aligned}$ | $\begin{gathered} 189, \\ 189 \end{gathered}$ | $\begin{gathered} 185, \\ 189 \end{gathered}$ | $\begin{gathered} 185, \\ 189 \end{gathered}$ | $\begin{aligned} & 185, \\ & 189 \end{aligned}$ | $\begin{aligned} & 185, \\ & 189 \end{aligned}$ | $\begin{gathered} 185, \\ 195 \end{gathered}$ | $\begin{aligned} & 189, \\ & 197 \end{aligned}$ |  | $\begin{gathered} 189, \\ 195 \end{gathered}$ | $\begin{aligned} & 189, \\ & 195 \end{aligned}$ | $\begin{aligned} & 189, \\ & 195 \end{aligned}$ |  | $\begin{aligned} & 189, \\ & 197 \end{aligned}$ | $\begin{aligned} & 195 \\ & 197 \end{aligned}$ | $\begin{aligned} & 189, \\ & 197 \end{aligned}$ |
| D17S784 | $\begin{gathered} 116 . \\ 86 \end{gathered}$ | $\begin{aligned} & 230 \\ & 234 \end{aligned}$ | $\begin{aligned} & 228, \\ & 232 \end{aligned}$ | $\begin{aligned} & 232, \\ & 234 \end{aligned}$ | $\begin{aligned} & 228 \\ & 234 \end{aligned}$ | $\begin{aligned} & 232, \\ & 234 \end{aligned}$ | $\begin{aligned} & 230 \\ & 232 \end{aligned}$ |  |  | $\begin{aligned} & 230 \\ & 234 \end{aligned}$ | $\begin{aligned} & 234, \\ & 236 \end{aligned}$ | $\begin{aligned} & 230 \\ & 236 \end{aligned}$ | $\begin{aligned} & 234, \\ & 234 \end{aligned}$ | $\begin{aligned} & 232, \\ & 234 \end{aligned}$ | $\begin{aligned} & 232, \\ & 234 \end{aligned}$ | $\begin{aligned} & 232, \\ & 234 \end{aligned}$ | $\begin{aligned} & 232 \\ & 234 \end{aligned}$ |  |  |


| D17S928 | $\begin{gathered} 126 . \\ 46 \end{gathered}$ | $\begin{aligned} & 149, \\ & 149 \end{aligned}$ | $\begin{array}{\|l} \hline 149, \\ 153 \\ \hline \end{array}$ | $\begin{aligned} & 149, \\ & 149 \end{aligned}$ | $\begin{aligned} & \hline 149, \\ & 153 \\ & \hline \end{aligned}$ | $\begin{aligned} & \hline 149, \\ & 149 \end{aligned}$ | $\begin{gathered} 149, \\ 149 \end{gathered}$ | $\begin{aligned} & 149, \\ & 153 \end{aligned}$ | $\begin{aligned} & \hline 149, \\ & 153 \end{aligned}$ | $\begin{aligned} & 145, \\ & 149 \end{aligned}$ | $\begin{aligned} & 151, \\ & 155 \end{aligned}$ |  | $\begin{aligned} & 145, \\ & 151 \end{aligned}$ | $\begin{aligned} & 145, \\ & 149 \end{aligned}$ | $\begin{aligned} & 145 \\ & 149 \end{aligned}$ |  | $\begin{aligned} & 145, \\ & 153 \end{aligned}$ | $\begin{aligned} & 145, \\ & 153 \end{aligned}$ | $\begin{aligned} & 149, \\ & 153 \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D18S63 | 8.30 | $\begin{aligned} & 255, \\ & 273 \end{aligned}$ | $\begin{aligned} & 277, \\ & 277 \end{aligned}$ | $\begin{aligned} & 273, \\ & 277 \end{aligned}$ | $\begin{aligned} & 255, \\ & 277 \end{aligned}$ |  | $\begin{aligned} & 255, \\ & 273 \end{aligned}$ | $\begin{aligned} & 255, \\ & 255 \end{aligned}$ | $\begin{aligned} & 255, \\ & 273 \end{aligned}$ | $\begin{aligned} & 255, \\ & 273 \end{aligned}$ | $\begin{aligned} & 255, \\ & 273 \end{aligned}$ | $\begin{aligned} & 255, \\ & 277 \end{aligned}$ | $\begin{aligned} & 255, \\ & 273 \end{aligned}$ | $\begin{aligned} & 269, \\ & 271 \end{aligned}$ | $\begin{aligned} & 255, \\ & 271 \end{aligned}$ | $\begin{aligned} & 255, \\ & 277 \end{aligned}$ | $\begin{aligned} & 273, \\ & 277 \end{aligned}$ |  |  |
| D18S53 | $\begin{gathered} 41.2 \\ 4 \end{gathered}$ | $\begin{gathered} 171, \\ 171 \end{gathered}$ | $\begin{aligned} & 171, \\ & 173 \end{aligned}$ | $\begin{aligned} & 171, \\ & 173 \end{aligned}$ | $\begin{gathered} 171, \\ 173 \end{gathered}$ | $\begin{aligned} & 171, \\ & 171 \end{aligned}$ | $\begin{gathered} 171, \\ 171 \end{gathered}$ | $\begin{aligned} & 171, \\ & 171 \end{aligned}$ | $\begin{aligned} & 171, \\ & 171 \end{aligned}$ | $\begin{gathered} 171, \\ 175 \end{gathered}$ | $\begin{gathered} 167, \\ 175 \end{gathered}$ | $\begin{gathered} 175, \\ 175 \end{gathered}$ | $\begin{aligned} & 167, \\ & 175 \end{aligned}$ | $\begin{aligned} & 167, \\ & 171 \end{aligned}$ | $\begin{aligned} & 171, \\ & 171 \end{aligned}$ | $\begin{gathered} 171, \\ 173 \end{gathered}$ | $\begin{gathered} 171, \\ 171 \end{gathered}$ | $\begin{aligned} & 171, \\ & 171 \end{aligned}$ | $\begin{gathered} 171, \\ 171 \end{gathered}$ |
| D18S452 | $\begin{gathered} 18.7 \\ 0 \end{gathered}$ | $\begin{aligned} & 135 \\ & 135 \end{aligned}$ | $\begin{aligned} & 133, \\ & 139 \end{aligned}$ | $\begin{aligned} & 135, \\ & 139 \end{aligned}$ | $\begin{gathered} 135, \\ 139 \end{gathered}$ | $\begin{gathered} 135, \\ 139 \end{gathered}$ | $\begin{aligned} & \hline 133, \\ & 135 \\ & \hline \end{aligned}$ | $\begin{aligned} & 131, \\ & 133 \end{aligned}$ |  | $\begin{aligned} & \hline 131, \\ & 135 \\ & \hline \end{aligned}$ | $\begin{gathered} 133, \\ 135 \end{gathered}$ | $\begin{aligned} & 133, \\ & 135 \end{aligned}$ | $\begin{aligned} & 133, \\ & 135 \end{aligned}$ | $\begin{gathered} 131, \\ 133 \end{gathered}$ | $\begin{aligned} & 131, \\ & 133 \end{aligned}$ | $\begin{gathered} 133, \\ 135 \end{gathered}$ | $\begin{aligned} & 133, \\ & 135 \end{aligned}$ | $\begin{aligned} & 131, \\ & 133 \end{aligned}$ | $\begin{gathered} 133, \\ 135 \end{gathered}$ |
| D18S464 | $\begin{gathered} 31.1 \\ 7 \end{gathered}$ | $\begin{aligned} & 289, \\ & 291 \end{aligned}$ | $\begin{aligned} & 287, \\ & 289 \end{aligned}$ | $\begin{aligned} & 287, \\ & 291 \end{aligned}$ | $\begin{gathered} 287, \\ 289 \end{gathered}$ | $\begin{aligned} & 289, \\ & 291 \end{aligned}$ | $\begin{aligned} & 289, \\ & 291 \end{aligned}$ | $\begin{aligned} & 289, \\ & 291 \end{aligned}$ | $\begin{aligned} & 289, \\ & 289 \end{aligned}$ | $\begin{aligned} & 289, \\ & 291 \end{aligned}$ | $\begin{gathered} 289, \\ 289 \end{gathered}$ | $\begin{aligned} & 289, \\ & 291 \end{aligned}$ | $\begin{aligned} & 289, \\ & 291 \end{aligned}$ | $\begin{aligned} & 289, \\ & 291 \end{aligned}$ | $\begin{aligned} & 289, \\ & 289 \end{aligned}$ | $\begin{aligned} & 289, \\ & 291 \end{aligned}$ | $\begin{aligned} & 289, \\ & 291 \end{aligned}$ | $\begin{aligned} & 289, \\ & 291 \end{aligned}$ | $\begin{gathered} 289, \\ 289 \end{gathered}$ |
| D18S53 | $\begin{gathered} 41.2 \\ 4 \end{gathered}$ | $\begin{aligned} & 171, \\ & 171 \end{aligned}$ | $\begin{gathered} 171, \\ 173 \end{gathered}$ | $\begin{aligned} & 171, \\ & 173 \end{aligned}$ | $\begin{aligned} & 171, \\ & 173 \end{aligned}$ | $\begin{aligned} & 171, \\ & 171 \end{aligned}$ | $\begin{aligned} & 171, \\ & 171 \end{aligned}$ | $\begin{aligned} & 171, \\ & 171 \end{aligned}$ | $\begin{aligned} & 171, \\ & 171 \end{aligned}$ | $\begin{aligned} & 171, \\ & 175 \end{aligned}$ | $\begin{gathered} 167, \\ 175 \end{gathered}$ | $\begin{aligned} & 175 \\ & 175 \end{aligned}$ | $\begin{gathered} 167, \\ 175 \end{gathered}$ | $\begin{aligned} & 167, \\ & 171 \end{aligned}$ | $\begin{aligned} & 171, \\ & 171 \end{aligned}$ | $\begin{gathered} 171, \\ 173 \end{gathered}$ | $\begin{aligned} & 171, \\ & 171 \end{aligned}$ | $\begin{aligned} & 171, \\ & 171 \end{aligned}$ | $\begin{aligned} & 171, \\ & 171 \end{aligned}$ |
| D181102 | $\begin{gathered} 62.8 \\ 4 \end{gathered}$ | $\begin{aligned} & \hline 214, \\ & 218 \end{aligned}$ | $\begin{aligned} & \hline 210, \\ & 210 \end{aligned}$ | $\begin{aligned} & 210 \\ & 218 \end{aligned}$ | $\begin{aligned} & 210, \\ & 214 \end{aligned}$ | $\begin{aligned} & 210, \\ & 218 \end{aligned}$ | $\begin{aligned} & 216, \\ & 216 \end{aligned}$ |  | $\begin{aligned} & 210, \\ & 216 \end{aligned}$ | $\begin{aligned} & 216, \\ & 218 \end{aligned}$ | $\begin{aligned} & 214, \\ & 218 \end{aligned}$ | $\begin{aligned} & 210, \\ & 218 \end{aligned}$ | $\begin{aligned} & 214, \\ & 216 \end{aligned}$ | $\begin{aligned} & 210, \\ & 214 \end{aligned}$ | $\begin{aligned} & 214, \\ & 214 \end{aligned}$ |  | $\begin{aligned} & \hline 210, \\ & 218 \end{aligned}$ | $\begin{aligned} & 214, \\ & 216 \end{aligned}$ | $\begin{aligned} & 214, \\ & 214 \end{aligned}$ |
| D18S1134 | $\begin{gathered} 88.6 \\ 2 \end{gathered}$ | $\begin{aligned} & 215 \\ & 215 \end{aligned}$ | $\begin{aligned} & 211, \\ & 213 \end{aligned}$ | $\begin{aligned} & 211, \\ & 215 \end{aligned}$ | $\begin{gathered} 211, \\ 215 \end{gathered}$ | $\begin{aligned} & 213, \\ & 215 \end{aligned}$ | $\begin{aligned} & 213, \\ & 215 \end{aligned}$ | $\begin{aligned} & 213, \\ & 215 \end{aligned}$ | $\begin{aligned} & 213, \\ & 215 \end{aligned}$ | $\begin{aligned} & 211, \\ & 215 \end{aligned}$ | $\begin{aligned} & \hline 211, \\ & 213 \end{aligned}$ | $\begin{aligned} & 213, \\ & 215 \end{aligned}$ | $\begin{aligned} & 213, \\ & 215 \end{aligned}$ | $\begin{aligned} & 211, \\ & 215 \end{aligned}$ | $\begin{aligned} & 211, \\ & 215 \end{aligned}$ | $\begin{aligned} & 209, \\ & 211 \end{aligned}$ | $\begin{aligned} & 209, \\ & 211 \end{aligned}$ |  |  |
| D18S1148 | $\begin{gathered} 90.6 \\ 0 \end{gathered}$ | $\begin{aligned} & \hline 144, \\ & 150 \end{aligned}$ | $\begin{aligned} & \hline 148, \\ & 150 \end{aligned}$ | $\begin{aligned} & 148, \\ & 150 \end{aligned}$ | $\begin{aligned} & 148, \\ & 150 \end{aligned}$ | $\begin{aligned} & \hline 144, \\ & 150 \end{aligned}$ | $\begin{gathered} 150, \\ 150 \end{gathered}$ | $\begin{aligned} & \hline 144, \\ & 150 \end{aligned}$ | $\begin{aligned} & 144, \\ & 150 \end{aligned}$ | $\begin{aligned} & \hline 148, \\ & 150 \end{aligned}$ | $\begin{gathered} 142, \\ 150 \end{gathered}$ | $\begin{aligned} & 150, \\ & 152 \end{aligned}$ | $\begin{aligned} & 150, \\ & 152 \end{aligned}$ | $\begin{aligned} & 148, \\ & 150 \end{aligned}$ | $\begin{aligned} & 144, \\ & 150 \end{aligned}$ | $\begin{aligned} & \hline 148, \\ & 150 \end{aligned}$ | $\begin{aligned} & \hline 148, \\ & 150 \end{aligned}$ |  |  |
| D18S1147 | $\begin{gathered} 90.6 \\ 0 \end{gathered}$ | $\begin{aligned} & 226 \\ & 226 \end{aligned}$ | $\begin{aligned} & 210, \\ & 226 \end{aligned}$ | $\begin{aligned} & 226, \\ & 226 \end{aligned}$ | $\begin{aligned} & 226 \\ & 226 \end{aligned}$ | $\begin{aligned} & 210, \\ & 226 \end{aligned}$ | $\begin{aligned} & 210, \\ & 226 \end{aligned}$ | $\begin{aligned} & 210, \\ & 226 \end{aligned}$ | $\begin{aligned} & 210 \\ & 226 \end{aligned}$ | $\begin{aligned} & 216, \\ & 226 \end{aligned}$ | $\begin{aligned} & 212, \\ & 226 \end{aligned}$ | $\begin{aligned} & 212, \\ & 226 \end{aligned}$ | $\begin{aligned} & 212, \\ & 226 \end{aligned}$ | $\begin{aligned} & 216 \\ & 226 \end{aligned}$ | $\begin{aligned} & 212, \\ & 226 \end{aligned}$ | $\begin{aligned} & 210, \\ & 220 \end{aligned}$ | $\begin{aligned} & 212, \\ & 220 \end{aligned}$ |  |  |
| D18S1148 | $\begin{gathered} 90.6 \\ 0 \end{gathered}$ | $\begin{aligned} & 144, \\ & 150 \end{aligned}$ | $\begin{aligned} & 148, \\ & 150 \end{aligned}$ | $\begin{gathered} 148, \\ 150 \end{gathered}$ | $\begin{gathered} 148, \\ 150 \end{gathered}$ | $\begin{gathered} 144, \\ 150 \end{gathered}$ | $\begin{gathered} 150, \\ 150 \end{gathered}$ | $\begin{aligned} & 144, \\ & 150 \end{aligned}$ | $\begin{aligned} & 144, \\ & 150 \end{aligned}$ | $\begin{gathered} 148, \\ 150 \end{gathered}$ | $\begin{aligned} & 142, \\ & 152 \end{aligned}$ | $\begin{aligned} & 150, \\ & 152 \end{aligned}$ | $\begin{aligned} & 150, \\ & 152 \end{aligned}$ | $\begin{aligned} & 148, \\ & 150 \end{aligned}$ | $\begin{aligned} & 144, \\ & 150 \end{aligned}$ | $\begin{gathered} 148, \\ 150 \end{gathered}$ | $\begin{aligned} & 148, \\ & 150 \end{aligned}$ |  |  |
| D18S1147 | $\begin{gathered} 90.6 \\ 0 \end{gathered}$ | $\begin{aligned} & 226, \\ & 226 \end{aligned}$ | $\begin{aligned} & 210, \\ & 226 \end{aligned}$ | $\begin{aligned} & 226, \\ & 226 \end{aligned}$ | $\begin{aligned} & 226 \\ & 226 \end{aligned}$ | $\begin{aligned} & 210, \\ & 226 \end{aligned}$ | $\begin{aligned} & 210, \\ & 226 \end{aligned}$ | $\begin{aligned} & 210, \\ & 226 \end{aligned}$ | $\begin{aligned} & 210, \\ & 226 \end{aligned}$ | $\begin{aligned} & 216, \\ & 226 \end{aligned}$ | $\begin{aligned} & 212, \\ & 226 \end{aligned}$ | $\begin{aligned} & 212, \\ & 226 \end{aligned}$ | $\begin{aligned} & 212, \\ & 226 \end{aligned}$ | $\begin{aligned} & 216 \\ & 226 \end{aligned}$ | $\begin{aligned} & 212, \\ & 226 \end{aligned}$ | $\begin{aligned} & 210, \\ & 220 \end{aligned}$ | $\begin{aligned} & 212, \\ & 220 \end{aligned}$ |  |  |
| D18S68 | $\begin{gathered} 96.4 \\ 8 \end{gathered}$ | $\begin{aligned} & 284, \\ & 288 \end{aligned}$ | $\begin{aligned} & 272, \\ & 288 \end{aligned}$ | $\begin{aligned} & 272, \\ & 284 \end{aligned}$ | $\begin{aligned} & 272, \\ & 284 \end{aligned}$ |  | $\begin{aligned} & 284, \\ & 284 \end{aligned}$ | $\begin{aligned} & 272, \\ & 280 \end{aligned}$ | $\begin{aligned} & 280, \\ & 284 \end{aligned}$ | $\begin{aligned} & 280, \\ & 284 \end{aligned}$ | $\begin{aligned} & 284, \\ & 284 \end{aligned}$ | $\begin{gathered} 284, \\ 288 \end{gathered}$ | $\begin{aligned} & 284, \\ & 288 \end{aligned}$ | $\begin{aligned} & 272, \\ & 284 \end{aligned}$ | $\begin{aligned} & 272, \\ & 284 \end{aligned}$ | $\begin{aligned} & 288, \\ & 288 \end{aligned}$ | $\begin{aligned} & 288, \\ & 288 \end{aligned}$ | $\begin{aligned} & 284, \\ & 288 \end{aligned}$ | $\begin{aligned} & 284, \\ & 284 \end{aligned}$ |
| D18S465 | $\begin{gathered} 100 . \\ 11 \end{gathered}$ | $\begin{aligned} & 239, \\ & 239 \end{aligned}$ | $\begin{aligned} & 239, \\ & 241 \end{aligned}$ | $\begin{aligned} & 239, \\ & 241 \end{aligned}$ | $\begin{aligned} & 239, \\ & 241 \end{aligned}$ | $\begin{aligned} & 239, \\ & 239 \end{aligned}$ | $\begin{aligned} & 239, \\ & 239 \end{aligned}$ | $\begin{aligned} & 239, \\ & 239 \end{aligned}$ | $\begin{aligned} & 239, \\ & 239 \end{aligned}$ | $\begin{aligned} & 239, \\ & 237 \end{aligned}$ | $\begin{aligned} & 243 \\ & 245 \end{aligned}$ | $\begin{aligned} & 237, \\ & 245 \end{aligned}$ | $\begin{aligned} & 237, \\ & 245 \end{aligned}$ | $\begin{aligned} & 237, \\ & 245 \end{aligned}$ | $\begin{aligned} & 239, \\ & 239 \end{aligned}$ | $\begin{aligned} & 239, \\ & 239 \end{aligned}$ | $\begin{aligned} & 239, \\ & 239 \end{aligned}$ |  |  |
| D18S61 | $\begin{gathered} 105 . \\ 03 \end{gathered}$ | $\begin{aligned} & 175 \\ & 179 \end{aligned}$ | $\begin{aligned} & 159 \\ & 175 \end{aligned}$ | $\begin{aligned} & 175 \\ & 175 \end{aligned}$ |  | $\begin{gathered} 159, \\ 179 \end{gathered}$ | $\begin{gathered} 159, \\ 179 \end{gathered}$ | $\begin{aligned} & 175 \\ & 175 \end{aligned}$ | $\begin{aligned} & 159 \\ & 175 \end{aligned}$ | $\begin{gathered} 159 \\ 175 \end{gathered}$ | $\begin{gathered} 175 \\ 175 \end{gathered}$ | $\begin{gathered} 159 \\ 175 \end{gathered}$ | $\begin{gathered} 159 \\ 175 \end{gathered}$ |  | $\begin{aligned} & 159, \\ & 175 \end{aligned}$ | $\begin{gathered} 159 \\ 179 \end{gathered}$ | $\begin{aligned} & 159, \\ & 179 \end{aligned}$ | $\begin{gathered} 177, \\ 179 \end{gathered}$ | $\begin{gathered} 159 \\ 177 \end{gathered}$ |


| D18S1161 | $\begin{gathered} 114 . \\ 26 \end{gathered}$ | $\begin{gathered} 82,9 \\ 4 \end{gathered}$ | $\begin{gathered} 94,9 \\ 4 \end{gathered}$ | $\begin{gathered} 82,9 \\ 4 \end{gathered}$ | $\begin{gathered} 94,9 \\ 4 \end{gathered}$ |  | $\begin{gathered} 94,1 \\ 04 \end{gathered}$ | $\begin{gathered} 94,9 \\ 4 \end{gathered}$ | $\begin{gathered} 94,1 \\ 04 \end{gathered}$ | $\begin{gathered} 94,1 \\ 04 \end{gathered}$ | $\begin{gathered} 82,9 \\ 4 \end{gathered}$ | $\begin{gathered} 94,9 \\ 4 \end{gathered}$ | $\begin{gathered} 94,1 \\ 04 \end{gathered}$ | $\begin{gathered} 82,9 \\ 0 \end{gathered}$ | $\begin{gathered} \hline 90,1 \\ 04 \end{gathered}$ | $\begin{gathered} 94,9 \\ 4 \end{gathered}$ | $\begin{gathered} 94,9 \\ 4 \end{gathered}$ | $\begin{gathered} 82,9 \\ 4 \end{gathered}$ | $\begin{gathered} 90,1 \\ 04 \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D18S462 | $\begin{gathered} 120 . \\ 05 \end{gathered}$ | $\begin{gathered} 187, \\ 193 \end{gathered}$ | $\begin{aligned} & 185, \\ & 185 \end{aligned}$ | $\begin{aligned} & 185, \\ & 193 \end{aligned}$ | $\begin{aligned} & 185, \\ & 187 \end{aligned}$ | $\begin{gathered} 185, \\ 187 \end{gathered}$ | $\begin{gathered} 185, \\ 187 \end{gathered}$ | $\begin{aligned} & 181, \\ & 187 \end{aligned}$ | $\begin{aligned} & 181, \\ & 185 \end{aligned}$ | $\begin{aligned} & 185, \\ & 187 \end{aligned}$ | $\begin{gathered} 187, \\ 193 \end{gathered}$ | $\begin{aligned} & 185 \\ & 187 \end{aligned}$ | $\begin{gathered} 185, \\ 187 \end{gathered}$ | $\begin{aligned} & 183, \\ & 187 \end{aligned}$ | $\begin{aligned} & 187, \\ & 187 \end{aligned}$ | $\begin{aligned} & 185, \\ & 187 \end{aligned}$ | $\begin{aligned} & 185, \\ & 187 \end{aligned}$ | $\begin{aligned} & 185, \\ & 187 \end{aligned}$ | $\begin{aligned} & 183, \\ & 185 \end{aligned}$ |
| D18S70 | $\begin{gathered} 126 . \\ 00 \end{gathered}$ | $\begin{aligned} & 112, \\ & 122 \end{aligned}$ | $\begin{aligned} & 106, \\ & 114 \end{aligned}$ | $\begin{aligned} & 106, \\ & 112 \end{aligned}$ | $\begin{aligned} & 106, \\ & 122 \end{aligned}$ | $\begin{aligned} & 114, \\ & 122 \end{aligned}$ | $\begin{aligned} & 112, \\ & 122 \end{aligned}$ | $\begin{aligned} & 114, \\ & 120 \end{aligned}$ | $\begin{aligned} & 114, \\ & 122 \end{aligned}$ | $\begin{aligned} & 120, \\ & 122 \end{aligned}$ | $\begin{aligned} & 112, \\ & 112 \end{aligned}$ | $\begin{aligned} & 114, \\ & 122 \end{aligned}$ | $\begin{aligned} & 112, \\ & 122 \end{aligned}$ |  | $\begin{aligned} & 112, \\ & 122 \end{aligned}$ | $\begin{aligned} & 114, \\ & 122 \end{aligned}$ | $\begin{aligned} & 106, \\ & 122 \end{aligned}$ | $\begin{aligned} & 112, \\ & 112 \end{aligned}$ | $\begin{aligned} & 120, \\ & 122 \end{aligned}$ |
| CA19S322 | 0.00 | $\begin{aligned} & 326, \\ & 326 \end{aligned}$ | $\begin{aligned} & 326, \\ & 326 \end{aligned}$ | $\begin{aligned} & 326, \\ & 326 \end{aligned}$ | $\begin{aligned} & 326, \\ & 326 \end{aligned}$ | $\begin{aligned} & 326, \\ & 326 \end{aligned}$ | $\begin{aligned} & 326, \\ & 326 \end{aligned}$ | $\begin{aligned} & 326, \\ & 326 \end{aligned}$ | $\begin{aligned} & 326, \\ & 326 \end{aligned}$ | $\begin{aligned} & 326, \\ & 326 \end{aligned}$ | $\begin{aligned} & 326, \\ & 326 \end{aligned}$ | $\begin{gathered} 326, \\ 326 \end{gathered}$ | $\begin{aligned} & 326, \\ & 326 \end{aligned}$ | $\begin{aligned} & 324, \\ & 326 \end{aligned}$ | $\begin{aligned} & 324, \\ & 326 \end{aligned}$ | $\begin{aligned} & 324, \\ & 326 \end{aligned}$ | $\begin{aligned} & 326, \\ & 326 \end{aligned}$ | $\begin{aligned} & 326, \\ & 326 \end{aligned}$ | $\begin{aligned} & 326, \\ & 326 \end{aligned}$ |
| D19S886 | 0.00 | $\begin{aligned} & 156, \\ & 158 \end{aligned}$ | $\begin{aligned} & 140, \\ & 160 \end{aligned}$ | $\begin{aligned} & 156, \\ & 160 \end{aligned}$ | $\begin{aligned} & 158, \\ & 160 \end{aligned}$ | $\begin{gathered} 156, \\ 160 \end{gathered}$ | $\begin{aligned} & 140, \\ & 156 \end{aligned}$ | $\begin{aligned} & 158, \\ & 160 \end{aligned}$ | $\begin{aligned} & 140, \\ & 158 \end{aligned}$ | $\begin{aligned} & 156, \\ & 158 \end{aligned}$ | $\begin{aligned} & 154, \\ & 156 \end{aligned}$ | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ | $\begin{gathered} 156, \\ 156 \end{gathered}$ | $\begin{aligned} & 156, \\ & 158 \end{aligned}$ | $\begin{aligned} & 156, \\ & 158 \end{aligned}$ | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ |
| GT19S949 | 0.00 | $\begin{aligned} & 272, \\ & 274 \end{aligned}$ | $\begin{aligned} & 256 \\ & 276 \end{aligned}$ | $\begin{aligned} & 272, \\ & 276 \end{aligned}$ | $\begin{aligned} & 274, \\ & 276 \end{aligned}$ | $\begin{aligned} & 272, \\ & 276 \end{aligned}$ | $\begin{aligned} & 256, \\ & 272 \end{aligned}$ | $\begin{aligned} & 274, \\ & 276 \end{aligned}$ | $\begin{aligned} & 256, \\ & 274 \end{aligned}$ | $\begin{aligned} & 272, \\ & 274 \end{aligned}$ | $\begin{aligned} & 270, \\ & 272 \end{aligned}$ | $\begin{aligned} & 272, \\ & 272 \end{aligned}$ | $\begin{aligned} & 272, \\ & 272 \end{aligned}$ | $\begin{aligned} & 272, \\ & 274 \end{aligned}$ | $\begin{aligned} & 272, \\ & 274 \end{aligned}$ | $\begin{aligned} & 272, \\ & 272 \end{aligned}$ | $\begin{aligned} & 272, \\ & 272 \end{aligned}$ | $\begin{aligned} & 272, \\ & 272 \end{aligned}$ | $\begin{aligned} & 272, \\ & 272 \end{aligned}$ |
| $\begin{gathered} \hline \text { ATA19S1 } \\ 001 \\ \hline \end{gathered}$ | 0.69 | $\begin{aligned} & 408, \\ & 411 \end{aligned}$ | $\begin{aligned} & 402, \\ & 411 \end{aligned}$ | $\begin{aligned} & 408, \\ & 411 \end{aligned}$ | $\begin{aligned} & \hline 411, \\ & 411 \end{aligned}$ | $\begin{aligned} & \hline 408, \\ & 411 \end{aligned}$ | $\begin{gathered} 402, \\ 408 \end{gathered}$ | $\begin{aligned} & 411, \\ & 411 \end{aligned}$ | $\begin{aligned} & 402, \\ & 411 \end{aligned}$ | $\begin{aligned} & \hline 408, \\ & 411 \end{aligned}$ | $\begin{aligned} & 393, \\ & 405 \end{aligned}$ | $\begin{gathered} 393, \\ 408 \end{gathered}$ | $\begin{aligned} & \hline 393, \\ & 408 \end{aligned}$ | $\begin{aligned} & \hline 402, \\ & 408 \end{aligned}$ | $\begin{gathered} 402, \\ 408 \end{gathered}$ | $\begin{aligned} & 389, \\ & 408 \end{aligned}$ | $\begin{gathered} 408, \\ 408 \end{gathered}$ | $\begin{aligned} & 408, \\ & 408 \end{aligned}$ | $\begin{aligned} & 389, \\ & 408 \end{aligned}$ |
| GT19S949 | 5.54 | $\begin{aligned} & 272, \\ & 274 \end{aligned}$ | $\begin{aligned} & 256 \\ & 276 \end{aligned}$ | $\begin{aligned} & 272, \\ & 276 \end{aligned}$ | $\begin{aligned} & 274, \\ & 276 \end{aligned}$ | $\begin{aligned} & 272, \\ & 276 \end{aligned}$ | $\begin{aligned} & 256, \\ & 272 \end{aligned}$ | $\begin{aligned} & 274, \\ & 276 \end{aligned}$ | $\begin{aligned} & 256 \\ & 274 \end{aligned}$ | $\begin{aligned} & 272, \\ & 274 \end{aligned}$ | $\begin{aligned} & 270, \\ & 272 \end{aligned}$ | $\begin{aligned} & 272, \\ & 272 \end{aligned}$ | $\begin{aligned} & 272, \\ & 272 \end{aligned}$ | $\begin{aligned} & 272, \\ & 274 \end{aligned}$ | $\begin{aligned} & 272, \\ & 274 \end{aligned}$ | $\begin{aligned} & 272, \\ & 272 \end{aligned}$ | $\begin{aligned} & 272, \\ & 272 \end{aligned}$ | $\begin{aligned} & 272, \\ & 272 \end{aligned}$ | $\begin{aligned} & 272, \\ & 272 \end{aligned}$ |
| $\begin{gathered} \text { ATA19S1 } \\ 001 \end{gathered}$ | 5.54 | $\begin{aligned} & 408, \\ & 411 \end{aligned}$ | $\begin{aligned} & 402, \\ & 411 \end{aligned}$ | $\begin{aligned} & 408, \\ & 411 \end{aligned}$ | $\begin{aligned} & \hline 411, \\ & 411 \end{aligned}$ | $\begin{gathered} 408, \\ 411 \end{gathered}$ | $\begin{gathered} 408, \\ 411 \end{gathered}$ | $\begin{aligned} & 411, \\ & 411 \end{aligned}$ | $\begin{aligned} & 402, \\ & 411 \end{aligned}$ | $\begin{aligned} & \hline 408, \\ & 411 \end{aligned}$ | $\begin{aligned} & \hline 393, \\ & 405 \end{aligned}$ | $\begin{gathered} \hline 393, \\ 408 \end{gathered}$ | $\begin{gathered} 393, \\ 408 \end{gathered}$ | $\begin{aligned} & 402, \\ & 408 \end{aligned}$ | $\begin{gathered} 402, \\ 408 \end{gathered}$ | $\begin{gathered} 389, \\ 408 \end{gathered}$ | $\begin{gathered} 408, \\ 408 \end{gathered}$ | $\begin{aligned} & 408, \\ & 408 \end{aligned}$ | $\begin{aligned} & 389, \\ & 408 \end{aligned}$ |
| $\begin{gathered} \text { CA19S231 } \\ 0 \end{gathered}$ | 6.57 | $\begin{aligned} & 270, \\ & 276 \end{aligned}$ | $\begin{aligned} & 272 \\ & 274 \end{aligned}$ | $\begin{aligned} & 274, \\ & 276 \end{aligned}$ | $\begin{aligned} & 270 \\ & 274 \end{aligned}$ | $\begin{aligned} & 274, \\ & 276 \end{aligned}$ | $\begin{aligned} & 272, \\ & 276 \end{aligned}$ | $\begin{aligned} & 270 \\ & 274 \end{aligned}$ | $\begin{aligned} & 270 \\ & 274 \end{aligned}$ | $\begin{aligned} & 270, \\ & 276 \end{aligned}$ | $\begin{aligned} & 266 \\ & 276 \end{aligned}$ | $\begin{aligned} & 276 \\ & 276 \end{aligned}$ | $\begin{aligned} & 276 \\ & 276 \end{aligned}$ | $\begin{aligned} & 266 \\ & 276 \end{aligned}$ | $\begin{aligned} & 266 \\ & 276 \end{aligned}$ | $\begin{aligned} & 268, \\ & 272 \end{aligned}$ | $\begin{aligned} & 268 \\ & 276 \end{aligned}$ | $\begin{aligned} & 268 \\ & 276 \end{aligned}$ | $\begin{aligned} & 266, \\ & 272 \end{aligned}$ |
| D19S878 | 6.57 | $\begin{aligned} & 212, \\ & 218 \end{aligned}$ | $\begin{aligned} & 214, \\ & 216 \end{aligned}$ | $\begin{aligned} & 216, \\ & 218 \end{aligned}$ | $\begin{aligned} & 212, \\ & 216 \end{aligned}$ | $\begin{aligned} & 216 \\ & 218 \end{aligned}$ | $\begin{aligned} & 214, \\ & 218 \end{aligned}$ | $\begin{aligned} & 212, \\ & 216 \end{aligned}$ | $\begin{aligned} & 212, \\ & 216 \end{aligned}$ | $\begin{aligned} & 212, \\ & 218 \end{aligned}$ | $\begin{gathered} 208, \\ 218 \end{gathered}$ | $\begin{aligned} & 218 \\ & 218 \end{aligned}$ | $\begin{aligned} & 218 \\ & 218 \end{aligned}$ | $\begin{aligned} & 208 \\ & 218 \end{aligned}$ | $\begin{aligned} & 208 \\ & 218 \end{aligned}$ | $\begin{aligned} & 210 \\ & 214 \end{aligned}$ | $\begin{aligned} & 210, \\ & 218 \end{aligned}$ | $\begin{aligned} & 210, \\ & 218 \end{aligned}$ | $\begin{aligned} & 208 \\ & 214 \end{aligned}$ |
| D19S565 | 6.57 | $\begin{aligned} & 166 \\ & 178 \end{aligned}$ | $\begin{aligned} & 186, \\ & 192 \end{aligned}$ | $\begin{aligned} & 178 \\ & 186 \end{aligned}$ | $\begin{gathered} 166 \\ 186 \end{gathered}$ | $\begin{aligned} & 178 \\ & 186 \end{aligned}$ | $\begin{aligned} & 178 \\ & 186 \end{aligned}$ | $\begin{aligned} & 166, \\ & 186 \end{aligned}$ | $\begin{aligned} & 166, \\ & 186 \end{aligned}$ | $\begin{aligned} & 166 \\ & 178 \end{aligned}$ | $\begin{gathered} 178, \\ 178 \end{gathered}$ | $\begin{gathered} 166 \\ 178 \end{gathered}$ | $\begin{gathered} 178 \\ 178 \end{gathered}$ | $\begin{aligned} & 176, \\ & 178 \end{aligned}$ | $\begin{aligned} & 176 \\ & 178 \end{aligned}$ | $\begin{gathered} 176 \\ 186 \end{gathered}$ | $\begin{gathered} 176 \\ 178 \end{gathered}$ | $\begin{aligned} & 176 \\ & 178 \end{aligned}$ | $\begin{gathered} 176 \\ 178 \end{gathered}$ |
| GT192518 | 6.57 | $\begin{aligned} & 293, \\ & 305 \end{aligned}$ | $\begin{aligned} & 315, \\ & 321 \end{aligned}$ | $\begin{aligned} & 305 \\ & 315 \end{aligned}$ | $\begin{gathered} 293, \\ 315 \end{gathered}$ | $\begin{aligned} & 305 \\ & 315 \end{aligned}$ | $\begin{gathered} 305, \\ 321 \end{gathered}$ | $\begin{aligned} & 293, \\ & 315 \end{aligned}$ | $\begin{aligned} & 293, \\ & 315 \end{aligned}$ | $\begin{gathered} 293, \\ 305 \end{gathered}$ | $\begin{gathered} 305 \\ 305 \end{gathered}$ | $\begin{gathered} 293 \\ 305 \end{gathered}$ | $\begin{gathered} 305, \\ 305 \end{gathered}$ | $\begin{aligned} & 303, \\ & 305 \end{aligned}$ | $\begin{gathered} 303, \\ 305 \end{gathered}$ | $\begin{gathered} 303, \\ 315 \end{gathered}$ | $\begin{gathered} 303, \\ 305 \end{gathered}$ | $\begin{aligned} & 303, \\ & 305 \end{aligned}$ | $\begin{aligned} & 303, \\ & 315 \end{aligned}$ |
| D19S591 | 9.84 | $\begin{gathered} 104 \\ 108 \end{gathered}$ | $\begin{aligned} & \hline 104, \\ & 108 \end{aligned}$ | $\begin{aligned} & 104, \\ & 104 \end{aligned}$ | $\begin{gathered} 108 \\ 108 \end{gathered}$ | $\begin{gathered} 104, \\ 108 \end{gathered}$ | $\begin{aligned} & \hline 104, \\ & 108 \end{aligned}$ | $\begin{aligned} & 104, \\ & 108 \end{aligned}$ | $\begin{aligned} & 104 \\ & 108 \end{aligned}$ | $\begin{gathered} 104, \\ 108 \end{gathered}$ | $\begin{gathered} 104, \\ 108 \end{gathered}$ | $\begin{gathered} 104, \\ 108 \end{gathered}$ | $\begin{aligned} & 104, \\ & 104 \end{aligned}$ | $\begin{aligned} & 100, \\ & 104 \end{aligned}$ | $\begin{aligned} & 100, \\ & 104 \end{aligned}$ | $\begin{gathered} 100, \\ 104 \end{gathered}$ | $\begin{aligned} & 104, \\ & 104 \end{aligned}$ | $\begin{aligned} & 104, \\ & 104 \end{aligned}$ | $\begin{aligned} & 100, \\ & 100 \end{aligned}$ |
| D19S424 | $\begin{gathered} 10.9 \\ 7 \end{gathered}$ | $\begin{aligned} & 147, \\ & 155 \end{aligned}$ | $\begin{aligned} & 155, \\ & 155 \end{aligned}$ | $\begin{aligned} & 147, \\ & 155 \end{aligned}$ | $\begin{aligned} & 155, \\ & 155 \end{aligned}$ | $\begin{gathered} 147, \\ 155 \end{gathered}$ | $\begin{gathered} 147, \\ 155 \end{gathered}$ | $\begin{aligned} & 155, \\ & 155 \end{aligned}$ | $\begin{aligned} & 155 \\ & 155 \end{aligned}$ | $\begin{aligned} & 147, \\ & 155 \end{aligned}$ | $\begin{aligned} & 153, \\ & 155 \end{aligned}$ | $\begin{gathered} 155 \\ 155 \end{gathered}$ | $\begin{gathered} 147, \\ 155 \end{gathered}$ | $\begin{aligned} & 147, \\ & 147 \end{aligned}$ | $\begin{aligned} & 147, \\ & 147 \end{aligned}$ | $\begin{aligned} & 147, \\ & 157 \end{aligned}$ | $\begin{aligned} & 147, \\ & 147 \end{aligned}$ | $\begin{aligned} & 147, \\ & 147 \end{aligned}$ | $\begin{aligned} & 147, \\ & 157 \end{aligned}$ |


| D19S209 | $\begin{gathered} 10.9 \\ 7 \end{gathered}$ | $\begin{aligned} & 262, \\ & 268 \end{aligned}$ | $\begin{aligned} & 264, \\ & 266 \end{aligned}$ | $\begin{aligned} & 262, \\ & 266 \end{aligned}$ | $\begin{gathered} 266, \\ 268 \end{gathered}$ | $\begin{aligned} & 262, \\ & 266 \end{aligned}$ | $\begin{aligned} & 262, \\ & 264 \end{aligned}$ | $\begin{aligned} & 266, \\ & 268 \end{aligned}$ | $\begin{aligned} & 266, \\ & 268 \end{aligned}$ | $\begin{gathered} 262, \\ 268 \end{gathered}$ | $\begin{aligned} & 270, \\ & 270 \end{aligned}$ | $\begin{aligned} & \hline 268, \\ & 270 \end{aligned}$ | $\begin{aligned} & 262, \\ & 270 \end{aligned}$ | $\begin{aligned} & 262, \\ & 272 \end{aligned}$ | $\begin{aligned} & 262, \\ & 272 \end{aligned}$ | $\begin{aligned} & 262, \\ & 264 \end{aligned}$ | $\begin{aligned} & 262, \\ & 262 \end{aligned}$ | $\begin{aligned} & 262, \\ & 262 \end{aligned}$ | $\begin{aligned} & 264, \\ & 272 \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D19S894 | $\begin{gathered} 15.5 \\ 5 \end{gathered}$ | $\begin{aligned} & 144, \\ & 154 \end{aligned}$ | $\begin{aligned} & 158, \\ & 160 \end{aligned}$ | $\begin{aligned} & 144, \\ & 160 \end{aligned}$ | $\begin{aligned} & 154, \\ & 160 \end{aligned}$ | $\begin{gathered} 144, \\ 160 \end{gathered}$ | $\begin{aligned} & 154, \\ & 158 \end{aligned}$ | $\begin{gathered} 154, \\ 160 \end{gathered}$ | $\begin{aligned} & 154, \\ & 160 \end{aligned}$ | $\begin{gathered} 144, \\ 154 \end{gathered}$ | $\begin{aligned} & 160, \\ & 162 \end{aligned}$ | $\begin{aligned} & 154, \\ & 160 \end{aligned}$ | $\begin{gathered} 144, \\ 160 \end{gathered}$ | $\begin{aligned} & 144, \\ & 144 \end{aligned}$ | $\begin{aligned} & 144, \\ & 144 \end{aligned}$ | $\begin{aligned} & 144, \\ & 146 \end{aligned}$ | $\begin{aligned} & 144, \\ & 146 \end{aligned}$ | $\begin{aligned} & 144, \\ & 146 \end{aligned}$ | $\begin{gathered} 144, \\ 144 \end{gathered}$ |
| D19S216 | $\begin{gathered} 20.0 \\ 1 \end{gathered}$ | $\begin{aligned} & 185, \\ & 187 \end{aligned}$ | $\begin{aligned} & 179, \\ & 187 \end{aligned}$ | $\begin{aligned} & 185, \\ & 187 \end{aligned}$ | $\begin{gathered} 187, \\ 187 \end{gathered}$ | $\begin{gathered} 185 \\ 187 \end{gathered}$ | $\begin{aligned} & \hline 179, \\ & 187 \end{aligned}$ | $\begin{aligned} & 187, \\ & 187 \end{aligned}$ | $\begin{aligned} & 187, \\ & 187 \end{aligned}$ | $\begin{gathered} 185, \\ 187 \end{gathered}$ | $\begin{gathered} 185, \\ 191 \end{gathered}$ |  | $\begin{gathered} 185, \\ 191 \end{gathered}$ | $\begin{aligned} & 179, \\ & 185 \end{aligned}$ | $\begin{aligned} & 179, \\ & 185 \end{aligned}$ | $\begin{aligned} & 179, \\ & 185 \end{aligned}$ | $\begin{aligned} & 185, \\ & 185 \end{aligned}$ | $\begin{aligned} & 185, \\ & 185 \end{aligned}$ | $\begin{gathered} 185, \\ 185 \end{gathered}$ |
| D19S1034 | $\begin{gathered} 20.7 \\ 5 \end{gathered}$ | $\begin{aligned} & 226, \\ & 234 \end{aligned}$ | $\begin{aligned} & 234, \\ & 234 \end{aligned}$ | $\begin{aligned} & 234, \\ & 234 \end{aligned}$ | $\begin{aligned} & 226, \\ & 234 \end{aligned}$ | $\begin{aligned} & 234, \\ & 234 \end{aligned}$ | $\begin{aligned} & 226, \\ & 234 \end{aligned}$ | $\begin{aligned} & 226, \\ & 234 \end{aligned}$ | $\begin{aligned} & 226, \\ & 234 \end{aligned}$ | $\begin{aligned} & 226, \\ & 234 \end{aligned}$ | $\begin{aligned} & 230, \\ & 234 \end{aligned}$ | $\begin{aligned} & 226 \\ & 234 \end{aligned}$ | $\begin{aligned} & 234, \\ & 234 \end{aligned}$ | $\begin{aligned} & 230, \\ & 234 \end{aligned}$ | $\begin{aligned} & 230, \\ & 234 \end{aligned}$ | $\begin{aligned} & 234, \\ & 238 \end{aligned}$ | $\begin{aligned} & 234, \\ & 238 \end{aligned}$ | $\begin{aligned} & 234, \\ & 238 \end{aligned}$ | $\begin{aligned} & 234, \\ & 234 \end{aligned}$ |
| D19S884 | $\begin{gathered} 26.3 \\ 7 \end{gathered}$ | $\begin{aligned} & 218, \\ & 226 \end{aligned}$ | $\begin{aligned} & 224, \\ & 224 \end{aligned}$ | $\begin{aligned} & 224, \\ & 226 \end{aligned}$ | $\begin{aligned} & 218, \\ & 224 \end{aligned}$ | $\begin{aligned} & 224, \\ & 226 \end{aligned}$ | $\begin{aligned} & 218, \\ & 224 \end{aligned}$ | $\begin{aligned} & 218, \\ & 224 \end{aligned}$ | $\begin{aligned} & 218, \\ & 224 \end{aligned}$ | $\begin{aligned} & 226, \\ & 226 \end{aligned}$ | $\begin{aligned} & 226, \\ & 234 \end{aligned}$ | $\begin{aligned} & 226, \\ & 226 \end{aligned}$ | $\begin{aligned} & 226, \\ & 226 \end{aligned}$ | $\begin{aligned} & 218, \\ & 236 \end{aligned}$ | $\begin{aligned} & 218, \\ & 226 \end{aligned}$ |  | $\begin{aligned} & 226, \\ & 234 \end{aligned}$ | $\begin{aligned} & 222, \\ & 226 \end{aligned}$ | $\begin{aligned} & 218, \\ & 234 \end{aligned}$ |
| D19S221 | $\begin{gathered} 36.2 \\ 2 \end{gathered}$ | $\begin{aligned} & 207, \\ & 207 \end{aligned}$ | $\begin{aligned} & 205, \\ & 207 \end{aligned}$ | $\begin{aligned} & 205, \\ & 207 \end{aligned}$ | $\begin{gathered} 205, \\ 207 \end{gathered}$ | $\begin{gathered} 205 \\ 207 \end{gathered}$ | $\begin{aligned} & 207, \\ & 207 \end{aligned}$ | $\begin{aligned} & 205 \\ & 207 \end{aligned}$ | $\begin{aligned} & 205, \\ & 207 \end{aligned}$ | $\begin{gathered} 207, \\ 207 \end{gathered}$ | $\begin{aligned} & 201, \\ & 203 \end{aligned}$ |  | $\begin{gathered} 203, \\ 207 \end{gathered}$ | $\begin{aligned} & 207, \\ & 207 \end{aligned}$ | $\begin{aligned} & 207, \\ & 207 \end{aligned}$ | $\begin{aligned} & 207, \\ & 207 \end{aligned}$ | $\begin{aligned} & 207, \\ & 207 \end{aligned}$ |  | $\begin{aligned} & 207, \\ & 207 \end{aligned}$ |
| D19S226 | $\begin{gathered} 42.2 \\ 8 \end{gathered}$ | $\begin{aligned} & 253, \\ & 243 \end{aligned}$ | $\begin{aligned} & 241, \\ & 243 \end{aligned}$ | $\begin{aligned} & 235, \\ & 243 \end{aligned}$ | $\begin{aligned} & 243, \\ & 243 \end{aligned}$ | $\begin{aligned} & 243, \\ & 235 \end{aligned}$ | $\begin{aligned} & 241, \\ & 243 \end{aligned}$ | $\begin{aligned} & 243, \\ & 243 \end{aligned}$ | $\begin{aligned} & 243, \\ & 243 \end{aligned}$ | $\begin{aligned} & 253, \\ & 235 \end{aligned}$ | $\begin{aligned} & 233, \\ & 235 \end{aligned}$ | $\begin{aligned} & 235, \\ & 253 \end{aligned}$ | $\begin{aligned} & 233, \\ & 235 \end{aligned}$ | $\begin{aligned} & 243, \\ & 245 \end{aligned}$ | $\begin{aligned} & 235, \\ & 243 \end{aligned}$ | $\begin{aligned} & 243, \\ & 245 \end{aligned}$ | $\begin{aligned} & 235, \\ & 243 \end{aligned}$ |  |  |
| D19S410 | $\begin{gathered} 45.4 \\ 8 \end{gathered}$ | $\begin{aligned} & 156, \\ & 170 \end{aligned}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ | $\begin{aligned} & 170, \\ & 170 \\ & \hline \end{aligned}$ | $\begin{aligned} & 156, \\ & 170 \end{aligned}$ | $\begin{aligned} & 170, \\ & 170 \end{aligned}$ | $\begin{aligned} & 156, \\ & 170 \end{aligned}$ | $\begin{aligned} & 156, \\ & 170 \end{aligned}$ | $\begin{aligned} & 156, \\ & 170 \end{aligned}$ | $\begin{gathered} 156, \\ 170 \end{gathered}$ | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ | $\begin{gathered} 156, \\ 170 \end{gathered}$ | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ | $\begin{aligned} & 156, \\ & 170 \end{aligned}$ | $\begin{aligned} & 156, \\ & 170 \end{aligned}$ | $\begin{aligned} & 156, \\ & 170 \end{aligned}$ |  |  |
| D19S414 | $\begin{gathered} 54.0 \\ 1 \end{gathered}$ | $\begin{aligned} & 163, \\ & 177 \end{aligned}$ | $\begin{aligned} & 179, \\ & 181 \end{aligned}$ | $\begin{aligned} & 177, \\ & 181 \end{aligned}$ | $\begin{gathered} 181, \\ 163 \end{gathered}$ | $\begin{gathered} \hline 181, \\ 163 \end{gathered}$ | $\begin{gathered} 163, \\ 179 \end{gathered}$ | $\begin{aligned} & 163, \\ & 181 \end{aligned}$ | $\begin{aligned} & 163, \\ & 179 \end{aligned}$ | $\begin{gathered} 177, \\ 163 \end{gathered}$ | $\begin{aligned} & 181, \\ & 181 \end{aligned}$ | $\begin{gathered} 181, \\ 163 \end{gathered}$ | $\begin{aligned} & \hline 177, \\ & 181 \end{aligned}$ | $\begin{aligned} & 163, \\ & 163 \end{aligned}$ | $\begin{aligned} & 163, \\ & 163 \end{aligned}$ | $\begin{aligned} & 163, \\ & 185 \end{aligned}$ | $\begin{aligned} & 163, \\ & 163 \end{aligned}$ |  |  |
| D19S220 | $\begin{gathered} 62.0 \\ 3 \end{gathered}$ | $\begin{aligned} & 273 \\ & 277 \end{aligned}$ | $\begin{aligned} & 275, \\ & 277 \end{aligned}$ | $\begin{aligned} & 273 \\ & 275 \end{aligned}$ |  | $\begin{aligned} & 275 \\ & 277 \end{aligned}$ | $\begin{aligned} & 277, \\ & 277 \end{aligned}$ | $\begin{aligned} & 275 \\ & 277 \end{aligned}$ | $\begin{aligned} & 277, \\ & 277 \end{aligned}$ | $\begin{aligned} & 273, \\ & 281 \end{aligned}$ | $\begin{aligned} & 275 \\ & 279 \end{aligned}$ | $\begin{aligned} & 273, \\ & 279 \end{aligned}$ | $\begin{aligned} & 273 \\ & 275 \end{aligned}$ | $\begin{aligned} & 267, \\ & 277 \end{aligned}$ | $\begin{aligned} & 267, \\ & 277 \end{aligned}$ |  | $\begin{aligned} & 277, \\ & 279 \end{aligned}$ |  | $\begin{aligned} & 277, \\ & 279 \end{aligned}$ |
| D19S420 | $\begin{gathered} 66.3 \\ 0 \end{gathered}$ | $\begin{aligned} & 255 \\ & 265 \end{aligned}$ | $\begin{aligned} & 259, \\ & 265 \end{aligned}$ | $\begin{aligned} & 259 \\ & 265 \end{aligned}$ | $\begin{aligned} & 255 \\ & 259 \end{aligned}$ | $\begin{aligned} & 255, \\ & 259 \end{aligned}$ | $\begin{aligned} & 255 \\ & 265 \end{aligned}$ | $\begin{aligned} & 255, \\ & 259 \end{aligned}$ | $\begin{aligned} & 255 \\ & 265 \end{aligned}$ | $\begin{aligned} & 257, \\ & 265 \end{aligned}$ | $\begin{aligned} & 263, \\ & 263 \end{aligned}$ | $\begin{aligned} & 257, \\ & 263 \end{aligned}$ | $\begin{aligned} & 263, \\ & 265 \end{aligned}$ | $\begin{aligned} & 255, \\ & 263 \end{aligned}$ | $\begin{aligned} & 255, \\ & 263 \end{aligned}$ | $\begin{aligned} & 257, \\ & 263 \end{aligned}$ | $\begin{aligned} & 255, \\ & 257 \end{aligned}$ | $\begin{aligned} & 255, \\ & 263 \end{aligned}$ | $\begin{aligned} & 255, \\ & 257 \end{aligned}$ |
| D19S902 | $\begin{gathered} 72.7 \\ 2 \end{gathered}$ | $\begin{aligned} & 209, \\ & 217 \end{aligned}$ | $\begin{aligned} & 203, \\ & 209 \end{aligned}$ | $\begin{aligned} & 203, \\ & 217 \end{aligned}$ | $\begin{aligned} & 203, \\ & 209 \end{aligned}$ | $\begin{aligned} & 203, \\ & 209 \end{aligned}$ | $\begin{aligned} & 203 \\ & 209 \end{aligned}$ | $\begin{aligned} & 203, \\ & 209 \end{aligned}$ | $\begin{aligned} & 209 \\ & 209 \end{aligned}$ | $\begin{aligned} & 213, \\ & 217 \end{aligned}$ | $\begin{aligned} & 209, \\ & 211 \end{aligned}$ | $\begin{aligned} & 209 \\ & 213 \end{aligned}$ | $\begin{aligned} & 211, \\ & 217 \end{aligned}$ | $\begin{aligned} & 209, \\ & 217 \end{aligned}$ | $\begin{aligned} & 209, \\ & 213 \end{aligned}$ |  | $\begin{aligned} & 209, \\ & 209 \end{aligned}$ | $\begin{aligned} & 209, \\ & 211 \end{aligned}$ | $\begin{aligned} & 209, \\ & 209 \end{aligned}$ |
| D19S210 | $\begin{gathered} 100 . \\ 01 \end{gathered}$ | $\begin{aligned} & 169, \\ & 175 \end{aligned}$ | $\begin{aligned} & 175 \\ & 175 \end{aligned}$ | $\begin{aligned} & 169, \\ & 175 \end{aligned}$ | $\begin{aligned} & 169 \\ & 175 \end{aligned}$ | $\begin{aligned} & 175 \\ & 175 \end{aligned}$ | $\begin{gathered} 175 \\ 175 \end{gathered}$ | $\begin{aligned} & 175, \\ & 175 \end{aligned}$ | $\begin{aligned} & 175, \\ & 175 \end{aligned}$ | $\begin{gathered} 169, \\ 169 \end{gathered}$ | $\begin{gathered} 165, \\ 169 \end{gathered}$ |  | $\begin{gathered} 165, \\ 169 \end{gathered}$ | $\begin{aligned} & 169, \\ & 175 \end{aligned}$ | $\begin{aligned} & 169, \\ & 175 \end{aligned}$ | $\begin{aligned} & 165, \\ & 165 \end{aligned}$ | $\begin{aligned} & 165, \\ & 175 \end{aligned}$ | $\begin{aligned} & 165, \\ & 169 \end{aligned}$ | $\begin{gathered} 165, \\ 175 \end{gathered}$ |
| D20S864 | 0.00 | $\begin{aligned} & 224, \\ & 226 \end{aligned}$ | $\begin{gathered} 224, \\ 26 \end{gathered}$ | $\begin{aligned} & 224, \\ & 226 \end{aligned}$ | $\begin{aligned} & 224, \\ & 226 \end{aligned}$ | $\begin{aligned} & 224, \\ & 226 \end{aligned}$ | $\begin{aligned} & 224, \\ & 226 \end{aligned}$ |  |  | $\begin{aligned} & 224, \\ & 228 \end{aligned}$ | $\begin{aligned} & 226, \\ & 228 \end{aligned}$ | $\begin{gathered} 226, \\ 228 \end{gathered}$ | $\begin{gathered} 226, \\ 228 \end{gathered}$ | $\begin{aligned} & 224, \\ & 226 \end{aligned}$ | $\begin{aligned} & 226, \\ & 228 \end{aligned}$ | $\begin{aligned} & 226, \\ & 228 \end{aligned}$ | $\begin{aligned} & 226, \\ & 228 \end{aligned}$ |  |  |
| D20S199 | 6.25 | $\begin{aligned} & 108, \\ & 120 \end{aligned}$ | $\begin{aligned} & 108, \\ & 120 \end{aligned}$ | $\begin{aligned} & 108, \\ & 108 \end{aligned}$ | $\begin{gathered} 108 \\ 108 \end{gathered}$ | $\begin{gathered} 108, \\ 108 \end{gathered}$ | $\begin{gathered} 108, \\ 120 \end{gathered}$ |  |  | $\begin{aligned} & 112, \\ & 116 \end{aligned}$ | $\begin{gathered} 108, \\ 120 \end{gathered}$ | $\begin{gathered} 108 \\ 116 \end{gathered}$ | $\begin{aligned} & 108 \\ & 116 \end{aligned}$ | $\begin{aligned} & 108, \\ & 120 \end{aligned}$ | $\begin{aligned} & 112, \\ & 116 \end{aligned}$ | $\begin{aligned} & 112, \\ & 114 \end{aligned}$ | $\begin{aligned} & 112, \\ & 112 \end{aligned}$ |  |  |


| D20S113 | 8.97 | $\begin{aligned} & 178, \\ & 178 \end{aligned}$ | $\begin{aligned} & 178, \\ & 178 \end{aligned}$ | $\begin{aligned} & 178, \\ & 178 \end{aligned}$ | $\begin{aligned} & 178, \\ & 178 \end{aligned}$ | $\begin{aligned} & 178 \\ & 178 \end{aligned}$ | $\begin{aligned} & 178, \\ & 178 \end{aligned}$ |  |  | $\begin{aligned} & 178, \\ & 178 \end{aligned}$ | $\begin{aligned} & 178, \\ & 186 \end{aligned}$ | $\begin{aligned} & \hline 178, \\ & 186 \end{aligned}$ | $\begin{aligned} & 178 \\ & 178 \end{aligned}$ | $\begin{aligned} & 178, \\ & 178 \end{aligned}$ | $\begin{aligned} & 178, \\ & 178 \end{aligned}$ | $\begin{aligned} & 178 \\ & 178 \end{aligned}$ | $\begin{aligned} & 178, \\ & 178 \end{aligned}$ |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D20S842 | 8.97 | $\begin{aligned} & 164, \\ & 168 \end{aligned}$ | $\begin{aligned} & 160, \\ & 164 \end{aligned}$ | $\begin{aligned} & 160, \\ & 168 \end{aligned}$ | $\begin{gathered} 160, \\ 168 \end{gathered}$ | $\begin{gathered} 160, \\ 164 \end{gathered}$ | $\begin{gathered} \hline 160, \\ 168 \end{gathered}$ |  |  | $\begin{gathered} 164, \\ 172 \end{gathered}$ | $\begin{gathered} 172, \\ 174 \end{gathered}$ | $\begin{gathered} 172, \\ 174 \end{gathered}$ | $\begin{aligned} & \hline 164, \\ & 172 \end{aligned}$ | $\begin{aligned} & 166, \\ & 172 \end{aligned}$ | $\begin{aligned} & \hline 164, \\ & 168 \end{aligned}$ | $\begin{gathered} 156 \\ 176 \end{gathered}$ | $\begin{aligned} & 156, \\ & 168 \end{aligned}$ |  |  |
| D20S181 | 9.53 | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ | $\begin{aligned} & 164, \\ & 164 \end{aligned}$ | $\begin{aligned} & 156, \\ & 164 \end{aligned}$ | $\begin{aligned} & 156, \\ & 164 \end{aligned}$ | $\begin{aligned} & 156, \\ & 164 \end{aligned}$ | $\begin{aligned} & \hline 156, \\ & 164 \end{aligned}$ |  |  | $\begin{gathered} 156, \\ 160 \end{gathered}$ | $\begin{aligned} & \hline 164, \\ & 164 \end{aligned}$ | $\begin{aligned} & 160, \\ & 164 \end{aligned}$ | $\begin{aligned} & 156, \\ & 164 \end{aligned}$ | $\begin{aligned} & 156, \\ & 160 \end{aligned}$ | $\begin{aligned} & 156, \\ & 156 \end{aligned}$ | $\begin{aligned} & 164, \\ & 164 \end{aligned}$ | $\begin{aligned} & 156, \\ & 164 \end{aligned}$ |  |  |
| D20S193 | 9.53 | $\begin{aligned} & 150, \\ & 154 \end{aligned}$ | $\begin{aligned} & 150, \\ & 158 \end{aligned}$ | $\begin{aligned} & 154 \\ & 158 \end{aligned}$ | $\begin{aligned} & 154, \\ & 158 \end{aligned}$ | $\begin{aligned} & 150, \\ & 158 \end{aligned}$ | $\begin{aligned} & 154, \\ & 158 \end{aligned}$ |  |  | $\begin{gathered} 150, \\ 150 \end{gathered}$ | $\begin{gathered} 150, \\ 150 \end{gathered}$ | $\begin{aligned} & 150 \\ & 150 \end{aligned}$ | $\begin{aligned} & 150, \\ & 150 \end{aligned}$ | $\begin{aligned} & 150, \\ & 150 \end{aligned}$ | $\begin{aligned} & 150, \\ & 154 \end{aligned}$ | $\begin{aligned} & 150, \\ & 154 \end{aligned}$ | $\begin{aligned} & 150, \\ & 154 \end{aligned}$ |  |  |
| D20S116 | $\begin{gathered} 11.2 \\ 0 \end{gathered}$ | $\begin{aligned} & 109 \\ & 109 \end{aligned}$ | $\begin{aligned} & 115, \\ & 119 \end{aligned}$ | $\begin{aligned} & 109, \\ & 119 \end{aligned}$ | $\begin{aligned} & 109 \\ & 119 \end{aligned}$ | $\begin{aligned} & 109 \\ & 119 \end{aligned}$ | $\begin{aligned} & 109, \\ & 110 \end{aligned}$ |  |  | $\begin{gathered} 109, \\ 121 \end{gathered}$ | $\begin{aligned} & 109, \\ & 111 \end{aligned}$ | $\begin{aligned} & 109 \\ & 121 \end{aligned}$ | $\begin{aligned} & \hline 109 \\ & 111 \end{aligned}$ | $\begin{aligned} & 113, \\ & 121 \end{aligned}$ | $\begin{aligned} & 109, \\ & 109 \end{aligned}$ | $\begin{aligned} & 109 \\ & 117 \end{aligned}$ | $\begin{aligned} & 109, \\ & 117 \end{aligned}$ |  |  |
| D20S889 | $\begin{gathered} 11.2 \\ 0 \end{gathered}$ | $\begin{aligned} & 266, \\ & 280 \end{aligned}$ | $\begin{aligned} & 272, \\ & 278 \end{aligned}$ | $\begin{aligned} & 266, \\ & 278 \end{aligned}$ | $\begin{aligned} & 266, \\ & 278 \end{aligned}$ | $\begin{aligned} & 278, \\ & 280 \end{aligned}$ | $\begin{aligned} & 266, \\ & 278 \end{aligned}$ | $\begin{aligned} & 278, \\ & 280 \end{aligned}$ | $\begin{aligned} & 266, \\ & 272 \end{aligned}$ | $\begin{aligned} & 280, \\ & 292 \end{aligned}$ | $\begin{aligned} & 272, \\ & 282 \end{aligned}$ |  | $\begin{aligned} & 272, \\ & 280 \end{aligned}$ | $\begin{aligned} & 290, \\ & 292 \end{aligned}$ | $\begin{aligned} & 266, \\ & 280 \end{aligned}$ |  | $\begin{aligned} & 266, \\ & 272 \end{aligned}$ |  | $\begin{aligned} & 280, \\ & 284 \end{aligned}$ |
| D20S849 | $\begin{gathered} 13.9 \\ 8 \end{gathered}$ | $\begin{aligned} & 214, \\ & 230 \end{aligned}$ | $\begin{aligned} & 214, \\ & 216 \end{aligned}$ | $\begin{aligned} & 214, \\ & 214 \end{aligned}$ | $\begin{aligned} & 214, \\ & 214 \end{aligned}$ | $\begin{aligned} & 214, \\ & 230 \end{aligned}$ | $\begin{aligned} & 214, \\ & 214 \end{aligned}$ |  |  | $\begin{aligned} & 214, \\ & 230 \end{aligned}$ | $\begin{aligned} & 214, \\ & 230 \end{aligned}$ | $\begin{aligned} & 214, \\ & 230 \end{aligned}$ | $\begin{aligned} & 214, \\ & 230 \end{aligned}$ | $\begin{aligned} & 214, \\ & 230 \end{aligned}$ | $\begin{aligned} & 214, \\ & 230 \end{aligned}$ | $\begin{aligned} & 214, \\ & 230 \end{aligned}$ | $\begin{aligned} & 214, \\ & 230 \end{aligned}$ |  |  |
| D20S895 | $\begin{gathered} 13.9 \\ 8 \end{gathered}$ | $\begin{aligned} & 216, \\ & 224 \end{aligned}$ | $\begin{aligned} & 216, \\ & 224 \end{aligned}$ | $\begin{aligned} & 216 \\ & 216 \end{aligned}$ | $\begin{aligned} & 216 \\ & 216 \end{aligned}$ | $\begin{aligned} & 216 \\ & 216 \end{aligned}$ | $\begin{aligned} & 216, \\ & 216 \end{aligned}$ |  |  | $\begin{aligned} & 224, \\ & 224 \end{aligned}$ | $\begin{aligned} & 216, \\ & 218 \end{aligned}$ | $\begin{aligned} & 216, \\ & 224 \end{aligned}$ | $\begin{aligned} & 218, \\ & 224 \end{aligned}$ | $\begin{aligned} & 224, \\ & 224 \end{aligned}$ | $\begin{aligned} & 216, \\ & 216 \end{aligned}$ | $\begin{aligned} & 220, \\ & 226 \end{aligned}$ | $\begin{aligned} & 216, \\ & 220 \end{aligned}$ |  |  |
| D20S882 | $\begin{gathered} 15.0 \\ 5 \end{gathered}$ | $\begin{aligned} & 120, \\ & 126 \end{aligned}$ | $\begin{aligned} & 120, \\ & 122 \end{aligned}$ | $\begin{aligned} & 120 \\ & 122 \end{aligned}$ | $\begin{aligned} & 120, \\ & 120 \end{aligned}$ | $\begin{aligned} & 120, \\ & 126 \end{aligned}$ | $\begin{aligned} & \hline 120, \\ & 120 \end{aligned}$ |  |  | $\begin{aligned} & 120 \\ & 126 \end{aligned}$ | $\begin{aligned} & 120, \\ & 120 \end{aligned}$ | $\begin{aligned} & 120, \\ & 120 \end{aligned}$ | $\begin{aligned} & 120, \\ & 126 \end{aligned}$ | $\begin{aligned} & 120, \\ & 126 \end{aligned}$ | $\begin{aligned} & 120, \\ & 126 \end{aligned}$ | $\begin{aligned} & 120, \\ & 126 \end{aligned}$ | $\begin{aligned} & 120, \\ & 120 \end{aligned}$ |  |  |
| D20S115 | $\begin{gathered} 21.1 \\ 5 \end{gathered}$ | $\begin{aligned} & 234, \\ & 236 \end{aligned}$ | $\begin{aligned} & 232, \\ & 236 \end{aligned}$ | $\begin{aligned} & 236, \\ & 236 \end{aligned}$ | $\begin{aligned} & 232, \\ & 236 \end{aligned}$ | $\begin{aligned} & 232, \\ & 234 \end{aligned}$ | $\begin{aligned} & 232, \\ & 236 \end{aligned}$ | $\begin{aligned} & 232, \\ & 234 \end{aligned}$ | $\begin{aligned} & 232, \\ & 236 \end{aligned}$ | $\begin{aligned} & 234, \\ & 236 \end{aligned}$ | $\begin{aligned} & 234, \\ & 234 \end{aligned}$ |  | $\begin{aligned} & 234, \\ & 236 \end{aligned}$ | $\begin{aligned} & 234, \\ & 236 \end{aligned}$ | $\begin{aligned} & 234, \\ & 236 \end{aligned}$ | $\begin{aligned} & 236 \\ & 236 \end{aligned}$ | $\begin{aligned} & 236, \\ & 236 \end{aligned}$ | $\begin{aligned} & 234, \\ & 236 \end{aligned}$ | $\begin{aligned} & 234, \\ & 236 \end{aligned}$ |
| D20S851 | $\begin{gathered} 24.7 \\ 0 \end{gathered}$ | $\begin{aligned} & 132, \\ & 132 \end{aligned}$ | $\begin{aligned} & 128, \\ & 136 \end{aligned}$ | $\begin{aligned} & 138 \\ & 132 \end{aligned}$ | $\begin{aligned} & 132, \\ & 136 \end{aligned}$ | $\begin{gathered} 132, \\ 136 \end{gathered}$ | $\begin{gathered} 132, \\ 136 \end{gathered}$ |  |  | $\begin{aligned} & 128, \\ & 132 \end{aligned}$ | $\begin{gathered} 132, \\ 146 \end{gathered}$ | $\begin{aligned} & 132, \\ & 146 \end{aligned}$ | $\begin{aligned} & 128, \\ & 132 \end{aligned}$ | $\begin{aligned} & 128, \\ & 132 \end{aligned}$ | $\begin{aligned} & 132, \\ & 132 \end{aligned}$ | $\begin{gathered} 132, \\ 134 \end{gathered}$ | $\begin{aligned} & 132, \\ & 134 \end{aligned}$ |  |  |
| D20S186 | $\begin{gathered} 32.3 \\ 0 \end{gathered}$ |  | $\begin{aligned} & 127, \\ & 131 \end{aligned}$ | $\begin{aligned} & 121, \\ & 131 \end{aligned}$ | $\begin{aligned} & 121, \\ & 127 \end{aligned}$ | $\begin{gathered} 125 \\ 127 \end{gathered}$ | $\begin{aligned} & 121, \\ & 127 \end{aligned}$ | $\begin{aligned} & 125 \\ & 127 \end{aligned}$ | $\begin{aligned} & 121, \\ & 127 \end{aligned}$ | $\begin{aligned} & 113, \\ & 131 \end{aligned}$ | $\begin{aligned} & 113, \\ & 119 \end{aligned}$ |  | $\begin{aligned} & 113, \\ & 131 \end{aligned}$ | $\begin{aligned} & 125, \\ & 131 \end{aligned}$ | $\begin{aligned} & 121, \\ & 125 \end{aligned}$ |  | $\begin{aligned} & 113, \\ & 121 \end{aligned}$ | $\begin{aligned} & 121, \\ & 125 \end{aligned}$ | $\begin{gathered} 121, \\ 125 \end{gathered}$ |
| D20S112 | $\begin{gathered} 39.2 \\ 5 \end{gathered}$ | $\begin{aligned} & 199, \\ & 211 \end{aligned}$ | $\begin{aligned} & 199, \\ & 207 \end{aligned}$ | $\begin{aligned} & 207, \\ & 211 \end{aligned}$ | $\begin{aligned} & 199, \\ & 211 \end{aligned}$ | $\begin{gathered} 199, \\ 199 \\ \hline \end{gathered}$ | $\begin{gathered} 199, \\ 199 \end{gathered}$ | $\begin{aligned} & 199, \\ & 211 \end{aligned}$ | $\begin{aligned} & 199, \\ & 211 \end{aligned}$ | $\begin{gathered} 207, \\ 213 \end{gathered}$ | $\begin{gathered} 209, \\ 219 \end{gathered}$ |  | $\begin{aligned} & 209, \\ & 213 \end{aligned}$ | $\begin{aligned} & 207, \\ & 213 \end{aligned}$ | $\begin{aligned} & 199, \\ & 211 \end{aligned}$ |  | $\begin{aligned} & 209, \\ & 211 \end{aligned}$ | $\begin{aligned} & 199, \\ & 209 \end{aligned}$ | $\begin{aligned} & 199, \\ & 209 \end{aligned}$ |
| D20S107 | $\begin{gathered} 55.7 \\ 4 \end{gathered}$ | $\begin{aligned} & 266, \\ & 274 \end{aligned}$ | $\begin{aligned} & 272, \\ & 274 \end{aligned}$ | $\begin{aligned} & 266, \\ & 274 \end{aligned}$ | $\begin{aligned} & \hline 266 \\ & 274 \end{aligned}$ | $\begin{aligned} & 266, \\ & 272 \end{aligned}$ | $\begin{aligned} & 266, \\ & 272 \end{aligned}$ | $\begin{aligned} & 266, \\ & 272 \end{aligned}$ | $\begin{aligned} & 266, \\ & 272 \end{aligned}$ | $\begin{aligned} & \hline 266, \\ & 270 \end{aligned}$ | $\begin{aligned} & 268, \\ & 270 \end{aligned}$ |  | $\begin{gathered} 26,2 \\ 70 \end{gathered}$ | $\begin{aligned} & 266, \\ & 270 \end{aligned}$ | $\begin{aligned} & 266, \\ & 270 \end{aligned}$ |  | $\begin{aligned} & 266, \\ & 270 \end{aligned}$ |  | $\begin{aligned} & 266, \\ & 270 \end{aligned}$ |
| D20S119 | $\begin{gathered} 61.7 \\ 7 \end{gathered}$ | $\begin{aligned} & 108, \\ & 118 \end{aligned}$ | $\begin{aligned} & 104, \\ & 110 \end{aligned}$ | $\begin{aligned} & 110, \\ & 118 \end{aligned}$ | $\begin{gathered} 110, \\ 118 \end{gathered}$ | $\begin{gathered} 104, \\ 108 \end{gathered}$ | $\begin{aligned} & 104, \\ & 118 \end{aligned}$ | $\begin{aligned} & 104, \\ & 118 \end{aligned}$ | $\begin{aligned} & 104, \\ & 118 \end{aligned}$ | $\begin{gathered} 112, \\ 118 \end{gathered}$ |  |  | $\begin{aligned} & 112, \\ & 114 \end{aligned}$ | $\begin{aligned} & 112, \\ & 118 \end{aligned}$ | $\begin{aligned} & 108, \\ & 118 \end{aligned}$ | $\begin{aligned} & 112, \\ & 116 \end{aligned}$ |  | $\begin{aligned} & 108, \\ & 116 \end{aligned}$ | $\begin{aligned} & 108 \\ & 112 \end{aligned}$ |


| D20S178 | $\begin{gathered} 66.1 \\ 6 \end{gathered}$ |  | $\begin{aligned} & 248, \\ & 252 \end{aligned}$ | $\begin{aligned} & 252, \\ & 252 \end{aligned}$ | $\begin{aligned} & 252, \\ & 252 \end{aligned}$ | $\begin{aligned} & 248, \\ & 252 \end{aligned}$ | $\begin{aligned} & 248, \\ & 252 \end{aligned}$ | $\begin{aligned} & 252, \\ & 252 \end{aligned}$ | $\begin{aligned} & 248, \\ & 252 \end{aligned}$ | $\begin{aligned} & 250, \\ & 252 \end{aligned}$ | $\begin{aligned} & \hline 248 \\ & 248 \end{aligned}$ | $\begin{aligned} & 248, \\ & 252 \end{aligned}$ | $\begin{aligned} & 248, \\ & 250 \end{aligned}$ | $\begin{aligned} & 250, \\ & 252 \end{aligned}$ | $\begin{aligned} & 244, \\ & 252 \end{aligned}$ |  | $\begin{aligned} & 244, \\ & 250 \end{aligned}$ | $\begin{aligned} & 244, \\ & 254 \end{aligned}$ | $\begin{aligned} & 244, \\ & 254 \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D20S100 | $\begin{gathered} 84.7 \\ 8 \end{gathered}$ |  | $\begin{aligned} & 206, \\ & 208 \end{aligned}$ | $\begin{aligned} & 208, \\ & 208 \end{aligned}$ | $\begin{aligned} & \hline 208, \\ & 208 \end{aligned}$ | $\begin{aligned} & 206, \\ & 208 \end{aligned}$ | $\begin{aligned} & 206, \\ & 208 \end{aligned}$ | $\begin{aligned} & 208, \\ & 208 \end{aligned}$ | $\begin{aligned} & 206, \\ & 208 \end{aligned}$ | $\begin{aligned} & 208, \\ & 210 \end{aligned}$ | $\begin{aligned} & 206 \\ & 206 \end{aligned}$ |  | $\begin{aligned} & 206, \\ & 210 \end{aligned}$ | $\begin{aligned} & 208, \\ & 208 \end{aligned}$ | $\begin{aligned} & 208, \\ & 208 \end{aligned}$ |  | $\begin{aligned} & 206 \\ & 208 \end{aligned}$ | $\begin{aligned} & 208 \\ & 214 \end{aligned}$ | $\begin{aligned} & 208, \\ & 214 \end{aligned}$ |
| D20S171 | $\begin{gathered} 95.7 \\ 0 \end{gathered}$ | $\begin{aligned} & 135, \\ & 139 \end{aligned}$ | $\begin{aligned} & 135, \\ & 141 \end{aligned}$ | $\begin{aligned} & 139, \\ & 141 \end{aligned}$ | $\begin{gathered} 139, \\ 141 \end{gathered}$ | $\begin{gathered} 135, \\ 135 \end{gathered}$ | $\begin{aligned} & 135, \\ & 139 \end{aligned}$ | $\begin{aligned} & 139 \\ & 141 \end{aligned}$ | $\begin{aligned} & 135, \\ & 139 \\ & \hline \end{aligned}$ | $\begin{aligned} & 137, \\ & 137 \end{aligned}$ | $\begin{gathered} 135, \\ 137 \end{gathered}$ | $\begin{aligned} & 135, \\ & 137 \end{aligned}$ |  | $\begin{aligned} & 135, \\ & 137 \end{aligned}$ | $\begin{aligned} & 135, \\ & 137 \end{aligned}$ |  | $\begin{aligned} & 137, \\ & 143 \end{aligned}$ | $\begin{aligned} & 135, \\ & 137 \end{aligned}$ | $\begin{aligned} & 137, \\ & 137 \end{aligned}$ |
| D20S173 | $\begin{gathered} 98.0 \\ 9 \end{gathered}$ | $\begin{aligned} & 185, \\ & 185 \end{aligned}$ | $\begin{aligned} & 175, \\ & 191 \end{aligned}$ | $\begin{gathered} 185, \\ 191 \end{gathered}$ | $\begin{gathered} 185, \\ 191 \end{gathered}$ | $\begin{aligned} & 185, \\ & 185 \end{aligned}$ | $\begin{gathered} 185, \\ 185 \end{gathered}$ | $\begin{gathered} 185, \\ 191 \end{gathered}$ | $\begin{aligned} & 185, \\ & 185 \end{aligned}$ | $\begin{gathered} 185, \\ 187 \end{gathered}$ | $\begin{gathered} 181, \\ 185 \end{gathered}$ | $\begin{aligned} & 175 \\ & 187 \end{aligned}$ | $\begin{aligned} & 181, \\ & 185 \end{aligned}$ | $\begin{aligned} & 185, \\ & 187 \end{aligned}$ | $\begin{aligned} & 185, \\ & 187 \end{aligned}$ |  | $\begin{aligned} & 187, \\ & 187 \end{aligned}$ |  | $\begin{gathered} 185, \\ 187 \end{gathered}$ |
| D21S1914 | $\begin{gathered} 19.3 \\ 9 \end{gathered}$ | $\begin{aligned} & 203, \\ & 207 \end{aligned}$ | $\begin{aligned} & 201, \\ & 205 \end{aligned}$ | $\begin{aligned} & 205, \\ & 207 \end{aligned}$ | $\begin{aligned} & 201, \\ & 207 \end{aligned}$ | $\begin{aligned} & 201, \\ & 207 \end{aligned}$ | $\begin{aligned} & 201, \\ & 203 \end{aligned}$ |  | $\begin{aligned} & 201, \\ & 203 \end{aligned}$ | $\begin{gathered} 203, \\ 207 \end{gathered}$ | $\begin{aligned} & 299, \\ & 209 \end{aligned}$ | $\begin{aligned} & 203, \\ & 209 \end{aligned}$ | $\begin{gathered} 207, \\ 209 \end{gathered}$ | $\begin{aligned} & 197, \\ & 203 \end{aligned}$ | $\begin{aligned} & 197, \\ & 207 \end{aligned}$ |  | $\begin{aligned} & 197, \\ & 205 \end{aligned}$ | $\begin{aligned} & 197, \\ & 203 \end{aligned}$ | $\begin{aligned} & 197, \\ & 203 \end{aligned}$ |
| D21S263 | $\begin{gathered} 27.4 \\ 0 \end{gathered}$ | $\begin{aligned} & 175, \\ & 179 \end{aligned}$ | $\begin{aligned} & \hline 179, \\ & 181 \\ & \hline \end{aligned}$ | $\begin{aligned} & 175, \\ & 179 \end{aligned}$ | $\begin{aligned} & 175 \\ & 179 \end{aligned}$ | $\begin{gathered} 175, \\ 179 \end{gathered}$ | $\begin{gathered} 179, \\ 179 \end{gathered}$ | $\begin{aligned} & 179, \\ & 179 \end{aligned}$ | $\begin{aligned} & 179, \\ & 179 \end{aligned}$ | $\begin{gathered} 175, \\ 179 \end{gathered}$ | $\begin{gathered} 175 \\ 181 \end{gathered}$ | $\begin{aligned} & 175 \\ & 175 \end{aligned}$ | $\begin{aligned} & 175 \\ & 175 \end{aligned}$ | $\begin{aligned} & 179, \\ & 179 \end{aligned}$ | $\begin{aligned} & 179, \\ & 199 \end{aligned}$ |  |  | $\begin{aligned} & 175 \\ & 199 \end{aligned}$ | $\begin{gathered} 175, \\ 199 \end{gathered}$ |
| D21S1252 | $\begin{gathered} 35.4 \\ 5 \end{gathered}$ | $\begin{aligned} & 231, \\ & 247 \end{aligned}$ | $\begin{aligned} & 231, \\ & 239 \end{aligned}$ | $\begin{aligned} & 239, \\ & 247 \end{aligned}$ | $\begin{aligned} & 239, \\ & 247 \end{aligned}$ | $\begin{aligned} & 239, \\ & 247 \end{aligned}$ | $\begin{aligned} & 231, \\ & 239 \end{aligned}$ | $\begin{aligned} & 231, \\ & 239 \end{aligned}$ | $\begin{aligned} & 231, \\ & 239 \end{aligned}$ | $\begin{aligned} & 231, \\ & 247 \end{aligned}$ |  |  | $\begin{aligned} & 247, \\ & 249 \end{aligned}$ | $\begin{aligned} & 231, \\ & 245 \end{aligned}$ | $\begin{aligned} & 231, \\ & 245 \end{aligned}$ |  | $\begin{aligned} & 245, \\ & 249 \end{aligned}$ | $\begin{aligned} & 231, \\ & 231 \end{aligned}$ | $\begin{aligned} & 245, \\ & 249 \end{aligned}$ |
| D21S2055 | $\begin{gathered} 40.4 \\ 9 \end{gathered}$ | $\begin{aligned} & \hline 127, \\ & 153 \end{aligned}$ | $\begin{aligned} & 127, \\ & 153 \end{aligned}$ | $\begin{aligned} & 127, \\ & 153 \end{aligned}$ | $\begin{aligned} & 127, \\ & 153 \end{aligned}$ | $\begin{gathered} 127, \\ 153 \end{gathered}$ | $\begin{aligned} & 153, \\ & 153 \end{aligned}$ |  |  | $\begin{gathered} 127, \\ 193 \end{gathered}$ | $\begin{aligned} & 127, \\ & 153 \end{aligned}$ | $\begin{gathered} 127, \\ 127 \end{gathered}$ | $\begin{aligned} & 127, \\ & 127 \end{aligned}$ | $\begin{gathered} 177, \\ 193 \end{gathered}$ | $\begin{aligned} & 177, \\ & 193 \end{aligned}$ | $\begin{gathered} 115, \\ 185 \end{gathered}$ | $\begin{aligned} & 193, \\ & 185 \end{aligned}$ |  |  |
| D21S266 | $\begin{gathered} 45.8 \\ 7 \end{gathered}$ | $\begin{aligned} & 157, \\ & 171 \end{aligned}$ | $\begin{aligned} & 157, \\ & 169 \end{aligned}$ | $\begin{aligned} & 169, \\ & 171 \end{aligned}$ | $\begin{aligned} & 169, \\ & 171 \end{aligned}$ | $\begin{aligned} & 169, \\ & 171 \end{aligned}$ | $\begin{gathered} 157, \\ 169 \end{gathered}$ | $\begin{aligned} & 157, \\ & 169 \end{aligned}$ | $\begin{aligned} & 157, \\ & 169 \end{aligned}$ | $\begin{aligned} & 157, \\ & 171 \end{aligned}$ |  | $\begin{aligned} & 171 \\ & 171 \end{aligned}$ | $\begin{aligned} & 171, \\ & 171 \end{aligned}$ | $\begin{aligned} & 157, \\ & 157 \end{aligned}$ | $\begin{aligned} & 157, \\ & 157 \end{aligned}$ |  | $\begin{aligned} & 157, \\ & 157 \end{aligned}$ | $\begin{aligned} & 157, \\ & 157 \end{aligned}$ | $\begin{aligned} & 157, \\ & 157 \end{aligned}$ |
| D22S420 | 4.06 | $\begin{aligned} & 152, \\ & 158 \end{aligned}$ | $\begin{aligned} & 148, \\ & 156 \end{aligned}$ | $\begin{aligned} & 148, \\ & 152 \end{aligned}$ | $\begin{aligned} & 148, \\ & 152 \end{aligned}$ | $\begin{gathered} 148 \\ 158 \end{gathered}$ | $\begin{aligned} & 152, \\ & 156 \end{aligned}$ | $\begin{aligned} & 148 \\ & 158 \end{aligned}$ | $\begin{aligned} & 152, \\ & 156 \end{aligned}$ | $\begin{array}{r} 150, \\ 152 \end{array}$ | $\begin{array}{r} 150, \\ 152 \end{array}$ | $\begin{gathered} 150, \\ 150 \end{gathered}$ | $\begin{gathered} 150, \\ 152 \end{gathered}$ | $\begin{aligned} & 150, \\ & 152 \end{aligned}$ | $\begin{aligned} & 150, \\ & 152 \end{aligned}$ |  | $\begin{aligned} & 152, \\ & 156 \end{aligned}$ |  | $\begin{aligned} & 152, \\ & 156 \end{aligned}$ |
| D22S539 | $\begin{gathered} 14.4 \\ 4 \end{gathered}$ | $\begin{aligned} & 123, \\ & 123 \end{aligned}$ | $\begin{aligned} & 123, \\ & 131 \end{aligned}$ | $\begin{aligned} & 123, \\ & 123 \end{aligned}$ | $\begin{aligned} & 123, \\ & 123 \end{aligned}$ | $\begin{aligned} & 123, \\ & 123 \end{aligned}$ | $\begin{gathered} 123, \\ 123 \end{gathered}$ | $\begin{aligned} & 123, \\ & 123 \end{aligned}$ | $\begin{aligned} & 123, \\ & 131 \end{aligned}$ | $\begin{gathered} 123, \\ 123 \end{gathered}$ | $\begin{gathered} 123, \\ 133 \end{gathered}$ | $\begin{aligned} & 123, \\ & 133 \end{aligned}$ | $\begin{aligned} & 123, \\ & 133 \end{aligned}$ | $\begin{aligned} & 123, \\ & 123 \end{aligned}$ | $\begin{aligned} & 123, \\ & 123 \end{aligned}$ |  | $\begin{aligned} & 123, \\ & 123 \end{aligned}$ | $\begin{aligned} & 123, \\ & 123 \end{aligned}$ | $\begin{aligned} & 123, \\ & 123 \end{aligned}$ |
| $\begin{gathered} \text { GCT10C1 } \\ 0 \end{gathered}$ | $\begin{gathered} 18.1 \\ 0 \end{gathered}$ | $\begin{aligned} & 195 \\ & 201 \end{aligned}$ | $\begin{aligned} & 192, \\ & 195 \end{aligned}$ | $\begin{aligned} & 192, \\ & 201 \end{aligned}$ | $\begin{aligned} & 192, \\ & 195 \end{aligned}$ | $\begin{aligned} & 192, \\ & 201 \end{aligned}$ | $\begin{aligned} & 195 \\ & 201 \end{aligned}$ |  |  | $\begin{aligned} & 195 \\ & 201 \end{aligned}$ | $\begin{gathered} 210 \\ 195 \end{gathered}$ | $\begin{aligned} & 195 \\ & 201 \end{aligned}$ | $\begin{aligned} & 201, \\ & 201 \end{aligned}$ | $\begin{aligned} & 195 \\ & 195 \end{aligned}$ | $\begin{aligned} & 195 \\ & 201 \end{aligned}$ | $\begin{gathered} 195 \\ 198 \end{gathered}$ | $\begin{aligned} & 195 \\ & 201 \end{aligned}$ |  |  |
| D22S315 | $\begin{gathered} 21.4 \\ 7 \end{gathered}$ | $\begin{aligned} & 193, \\ & 195 \end{aligned}$ | $\begin{aligned} & 195, \\ & 195 \end{aligned}$ | $\begin{aligned} & 195, \\ & 195 \end{aligned}$ | $\begin{gathered} 195, \\ 195 \end{gathered}$ | $\begin{gathered} 195 \\ 195 \end{gathered}$ | $\begin{gathered} 195, \\ 195 \end{gathered}$ | $\begin{aligned} & 195, \\ & 195 \end{aligned}$ | $\begin{aligned} & 195, \\ & 195 \end{aligned}$ | $\begin{gathered} 195, \\ 195 \end{gathered}$ | $\begin{gathered} 195 \\ 195 \end{gathered}$ | $\begin{gathered} 195 \\ 195 \end{gathered}$ | $\begin{gathered} 195 \\ 195 \end{gathered}$ | $\begin{aligned} & 193, \\ & 197 \end{aligned}$ | $\begin{aligned} & 193, \\ & 197 \end{aligned}$ |  | $\begin{aligned} & 193, \\ & 197 \end{aligned}$ | $\begin{aligned} & 193, \\ & 197 \end{aligned}$ | $\begin{gathered} 197, \\ 197 \end{gathered}$ |
| D22S280 | $\begin{gathered} 31.3 \\ 0 \end{gathered}$ | $\begin{aligned} & 216 \\ & 216 \end{aligned}$ | $\begin{aligned} & 210, \\ & 216 \end{aligned}$ | $\begin{aligned} & 216 \\ & 216 \end{aligned}$ | $\begin{aligned} & 216 \\ & 216 \end{aligned}$ | $\begin{gathered} 216, \\ 216 \end{gathered}$ | $\begin{array}{\|l\|} \hline 210, \\ 216 \end{array}$ | $\begin{aligned} & 216, \\ & 216 \end{aligned}$ | $\begin{aligned} & 216, \\ & 216 \end{aligned}$ | $\begin{aligned} & 216 \\ & 216 \end{aligned}$ | $\begin{aligned} & 216 \\ & 218 \end{aligned}$ | $\begin{aligned} & 216, \\ & 216 \end{aligned}$ | $\begin{aligned} & 216, \\ & 216 \end{aligned}$ | $\begin{aligned} & 214, \\ & 214 \end{aligned}$ | $\begin{aligned} & 212, \\ & 214 \end{aligned}$ |  | $\begin{aligned} & 212, \\ & 216 \end{aligned}$ | $\begin{aligned} & 212, \\ & 216 \end{aligned}$ | $\begin{aligned} & 214, \\ & 214 \end{aligned}$ |
| D22S283 | $\begin{gathered} 38.6 \\ 2 \end{gathered}$ | $\begin{aligned} & 130 \\ & 154 \end{aligned}$ | $\begin{aligned} & 146 \\ & 148 \end{aligned}$ | $\begin{aligned} & 146 \\ & 154 \end{aligned}$ | $\begin{gathered} 146 \\ 154 \end{gathered}$ | $\begin{gathered} 130, \\ 146 \end{gathered}$ | $\begin{aligned} & 130, \\ & 148 \end{aligned}$ | $\begin{aligned} & 146, \\ & 154 \end{aligned}$ | $\begin{aligned} & 146, \\ & 154 \end{aligned}$ | $\begin{gathered} 130 \\ 154 \end{gathered}$ | $\begin{aligned} & 144, \\ & 148 \end{aligned}$ | $\begin{aligned} & 144, \\ & 154 \end{aligned}$ | $\begin{gathered} 144, \\ 154 \end{gathered}$ | $\begin{aligned} & 140, \\ & 150 \end{aligned}$ | $\begin{aligned} & 140, \\ & 150 \end{aligned}$ |  | $\begin{aligned} & 140, \\ & 142 \end{aligned}$ | $\begin{aligned} & 140 \\ & 142 \end{aligned}$ | $\begin{gathered} 140, \\ 150 \end{gathered}$ |


|  | 46.4 | 152, | 148, | 148, | 148, | 148, | 152, | 148, | 152, | 150, | 150, | 150, | 150, | 150, | 150, |  | 152, |  | 152, |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| D22S423 | 2 | 158 | 156 | 152 | 152 | 158 | 156 | 158 | 156 | 152 | 152 | 150 | 152 | 152 | 152 |  | 156 |  | 156 |
|  | 51.5 | 206, | 200, | 206, | 206, | 206, | 200, | 206, | 206, | 206, | 204, | 204, | 204, | 206, | 202, |  | 202, | 202, | 206, |
| D22S274 | 4 | 206 | 214 | 214 | 214 | 214 | 206 | 214 | 214 | 206 | 206 | 206 | 206 | 206 | 210 |  | 202 | 202 | 210 |

