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Title	Stringency of start codon selection modulates autoregulation of translation initiation factor eIF5
Authors	Loughran, Gary;Sachs, Matthew S.;Atkins, John F.;Ivanov, Ivaylo P.
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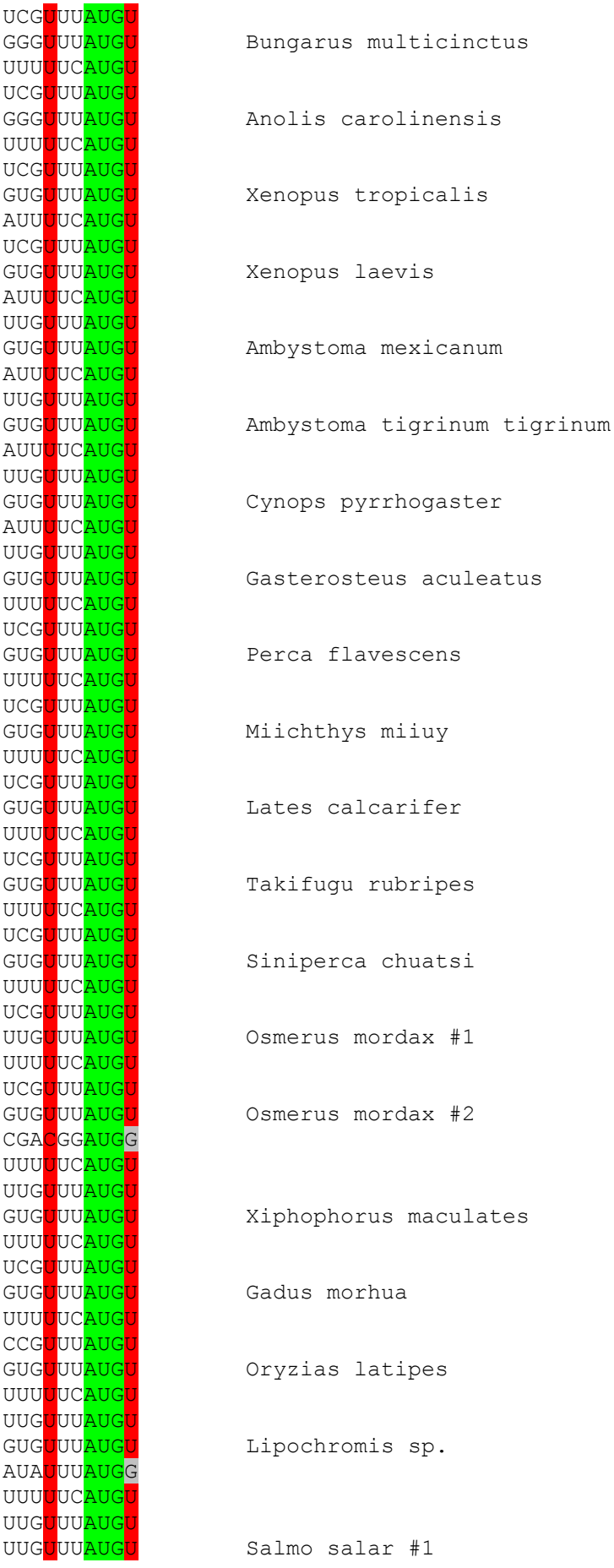
Supplementary Data

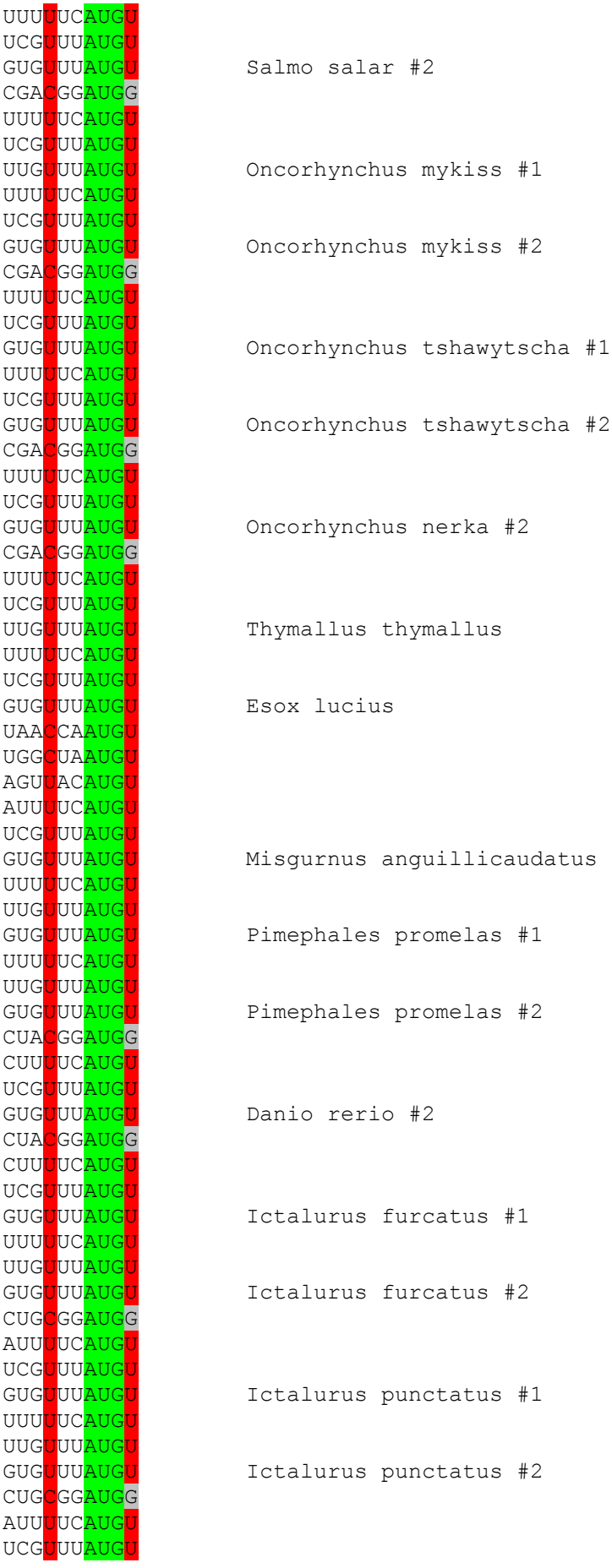
Supplementary Figure S1. Nucleotide sequences used for generating logograms. Alignments of the 10 nucleotides surrounding the uAUG start codons of eIF5 that define their initiation contexts are shown for: (*A*) vertebrates; (*B*) arthropods; (*C*) nematodes; (*D*) molluscs; (*E*) Pezyzomycotina; (*F*) Basidiomycota; (*G*) fungi other than Pezyzomycotina or Basidiomycota; (*H*) plants. Species names are on the right. Species names in quotation marks specify presumed contaminant sequences that cluster with eIF5 sequences from an unrelated taxonomic group. Some plants have more than one paralog of eIF5. Here they are arbitrarily labelled "#1", "#2", "#3" or "#4" and their placement in one of the groups does not imply orthologous clusters. For each species the sequence on top is the context of the first uAUG and subsequent uAUG contexts are shown in descending order. The initiation codon is highlighted in green. Nucleotides in positions -3 and +4 are highlighted in red if they deviate from the Kozak consensus and in gray if they comply with it. Species names in quotation marks represent sequences derived from Expressed Sequence Tags that by similarity analysis of the main open reading frame do not match eIF5 homologs from the phylum to which the species belongs and instead likely belong to contaminating sequences of unknown species. These sequences are grouped with the taxonomic group to which they actually belong determined by reconstructed phylogenetic trees with the entire sequence.

A)

Vertebrata

```
GGGUUUAUGU          Homo sapiens
UUUUUCAUGU
UCGUUUAUGU
GGGUUUAUGU          Pan troglodytes verus
UCGUUUAUGU
UUUUUCAUGU
GGGUUUAUGU          Macaca fascicularis
UUUUUCAUGU
UCGUUUAUGU
GGGUUUAUGU          Macaca mulatta
UUUUUCAUGU
UCGUUUAUGU
GGGUUUAUGU          Pongo abelii
UUUUUCAUGU
UCGUUUAUGU
GGGUUUAUGU          Canis lupus familiaris
UUUUUCAUGU
UCGUUUAUGU
GGGUUUAUGU          Ursus americanus
UUUUUCAUGU
UCGUUUAUGU
GGGUUUAUGU          Equus caballus
UUUUUCAUGU
UCGUUUAUGU
GGGUUUAUGU          Peromyscus polionotus
UUUUUCAUGU
UCGUUUAUGU
GGGUUUAUGU          Bos taurus
UUUUUCAUGU
UCGUUUAUGU
GGGUUUAUGU          Sus scrofa
UUUUUCAUGU
UCGUUUAUGU
GGGUUUAUGU          Rattus norvegicus
UUUUUCAUGU
UCGUUUAUGU
GGGUUUAUGU          Mus musculus
UUUUUCAUGU
UCGUUUAUGU
GGGUUUAUGU          Cavia porcellus
UUUUUCAUGU
UCGUUUAUGU
GGGUUUAUGU          Ovis aries
UUUUUCAUGU
UCGUUUAUGU
GGGUUUAUGU          Oryctolagus cuniculus
UUUUUCAUGU
UCGUUUAUGU
GGGUUUAUGU          Taeniopygia guttata
UUUUUCAUGU
UCGUUUAUGU
GGGUUUAUGU          Gallus gallus
UUUUUCAUGU
UCGUUUAUGU
GGGUUUAUGU          Lonchura striata domestica
UUUUUCAUGU
UCGUUUAUGU
GGGUUUAUGU          Meleagris gallopavo
UUUUUCAUGU
```


UCGUUUAUGU
GGGUUUAUGU
Bungarus multicinctus
UUUUUCAUGU
UCGUUUAUGU
GGGUUUAUGU
Anolis carolinensis
UUUUUCAUGU
UCGUUUAUGU
GUGUUUAUGU
Xenopus tropicalis
AUUUUCAUGU
UCGUUUAUGU
GUGUUUAUGU
Xenopus laevis
AUUUUCAUGU
UUGUUUAUGU
GUGUUUAUGU
Ambystoma mexicanum
AUUUUCAUGU
UUGUUUAUGU
GUGUUUAUGU
Ambystoma tigrinum tigrinum
AUUUUCAUGU
UUGUUUAUGU
GUGUUUAUGU
Cynops pyrrhogaster
AUUUUCAUGU
UUGUUUAUGU
GUGUUUAUGU
Gasterosteus aculeatus
UUUUUCAUGU
UCGUUUAUGU
GUGUUUAUGU
Perca flavescens
UUUUUCAUGU
UCGUUUAUGU
GUGUUUAUGU
Miichthys miiuy
UUUUUCAUGU
UCGUUUAUGU
GUGUUUAUGU
Lates calcarifer
UUUUUCAUGU
UCGUUUAUGU
GUGUUUAUGU
Takifugu rubripes
UUUUUCAUGU
UCGUUUAUGU
GUGUUUAUGU
Siniperca chuatsi
UUUUUCAUGU
UCGUUUAUGU
UUGUUUAUGU
Osmerus mordax #1
UUUUUCAUGU
UCGUUUAUGU
GUGUUUAUGU
Osmerus mordax #2
CGACGGAUGG
UUUUUCAUGU
UUGUUUAUGU
GUGUUUAUGU
Xiphophorus maculates
UUUUUCAUGU
UCGUUUAUGU
GUGUUUAUGU
Gadus morhua
UUUUUCAUGU
CCGUUUAUGU
GUGUUUAUGU
Oryzias latipes
UUUUUCAUGU
UUGUUUAUGU
GUGUUUAUGU
Lipochromis sp.
AUAUUUAUGG
UUUUUCAUGU
UUGUUUAUGU
UUGUUUAUGU
Salmo salar #1


Salmo salar #2
Oncorhynchus mykiss #1
Oncorhynchus mykiss #2
Oncorhynchus tshawytscha #1
Oncorhynchus tshawytscha #2
Oncorhynchus nerka #2
Thymallus thymallus
Esox lucius
Misgurnus anguillicaudatus
Pimephales promelas #1
Pimephales promelas #2
Danio rerio #2
Ictalurus furcatus #1
Ictalurus furcatus #2
Ictalurus punctatus #1
Ictalurus punctatus #2

GUGUUUAUGU Cynoglossus semilaevis
UUUUUCAUGU
UCGUUUAUGU
GUGUUUAUGU Squalus acanthias
UUUUUCAUGU
UUGUUUAUGU
GUGUUUAUGU Leucoraja erinacea
UUUUUCAUGU
UUGUUUAUGU
GGUUUUAUGU Petromyzon marinus
AUUUUCAUGU
GAUUUUAUGU Eptatretus burgeri
AUUUUCAUGU
CAGUUUAUGU

B)
Arthropoda

UUUUAUAUGA Tribolium castaneum
AGGUGUAUGA
UUUUAUAUGA Dendroctonus ponderosae
AGGUGUAUGA
UUUUAUAUGA Aedes aegypti
UUGUGCAUGA
UUUUAUAUGA Anopheles gambiae
ACGUGCAUGA
UCUUAUAUGA Drosophila melanogaster
CUGUGCAUGA
GUACACAUGA
UCUUAUAUGA Drosophila willistoni
CUGUGCAUGA
UCUUAUAUGA Drosophila serrata
CUGUGCAUGA
GUACACAUGA
UCUUAUAUGA Drosophila erecta
CUGUGCAUGA
GUACACAUGA
AAUCUUAUGA Drosophila ananassae
UGACUUAUGA
UGGUGCAUGA
GUACACAUGA
UCUUAUAUGA Phlebotomus papatasi
UCGUGCAUGA
UCUUAUAUGA Phlebotomus sergenti
UCGUGCAUGA
AUUUAUAUGA Acyrthosiphon pisum
AGGUGCAUGA
UUUUAUAUGA Schistocerca gregaria
ACCUACAUGC
AGGUGUAUGA
UUUUAUAUGA Teleopsis dalmanni
UCGUGCAUGA
UUUUAUAUGA "Quercus robur"
UCGUGCAUGA
GCGUGUAUGA Bombus terrestris
UUUUAUAUGA Nilaparvata lugens
AGGUGUAUGA
UUUUAUAUGA Parasteatoda tepidariorum
AGGUUUAUGC
UUUUAUAUGA "Selaginella moellendorffii"
AGGUUUAUGC

```
UUUUAUAUGA              Limulus polyphemus #1
CCGUUUAUGA
UUUUAUAUGA              Limulus polyphemus #2
UUGUUUAUGA
UUUUAUAUGA              Rhipicephalus appendiculatus
AGGUUUAUGC
CUUGUAAUGC
UUUUAUAUGA              Amblyomma variegatum
AGGUUUAUGC
UUUGUAAUGC
UUUUAUAUGA              Loxosceles laeta
AGGUUUAUGC
UUUUGUAUGC              Campodea fragilis
GAAUUGAUGC
AUUUAUAUGA              Aphis gossypii
AGGUGCAUGA
UUUUAUAUGA              Locusta migratoria
GGGUGCAUGA
CUGUGUAUGU              Calanus finmarchicus
GAUUUUAUGA              Polypedilum vanderplanki
AAGUGCAUGU
UUUUUCAUGA              Petrolisthes cinctipes
UCUUGUAUGU
ACUUUCAUGA              Penaeus monodon
UCUUGUAUGU
ACUUUCAUGA              Callinectes sapidus
UCUUGUAUGU
UUUUAUAUGA              Glossina morsitans
UCGUGCAUGA
UUUUAUAUGA              Ceratitis capitata
UCGUGCAUGA
UCUUAUAUGA              Rhynchosciara americana
CCUUUUAUGA              Chironomus tentans
CAGUGCAUGA
UUUUAUAUGA              Spodoptera frugiperda
GCGUGCAUGA
UUUUAUAUGA              Spodoptera litura
GCGUGCAUGA
UUUUAUAUGA              Gryllus bimaculatus
ACCUACAUGC
AGGUGUAUGA
AAACUUAUGG              Pediculus humanus capitis
ACGUGUAUGA
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C)
Nematoda

```
UUGUGCAUGU          Caenorhabditis remanei
AAUUUUAUGA
CGGUGUAUGC
UUGUGCAUGU          Caenorhabditis elegans
AAUUUUAUGA
UAGUGUAUGC
UUGUGCAUGU          Caenorhabditis japonica
AAUUAUAUGA
CAGUGUAUGC
UUGUGCAUGU          Heterorhabditis bacteriophora
GAUUUUAUGA
CAUCGUAUGC
AGUUGCAUGU          Wuchereria bancrofti
GAUUUUAUGG
```

UCACGUAUGC
AGUUGCAUGU Brugia malayi
GAUUUUAUGG
UCACGUAUGC
AGUUGUAUGU Ascaris suum
UAUUUUAUGG
CCCCGUAUGC
UUGUGCAUGU Angiostrongylus cantonensis
AAUUUUAUGC
CAUCGUAUGC
UUGUGCAUGU Ancylostoma ceylanicum
GAUUUUAUGC
UAUCGUAUGC
AGGUGUAUGA Bursaphelenchus mucronatus
CGUUUUAUGG
UAACGUAUGC

D)
Mollusca

GUUUUUAUGA Lottia gigantean
AGGUUUAUGA
AUUUUUAUGA Aplysia californica
GCGUUUAUGA
AUUUUUAUGA Aplysia kurodai
GCGUUUAUGA
AUUUUUAUGA Lymnaea stagnalis
GCGUAUAUGA
GUUUUUAUGA Crassostrea gigas
GGGUUUAUGA
GGUUUUAUGA Crassostrea virginica
GGGUUUAUGA
AGUCGUAUGA Mytilus californianus
AGUUUUAUGA

E)
Pezizomycotina

UUGUUUAUGU Neurospora crassa
GCGUUAAUGC
UACCCUAUGU
UUGUUUAUGU Podospora anserine
CUGCCCAUGU
CACCACAUGU
GAGUUUAUGC Mycosphaerella graminicola
GAGUUUAUGC
UGCUGCAUGU
CAGUUUAUGU Aureobasidium pullulans
AAUUGCAUGU
CUGUCUAUGU Trichophyton rubrum
CUACGCAUGU
UUGUUUAUGU Hypocrea jecorina
GAGUUCAUGC
CGCCACAUGU
CUGUUUAUGU Gibberella moniliformis
UCGUCUAUGC
UGCCACAUGU
CUAUACAUGA
UUGUUUAUGU Thielavia terrestris
GCGUUAAUGC
UAAUGCAUGC
UACCCUAUGU

CUGUCUAUGU Coccidioides posadasii
CUACGCAUGU
UUGUCUAUGU Aspergillus niger
CCACGCAUGU
CUGUUUAUGU Trichoderma atroviride
GAGUUCAUGC
CUCCCUAUGU
CGCUUCAUGC
UUGUUUAUGU Trichoderma virens
UCGUUCAUGC
CGCCCUAUGU
CUCUUGAUGC
CGUUAUAUGU Geomyces pannorum
CAACACAUGU
CUCUAUAUGU
GAGUAUAUGU Cochliobolus heterostrophus
CGCUGUAUGC
UUGCGCAUGU
UUGUUUAUGU Ophiostoma piliferum
CAGCACAUGC
UUGUUCAUGA Tuber melanosporum
CAGUUCAUGC
GGGUUUAUGU Cercospora zeae-maydis
UACCGCAUGU
CGUUAUAUGU Amorphotheca resinae
CUUCGAAUGU
UUCUGCAUGU
UGUUCCAUGU
UUGUUUAUGU “Panicum virgatum”
GCGUUAAUGC
UACCCUAUGU
CGUCUCAUGA
UUGUUUAUGU Myceliophthora thermophila
GCGUUAAUGC
UACCCUAUGU
UUGUUUAUGU Thielavia heterothallica
GCGUUAAUGC
UACCCUAUGU
CUGUUUAUGU Fusarium sporotrichioides
UUGUCUAUGC
UUGUUUAUGU “Wrightia tinctoria”
GCGUUAAUGC
UAAUGCAUGC
UACCCUAUGU
UUGUUUAUGU Cordyceps militaris
GAGUUCAUGC
AACCUCAUGU
GUCUUCAUGA
UUGUUCAUGA “Picea glauca”
CGGUUCAUGC
UUGUUUAUGU Sordaria macrospora
GCGUUAAUGC
UACCCUAUGU
CUGUUUAUGU Magnaporthe oryzae
GCGUUCAUGC
UCCCCUAUGU
CAGUCUAUGU
CUGUUUAUGU Magnaporthe poae
GCGUUCAUGC
UCCCCUAUGU
AAGUUCAUGU

CUGUUUAUGU Gaeumannomyces graminis
GCGUUCAUGC
UCCCCUAUGU
AAGUUCAUGU
UUGUUUAUGU Trichoderma reesei
GAGUUCAUGC
CGCCACAUGU
CUCUUGAUGC
CUGUUUAUGU Nectria haematococca
UUGUCUAUGC
UGCCACAUGC
CUCUUCAUGA
CAGUUUAUGU Glomerella graminicola
GAGUUCAUGA
UACCACAUGU
UUCUUAAUGC
CUGUUUAUGU Gibberella zeae
CUGUCUAUGC
UGCCACAUGA
CUAUACAUGA
UUGUCUAUGU Neosartorya fischeri
CUACGCAUGU
CUGUUUAUGU Fusarium oxysporum
UCGUCUAUGC
UGCCACAUGU
CUAUACAUGA
CUGUUUAUGU Verticillium dahliae
GAGUUCAUGU
UACCCCAUGU
UCUUCCAUGU
UUGUCUAUGU Aspergillus fumigatus
UGCUGUAUGA
CUACGCAUGU
UUGUUUAUGU Grosmannia clavigera
GCGUUCAUGC
UCCCUUAUGU
UUCUUCAUGC
UUGUCUAUGU Aspergillus clavatus
CUACGCAUGU
CGUUAUAUGU Geomyces destructans
CAACACAUGU
CUCUAUAUGU
CUGUUUAUGU Metarhizium acridum
GAGUUCAUGC
UGCCCCAUGU
ACCUUCAUGC
CUGUUCAUGU Talaromyces stipitatus
UACCGCAUGU
UUGUCUAUGU Aspergillus terreus
UGACGCAUGU
CGUUAUAUGU Botryotinia fuckeliana
AUUCGAAUGU
UUCUGCAUGU
UUGUCUAUGU Aspergillus nidulans
CGGCGUAUGU
CGUUAUAUGU Blumeria graminis
CGGCGAAUGU
CAUCAUAUGU
UUGUCUAUGU Aspergillus flavus
AUACGCAUGU
GAGUAUAUGU Pyrenophora teres

CGCUGUAUGC
CUGCGCAUGU
GAGUAUAUGU Pyrenophora tritici-repentis
CGCUGUAUGC
CUGCGCAUGU
CUGUUCAUGU Penicillium marneffei
UACCGCAUGU
CUGUUUAUGU Epichloe festucae
GAGUUCAUGC
UGCCCUAUGU
GUGUUUAUGU
CGGUAUAUGU Phaeosphaeria nodorum
UACUGCAUGU
CGUUAUAUGU Sclerotinia sclerotiorum
AUUCGAAUGU
UUCUGCAUGU
CUGUUUAUGU Verticillium albo-atrum
GAGUUCAUGU
UACCCCAUGU
UCUUCCAUGU
CUGUUUAUGU Metarhizium anisopliae
GAGUUCAUGC
UGCCCCAUGU
AUCUUCAUGC

F)
Basidiomycota

GGAUACAUGC Postia placenta
GGAUUCAUGC Trametes versicolor
GGAUUCAUGC Phanerochaete chrysosporium
GGAUUCAUGC Heterobasidion annosum
GGAUUCAUGC Pleurotus ostreatus
GGAUUCAUGC Schizophyllum commune
GGAUUCAUGC Lentinula edodes
GAAUUCAUGC Coprinopsis cinerea
GGAUUCAUGC Gloeophyllum trabeum
CGGUUCAUGU Leucosporidium scottii
CAGCUUAUGU Microbotryum violaceum
UCGUUCAUGU Melampsora larici-populina
GAGUUCAUGC Tremella mesenterica
GAGUUCAUGC Cryptococcus neoformans
UUGUUCAUGC Cryptococcus laurentii
UCGUAUAUGU Phakopsora pachyrhizi
GUUUUUAUGU Ustilago maydis
AAUUUCAUGC Paxillus involutus

G)
Other fungi

UAGUUCAUGU Cunninghamella elegans
UUGUACAUGU Mucor circinelloides
CUGUUCAUGU Phycomyces blakesleeanus
CGCUUCAUGU Glomus intraradices
CUGUACAUGU Piromyces sp.
CGCUUCAUGA Spizellomyces punctatus

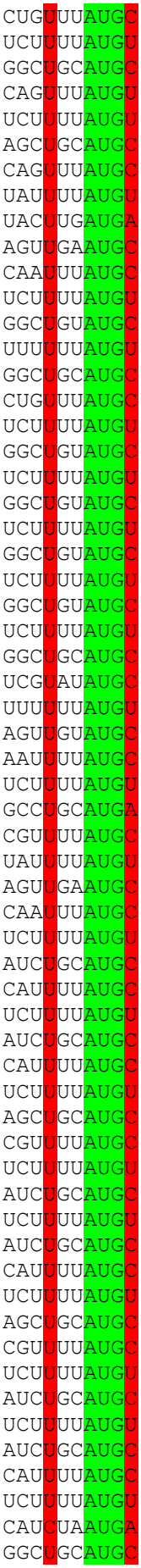
H)
Plants

UCUUUUAUGU Oryza sativa #1
UGCUGCAUGC

```
CAAUUUAUGC
UCUUUUAUGU          Oryza sativa  #2
UCUUUUAUGU          Sorghum bicolour #1
CACUGCAUGC
UCUUUUAUGU          Sorghum bicolour #2
CACUGCAUGC
UCUUUUAUGU          Populus trichocarpa  #1
GGCUGCAUGC
GCUUUUAUGU          Populus trichocarpa #2
AGCUGUAUGC
UUUUUUAUGU          Populus trichocarpa  #3
ACACAGAUGC
UCUUUUAUGU          Populus tremula #1
GGCUGUAUGC
UGUUUUAUGA
GCUUUUAUGU          Populus tremula #2
AGCUGUAUGC
UCUUUUAUGU          Populus deltoids #1
GGCUGCAUGC
UUUUUUAUGU          Populus deltoides #3
AGCUAUAUGC
UCUUUUAUGU          Populus nigra #1
GGCUGCAUGC
UUUUUUAUGU          Populus nigra #3
ACACAGAUGC
AGCUAUAUGC
GCUUUUAUGU          Populus fremontii #2
AGCUGUAUGC
UCUUUUAUGU          Zea mays #1
CACUGCAUGC
UCUUUUAUGU          Zea mays #2
CACUGCAUGC
UCUUUUAUGU          Arabidopsis thaliana
CAGUUUAUGC
UCUUUUAUGU          Vitis vinifera #1
AGCUGCAUGC
AGGUUUAUGU
UCUUUUAUGU          Vitis vinifera #2
GACUGUAUGC
AGCUGGAUGC
UCUUUUAUGU          Vitis shuttleworthii #1
AGCUGCAUGC
AGGUUUAUGU
UCUUUUAUGU          Vitis shuttleworthii #2
GACUGUAUGC
AGCUGGAUGC
GCUUUUAUGU          Crocus sativus
GACUGCAUGA
UCGUUCAUGC
UCUUUUAUGU          Nicotiana tabacum #1
GACUGUAUGC
UCUUUUAUGU          Nicotiana tabacum #2
GACUGUAUGC
UCUUUUAUGU          Nicotiana tabacum #3
GGCUGCAUGC
UCUUUUAUGU          Nicotiana benthamiana #2
GACUUUAUGC
UUUUUUAUGU          Nicotiana benthamiana #3
GGCUGCAUGC
UCUUUUAUGU          Amborella trichopoda
ACUCAGAUGC
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```
AGAUGCAUGC
ACUUUAAUGC
UCUUUUAUGU          Quercus petraea
GGCUGCAUGC
UGGUAAAUGA
UCUUUUAUGU          Petunia axillaris
GACUGUAUGC
UCUUUUAUGU          Petunia x hybrida
GACUGUAUGC
UCUUUUAUGU          Quercus robur
GGCUGUAUGC
AGUUUGAUGG
UCUUUUAUGU          Triticum aestivum #1
CCCUGCAUGC
UCUUUUAUGU          Triticum aestivum #2
CCCUGCAUGC
UCUUUUAUGU          Triticum aestivum #3
CCCUGCAUGC
UCUUUUAUGU          Lolium multiflorum
CCCUGUAUGC
UCUUUUAUGU          Lolium perenne
CCCUGUAUGC
UCUUUUAUGU          Lolium temulentum
CCCUGUAUGC
UCUUUUAUGU          Avena sativa #1
CCCUGUAUGC
UCUUUUAUGU          Avena sativa #2
CCCUGUAUGC
UCUUUUAUGU          Avena barbata
CCCUGUAUGC
UCUUUUAUGU          Festuca pratensis
CCCUGUAUGC
UCUUUUAUGU          Festuca arundinacea
CCCUGUAUGC
UCUUUUAUGU          Hordeum vulgare #1
CCCUGCAUGC
UCUUUUAUGU          Hordeum vulgare #2
CCCUGCAUGC
UCUUUUAUGU          Brachypodium distachyon
CCCUGCAUGC
UUUUUUAUGU          Lactuca sativa #1
CUUUAUAUGC
AUUUUUAUGU          Lactuca sativa #2
ACCUGUAUGC
GCUUUUAUGU          Iris brevicaulis
CGCUGCAUGC
UCGUUUAUGC
UCUUUUAUGU          Cynodon dactylon
CACUGCAUGC
UCUUUUAUGU          Zostera marina
UGUUGCAUGA
AGUUGGAUGC
UCUUUUAUGU          Dioscorea alata
GGCUGCAUGC
UCUUUUAUGU          Prunus persica #1
AGCUGUAUGC
AGUUUCAUGG
UAUUUUAUGU          Prunus persica #2
AUUUGGAUGC
GGAUGCAUGC
CGGUUUAUGC
```

```
UCUUUUAUGU          Solanum torvum
GGCUGUAUGC
UCUUUUAUGU          Solanum lycopersicum #1
GACUGUAUGC
UCUUUUAUGU          Solanum lycopersicum #3
UAUUGUAUGU
GGGUUGAUGA
GACUGUAUGC
UCUUUUAUGU          Solanum tuberosum #1
AACUGUAUGC
UCUUUUAUGU          Solanum tuberosum #2
GACUGUAUGC
UCUUUUAUGU          Solanum tuberosum #3
GACUGUAUGC
UCUUUUAUGU          Solanum chacoense
GACUGUAUGC
UCUUUUAUGU          Actinidia deliciosa #1
GACUGUAUGC
UCUUUUAUGU          Actinidia arguta #2
GACUGUAUGC
UCUUUUAUGU          Actinidia arguta #3
GACUGUAUGC
UCUUUUAUGU          Actinidia chinensis #1
GACUGUAUGC
UCUUUUAUGU          Actinidia chinensis #2
GACUGUAUGC
UCUUUUAUGU          Liriodendron tulipifera
GGUGUCAUGC
AGUUGCAUGC
UCUUUUAUGU          Agrostis capillaris
CCCUGUAUGC
UUUUUUAUGU          Jatropha curcas
GGCUGCAUGC
CAGUUUAUGC
UCUUUUAUGU          Ricinus communis
GCCUGCAUGC
CAGUUUAUGC
UUUUCCAUGC          Limnanthes alba
UCUUUUAUGU
UGCUGUAUGC
AUUUUAAUGG
UUUUUUAUGU          Carthamus tinctorius
GGCUGUAUGC
UUUUUUAUGU          Euphorbia esula
AUCUGCAUGC
CAGUUUAUGC
UCUUUUAUGU          Coffea arabica
GACUGCAUGC
AGUUGUAUGA
UCUUUUAUGU          Coffea canephora
GACUGCAUGC
AGUUGUAUGA
UCUUUUAUGU          Theobroma cacao
AUCUGCAUGC
UUUUUUAUGU          Fagus sylvatica
GGCUGUAUGC
AGUUUGAUGG
UCUUUUAUGU          Triphysaria pusilla
CCGUAUAUGU
UCUUUUAUGU          Ficus elastica
AGCUGUAUGC
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Manihot esculenta

Carica papaya

Malus x domestica

Citrus clementina #1

Citrus clementina #2

Citrus sinensis

Citrus reticulata

Citrus aurantium

Citrus trifoliata

Diospyros kaki

Beta vulgaris

Euonymus alatus

Rubus ulmifolius

Gossypium raimondii

Gossypium hirsutum #1

Gossypium hirsutum #2

Gossypium hirsutum #3

Gossypium arboreum #1

Gossypium arboreum #2

Gossypium arboreum #3

Gossypium barbadense

Panax ginseng

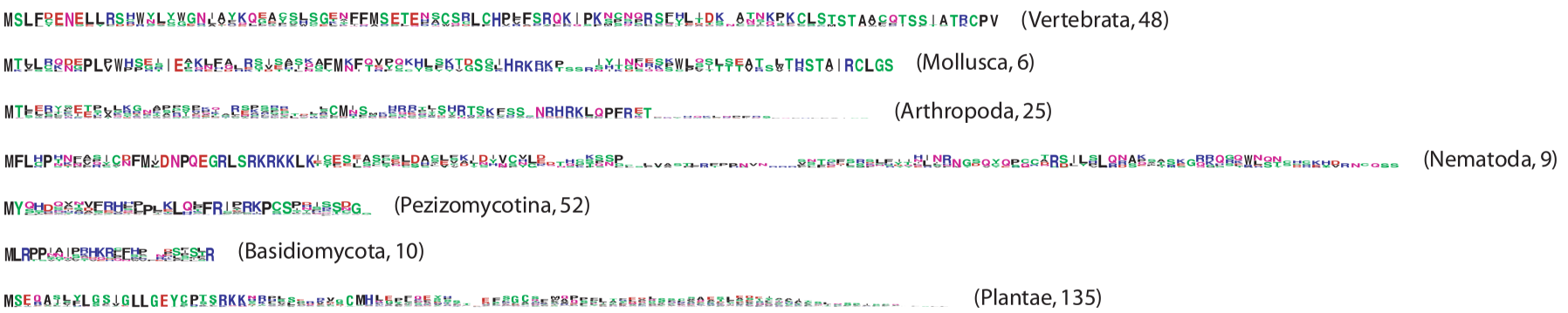
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UCUUUUAUGU          Catharanthus roseus
GACUGUAUGC
UAUUUUAUGU          Cannabis sativa
GGCUGUAUGC
AAGUGUAUGC
UCUUUUAUGU          Aquilegia formosa
GACUGCAUGC
UAUUUUAUGC
CCUUUUAUGU          Ipomoea nil
GGCUGCAUGC
UCUUUUAUGU          Striga hermonthica
GGCUGCAUGC
CAUUUUAUGU
CCUUUUAUGU          Cucumis melo
GGCUGCAUGC
CAUUUUAUGC
UUUUUUAUGU          Barnadesia spinosa #1
GGCUGUAUGC
UCUUUUAUGU          Barnadesia spinosa #2
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GGCUGCAUGC
UAUUGUAUGC
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GACUGUAUGC
AGUUUCAUGG
UUUUUUAUGU          Cynara cardunculus
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ACCUGCAUGC
UCUUUUAUGU          Mimulus guttatus
AGCUGCAUGA
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UCUUUUAUGU          Brassica napus #2
UCUUUUAUGU          Brassica oleracea #2
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UCUUUUAUGU          Eucalyptus globulus
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GGGUUUAUGC
AUUUUUAUGU          Helianthus petiolaris
GGCUGUAUGC
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UCUUUCAUGU          Glycine max #1
AGCUGUAUGC
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AGUUGUAUGA
UCUUUCAUGU          Glycine max #3
AGUUGUAUGA
UCUUUCAUGU          Glycine max #4
ACCUGUAUGC
UCUUUCAUGU          Phaseolus coccineus
AGCUUUAUGC
UCUUUCAUGU          Phaseolus vulgaris #1
AGCUGUAUGC
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AGCUGUAUGC
UCUUUCAUGU          Phaseolus acutifolius #1
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AGCUGCAUGC
UCUUUCAUGU          Phaseolus acutifolius #2
AGCUGUAUGC
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GGCUGUAUGC
UCUUUCAUGU          Vigna unguiculata
AUCUGUAUGC
UCUUUUAUGU          Leymus cinereus
CCCUGCAUGC
UCUUUUAUGU          Pinus taeda
GGUUGGAUGC
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UCUUUUAUGU          Pinus radiate
GGUUGGAUGC
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UUGGUUAUGU
GAAUAAAUGU
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Supplementary Figure S2. Weblogo representation of amino acid conservation in eIF5 uORF1 encoded peptides.

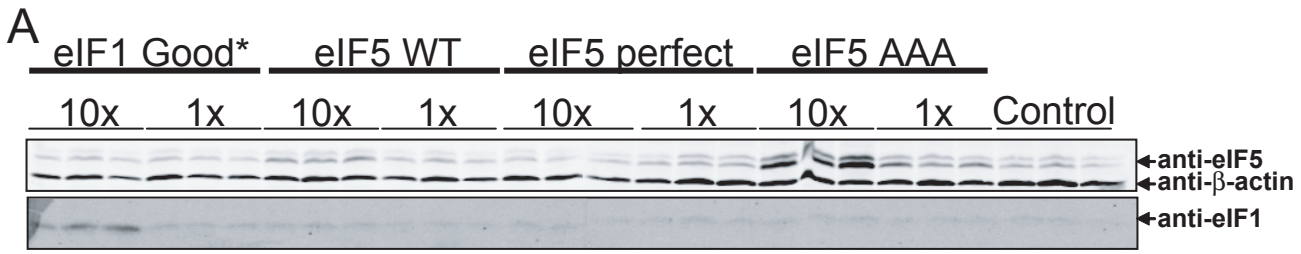
Letter heights are proportional to the frequency of conservation of each amino acid at each position. Each line represents a different eukaryotic branch which is indicated in parentheses on the right followed by the number of sequences used in each alignment. The sequences used to generate the alignment presented in this figure are available upon request.

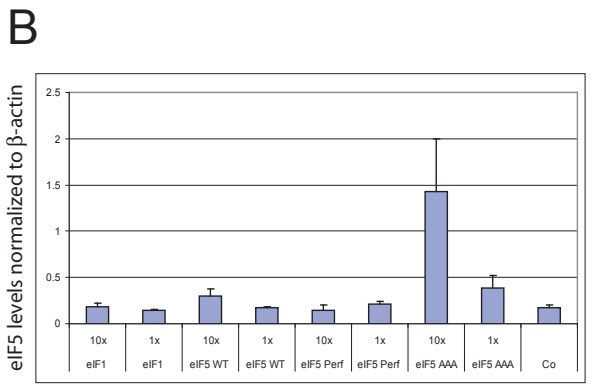
Supplementary Figure S2

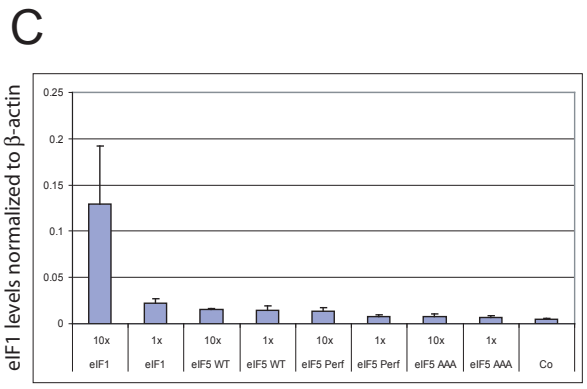


Supplementary Figure S3. Removing eIF5 uORFs results in constitutive eIF5 expression. *(A)* Western blots of protein lysates from cells transfected in triplicate with the eIF5 or eIF1 overexpression constructs indicated in Figure 2B of the main text. The eIF1 overexpression construct is the "eIF1 good*" described previously (2). In lanes marked "10×", 10-fold more vector with insert was transfected compared with lanes marked "1×," where the difference in the amount of transfecting DNA is made up with the inert vector pcDNA3 (2). The control cells are transfected with "10×" amount of pcDNA3. The blot shown was probed with anti-eIF5 and anti-β-actin followed by anti-eIF1. The corresponding detected proteins are indicated by arrows. *(B & C)* Densitometry analysis of the western blot shown in *A*. Relative eIF5 levels *(B)* and eIF1 levels *(C)* were determined by normalizing to β-actin levels.

Supplementary Figure S3







Supplementary Table S1.

Oligonucleotides used in this study.

1	eIF5 wt S PstI	ATAACTGCAGCCAGCCAATGGGCAGTGAGG
2	eIF5 wt AS 5' intron	AACCTTGATACTTACCGTTTTCGTCAAATAAAGACATAAACCCAACGC
3	eIF5 wt S 3' intron	TTTCTCTCCACAGAGCTGTTGCGCAGCCATTGG
4	eIF5 wt AS BamHI	TTATGGATCCAGACATTTTGGCTTATTAGTGG
5	eIF5 wt S 5' intron	TTTATTTGACGAAAACGGTAAGTATCAAGGTTAC
6	eIF5 wt AS 3' intron	AATGGCTGCGCAACAGCTCTGTGGAGAGAAAGG
7	eIF5 wt S	AAGCCAAAATGTCTGTCAATGTCAACCGC
8	eIF5 wt stop AS XbaI	TTATTCTAGACCTTTAAATGGCATCAATATCG
9	eIF5 wt start S SacI	ATAAGAGCTCGTTTAGTGAACCGCCAGCCAATGGGCAGTGAGG
10	eIF5 wt AS	AGACATTTTGGCTTATTAGTGG
11	eIF5 uORF1 perfect AS 5' intron	AACCTTGATACTTACCGTTTTCGTCAAATAAAGCCATGGTGGCAACGCTGCTCGCCCGGG
12	eIF5 uORF1 perfect S 5' intron	CCCGGGCGAGCAGCGTTGCCACCATGGCTTTATTTGACGAAAACGGTAAGTATCAAGGTT
13	eIF5 uAUG1 S	GTAGGGGTTTATGTCCG
14	eIF5 uAUG1 AS	GATCCGGACATAAACCCCTACTGCA
15	eIF5 uAUG2 S	GTAGTTTTTCATGTCCG
16	eIF5 uAUG2 AS	GATCCGGACATGAAAAACTACTGCA
17	eIF5 uAUG3 S	GTAGTCGTTTATGTCCG
18	eIF5 uAUG3 AS	GATCCGGACATAAACGACTACTGCA
19	eIF5 mAUG S	GTAGGCCAAAATGTCCG
20	eIF5 mAUG AS	GATCCGGACATTTTGGCCTACTGCA
21	Optimal context S	GTAGGCCACCATGGCCG
22	Optimal context A	GATCCGGCCATGGTGGCCTACTGCA
23	GG/S in worst context	GTAGTTTGTTATGGCCG
24	GG/A in worst context	GATCCGGCCATAACAAACTACTGCA
25	CG/S in worst context	GTAGTTTCTTATGGCCG
26	CG/A in worst context	GATCCGGCCATAAGAAACTACTGCA
27	TG/S in worst context	GTAGTTTTTTATGGCCG
28	TG/A in worst context	GATCCGGCCATAAAAAACTACTGCA
29	AA/S in worst context	GTAGTTTATTATGACCG
30	AA/A in worst context	GATCCGGTCATAATAAACTACTGCA
31	AC/S in worst context	GTAGTTTATTATGCCCG
32	AC/A in worst context	GATCCGGGCATAATAAACTACTGCA
33	AT/S in worst context	GTAGTTTATTATGTCCG
34	AT/A in worst context	GATCCGGACATAATAAACTACTGCA
35	GA/S in worst context	GTAGTTTGTTATGACCG
36	GA/A in worst context	GATCCGGTCATAACAAACTACTGCA
37	GC/S in worst context	GTAGTTTGTTATGCCCG
38	GC/A in worst context	GATCCGGGCATAACAAACTACTGCA
39	GT/S in worst context	GTAGTTTGTTATGTCCG
40	GT/A in worst context	GATCCGGACATAACAAACTACTGCA

41	CA/S in worst context	GTAGTTTCTTATGACCG
42	CA/A in worst context	GATCCGGTCATAAGAAACTACTGCA
43	CC/S in worst context	GTAGTTTCTTATGCCCG
44	CC/A in worst context	GATCCGGGCATAAGAAACTACTGCA
45	CT/S in worst context	GTAGTTTCTTATGTCCG
46	CT/A in worst context	GATCCGGACATAAGAAACTACTGCA
47	TA/S in worst context	GTAGTTTTTTATGACCG
48	TA/A in worst context	GATCCGGTCATAAAAAACTACTGCA
49	TC/S in worst context	GTAGTTTTTTATGCCCG
50	TC/A in worst context	GATCCGGGCATAAAAAACTACTGCA
51	TT/S in worst context	GTAGTTTTTTATGTCCG
52	TT/A in worst context	GATCCGGACATAAAAAACTACTGCA
53	AG/S in worst context	GTAGTTTATTATGGCCG
54	AG/A in worst context	GATCCGGCCATAATAAACTACTGCA