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| <b>Title</b>                       | Cas9/gRNA targeted excision of cystic fibrosis-causing deep-intronic splicing mutations restores normal splicing of CFTR mRNA  |
| <b>Author(s)</b>                   | Sanz, David J.; Hollywood, Jennifer A.; Scallan, Martina F.; Harrison, Patrick T.  |
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| Oligo Name                              | Sequence (5'>3')                      | Cloning site |
|---|---------------------------------------|--------------|
| <b><i>gRNA oligos cloning</i></b>       |                                       |              |
| g-in12U-1fw                             | caccgGAAACTGTGTACATTTTGAT             |              |
| g-in12U-1rv                             | aaacATCAAAATGTACACAGTTTCc             |              |
| g-in12U-2fw                             | caccgAGTATGCAAGAGCTACATAA             |              |
| g-in12U-2rv                             | aaacTTATGTAGCTCTTGCATACTc             |              |
| g-in12U-3fw                             | caccgGACTTTTTAAAGTTTTGCCAT            |              |
| g-in12U-3rv                             | aaacATGGCAAACTTTAAAAGTCc              |              |
| g-in12D-1fw                             | caccgATGTACTTGAGATGTAAGTA             |              |
| g-in12D-1rv                             | aaacTACTTACATCTCAAGTACATc             |              |
| g-in12D-2fw                             | caccgTTACTCATACTTTCCTTATT             |              |
| g-in12D-2rv                             | aaacAATAAGGAAAGTATGAGTAAc             |              |
| g-in12D-3                               | caccgTTATCTCATTCTATTAATA              |              |
| g-in12D-3rv                             | aaacTATTAATAGAAATGAGATAAc             |              |
| g-in19U-1                               | caccgTTTACTTGGCTACCAGAGAT             |              |
| g-in19U-1rv                             | aaacATCTCTGGTAGCCAAGTAAAc             |              |
| g-in19U-2                               | caccgAGTTACCCTCTTTTTTTACT             |              |
| g-in19U-2rv                             | aaacAGTAAAAAAGAGGGTAACTc              |              |
| g-in19D-1                               | caccgGTTATTTGCAGTGTTCCTA              |              |
| g-in19D-1rv                             | aaacTAGAAAACACTGCAAATAACc             |              |
| g-in19D-2                               | caccgTTTCTATGGAAATATTTTAC             |              |
| g-in19D-2rv                             | aaacGTGAAATATTTCCATAGAAAc             |              |
| g-in22U-1                               | caccgCATTTTAATACTGCAACAGA             |              |
| g-in22U-1rv                             | aaacTCTGTTGCAGTATTAATGc               |              |
| g-in22U-2                               | caccgCATCTGTTGCAGTATTAATA             |              |
| g-in22U-2rv                             | aaacTTTTAATACTGCAACAGATGc             |              |
| g-in22U-3                               | caccgCTTGATTTCTGGAGACCACA             |              |
| g-in22U-3rv                             | aaacTGTGGTCTCCAGAAATCAAGc             |              |
| g-in22D-1                               | caccgTTGATCCAACATTCTCAGGG             |              |
| g-in22D-1rv                             | aaacCCCTGAGAATGTTGGATCAAc             |              |
| g-in22D-2                               | caccgATCCAACATTCTCAGGGAGG             |              |
| g-in22D-2rv                             | aaacCCTCCCTGAGAATGTTGGATc             |              |
| <b><i>Minigenes cloning primers</i></b> |                                       |              |
| MGint12-int13FW                         | cacacactcgagTGTGTTGTCCAGTTTTGGATGA    | <i>XhoI</i>  |
| MGint12-int13RV                         | ctcacagctagcACTGGTTTAGCATGAGGCGG      | <i>NheI</i>  |
| MGint18-int20FW                         | ctcactctcgagTGACTAGGAATAGAATGGGGAGAG  | <i>XhoI</i>  |
| MGint18-int20RV                         | cacacagctagcACAATGGAAATTCAAAGAAATCACT | <i>NheI</i>  |
| MGint22-int23FW                         | cacacactcgagACAGTACTGGATAGTCCTCTGA    | <i>XhoI</i>  |
| MGint22-int23RV                         | cacacatctagaGCCTATGAGAAAAGTGCCTGG     | <i>XbaI</i>  |

|                                    |  |  |
|------------------------------------|--|--|
| <b><i>Directed Mutagenesis</i></b> |  |  |
|------------------------------------|--|--|

|                                       |  |  |
|---------------------------------------|--|--|
| MD1811+1.6kbA>Gfw<br>(c.1679+1634A>G) | CCTATGTACTTGGAGATGTAAGTAAGGTTACTATC      |  |
| MD1811+1.6kbA>Grv<br>(c.1679+1634A>G) | GATAGTAACCTTACTTACATCTCAAGTACATAGG       |  |
| MD3272-26A>Gfw<br>(c.3140-26A>G)      | GTGTTTATGTTATTTGTCAGTGTTTTCTATGG         |  |
| MD3272-26A>Grv<br>(c.3140-26A>G)      | CCATAGAAAACACTGCAAATAACATAAACAC          |  |
| MD3849+10kbC>Tfw<br>(c.3717-12191C>T) | CTGTTGCAGTATTAAAATGGtGAGTAAGACACCCTGAAAG |  |
| MD3849+10kbC>Trv<br>(c.3717-12191C>T) | CTTTCAGGGTGTCTTACTCaCCATTTTAATACTGCAACAG |  |
|                                       |  |  |
| <b>Sequencing Primers</b>             |  |  |
| pspSEQfw                              | TCACAGTCTATTATGGGGTACGG                  |  |
| pspSEQrv                              | AATTTCTGGGTCCCCTCCTGA                    |  |
| pcas9SEQfw                            | GAGGGCCTATTTCCCATGATTCC                  |  |
| pcas9SEQrv                            | GTCTGCAGAATTGGCGCAC                      |  |
| pcas9tandemSEQfw                      | TTTGTGATGCTCGTCAGGGG                     |  |
| pcas9tandemSEQrv                      | TGGAAAGTCCCTATTGGCGT                     |  |

|                                   |                                    |             |
|-----------------------------------|------------------------------------|-------------|
| <b>Tandem gRNA Cloning</b>        |                                    |             |
| pcas9TANDfw                       | CACACAGCTAGCGAGGGCCTATTTCCCATGATT  | <i>NheI</i> |
| pcas9TANDrv                       | CACACATCTAGATCTAGCTCTAAAACAAAAAAGC | <i>XbaI</i> |
|                                   |                                    |             |
| <b>Deletion Screening Primers</b> |                                    |             |
| in12Ufw                           | AAGAACAGAGTGTGGGGAAGA              |             |
| in12Drv                           | GCATTTTGTTCCTTGTAGTTTCAG           |             |
| in19Ufw                           | ACTCCCAGTGGTAGCCAAGA               |             |
| in19Drv                           | GCACGAAGTGTCCATAGTCCT              |             |
| in22Ufw                           | TCATTCAGTGGGTATAAGCAGCA            |             |
| in22Drv                           | GACATACCCTAAATCTAAGTCAGTG          |             |
|                                   |                                    |             |
| <b>RT-PCR</b>                     |                                    |             |
| pspRTfw                           | TCTGAGTCACCTGGACAACC               |             |
| pspRTrv                           | ATCTCAGTGGTATTTGTGAGC              |             |
| RTex13rv                          | ACATCTAGGTATCCAAAAGGAGAGT          |             |
| RTex20rv                          | GCACGAAGTGTCCATAGTCCT              |             |
|                                   |                                    |             |