| (4906 .. 4925) gRNA Protospacer <br> (4891 .. 4957) Donor Template WT -> SNV (4848 .. 4867) PCR Forward ) | PCR Reverse (5381 .. 5400) <br> Sanger Sequencing Primer (5381 .. 5400) $\square$ |  |
| :---: | :---: | :---: |
| 25001 50001 | 75001 | 10,000 |
| FUS |  |  |
| FLS-201 |  |  |
|  | FUS-201 <br> SNV <br> A_INK2SO0106_FUS_R216C_SNVSNV | $=\square$ |

 en | $\square$ | FUS |
| :---: | :---: |
| $\square U S-201$ |  | fus-201




$\square$ NUS 201

FUS-201




| FUS |  |
| :---: | :---: |
| FUS-201 |  |
|  | US-201 |
| cccccaictcccgaccccgcgctcgagcccgctttgtcgcagtactgcatccgagcactcgcgac <br> GGGGGTTGAGGGCCGGGGCGCGAGCTCGGGCGAAACAGCGTCACGACGTAGGCCCGTGAGCGCCGC | CGCACGCGCTCTGCGGGCCCCTCCCCCTTCGCGGCGCGGGTACCCCTTCCCCGCCTCGTGTTGGTTCAG <br>  GCGTGCGCGAGACGCCCGGGGAGGGGGAAGCGCCGCGCCCATGGGGAAGGGGCGGAGCACAACCAAGTC |
| $\square$ | FUS |
| - | US-201 |






## FUS-201



 FUS
FUS-201

FUS-201






 FUS-201



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FUS-201
FUS-201


 FUS
FUS-201

FUS-201


 FUS-201
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FUS-201


 FUS


US-201





FUS-201





FUS-201


 FUS $\quad$ FUS-201 $\ggg>$


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## FUS-201




FUS

## FUS-201



 fus
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 FUS-201











## FUS-201







 FUS
















FUS-201
TGGCTACTAGGGTGAG GACC
PCR Reverse
SGGCTACTAGGGTGAG GACC
Sanger Sequencing Primer











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| FUS 201 |
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## FUS-201














FUS-201







| Feature |  | Location | Size | $\square$ | 4 | Type |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| FUS |  | 1 .. 11,468 | 11,468 bp | $\square$ | $\rightarrow$ | gene |
| /note | $\begin{aligned} & =\begin{array}{l} \text { gene ENSG00000089280 } \\ \text { Protein coding } \end{array} \end{aligned}$ |  |  |  |  |  |
| FUS-209 |  | 1 .. 11,465 | 11,465 bp | $\square$ | $\rightarrow$ | prim_transcript |
| /note | $=$ primary transcript ENST00000566605 <br> Nonsense mediated decay |  |  |  |  |  |
| FUS-201 |  | 2 .. 11,468 | $11,467 \mathrm{bp}$ | $\square$ | $\rightarrow$ | prim_transcript |
| /note | $=$ primary transcript ENST00000254108 |  |  |  |  |  |
| FUS-202 |  | 2 .. 11,465 | 11,464 bp | $\square$ | $\rightarrow$ | prim_transcript |
| /note | $=$ primary transcript ENST00000380244 |  |  |  |  |  |
| FUS-205 |  | 2 .. 3213 | 3212 bp | $\square$ | $\rightarrow$ | prim_transcript |
| /note | $\begin{aligned} & =\begin{array}{l} \text { primary transcript ENST00000487045 } \\ \text { Retained intron } \end{array} \end{aligned}$ |  |  |  |  |  |
| FUS-206 |  | 13 .. 11,465 | 11,453 bp | $\square$ | $\rightarrow$ | prim_transcript |
| /note | $=$ primary transcript ENST00000487509 <br> Retained intron |  |  |  |  |  |
| FUS-210 |  | $14 . .11,438$ | 11,425 bp | $\square$ | $\rightarrow$ | prim_transcript |
| /note | $=$ primary transcript ENST00000568685 |  |  |  |  |  |
| FUS-201 |  | $78 . .11,301$ | 11,224 bp | $\square$ | $\rightarrow$ | CDS |

- 15 segments $=1581 \mathrm{~b}$
/note $\quad=$ coding sequence ENSP00000254108
 SYGGQQQSYGQQQSYNPPQGYGQQNQYNSSSGGGGGGGGG,,GNYGQDQSSMSSGGGSGGGYGNQDQSGGGGSGGYGQQDRGGRGRGGSGGGGGGGGGGYNRSSGGYEPRGRGGGRGGRGGMG,,GSDRGGFNK G,,GPRDQ GSRHDS,,EQDNSDNNTIFVQGLGENVTIESVADYFKQIGIIK,,TNKKTGQPMINLYTDRETGKLKGEATVSFDDPPSAKAAIDWFD,,GKEFSGNPIKVSFATRRADFNRGGGNGRGGRGRG,,GPMGRGGYGGGGS GGGGRGGFPSGGGGGGGQQRAGDWKCPNP,,TCENMNFSWRNECNQCKAPKPDGPGGGPGGSHM,,GGNYGDD२RGGRGGYDRGGYRGRGGDRGGFRGGRGGGDRGGFGPGKMDSR,,GEHRQDRRERPY* 526 amino acids $=53.4 \mathrm{kDa}$
FUS-202 78 .. $11,301 \quad 11,224 \mathrm{bp} \quad \square \rightarrow \mathrm{CDS}$
- 15 segments $=1578 \mathrm{bp}$

| /note | coding sequence ENSP00000369594 |  |  |
| :---: | :---: | :---: | :---: |
| /translation | $=\quad M A S N,, D Y T Q Q A T Q S$, ,YGAYPTQPGQGYSQQSSQPYGQQSYSGYSQSTDTSGYGQSSYSSYGQSQN,,SYGTQSTPQGYGSTGGYGSSQSSQSSYGQQSSYPGYGQQPAPSSTSGS,,YGSSSQSSSSYGQPQSGSYSQQP SYGGQQQSYGQQQSYNPPQGYGQQNQYNSSSGGGGGGGGG,,GNYGQDQSSMSSGGGSGGGYGNQDQSGGGGSGGYGQQDRGGRGRGGSGGGGGGGGGGYNRSSGGYEPRGRGGGRGGRGGMG,,GSDRGGFNKFG ,,GPRDQGSRHDS,,EQDNSDNNTIFVQGLGENVTIESVADYFKQIGIIK,,TNKKTGQPMINLYTDRETGKLKGEATVSFDDPPSAKAAIDWFD,,GKEFSGNPIKVSFATRRADFNRGGGNGRGGRGRG,,GPMGRGGYGGGGSG GGGRGGFPSGGGGGGGQQRAGDWKCPNP,,TCENMNFSWRNECNQCKAPKPDGPGGGPGGSHM,,GGNYGDDRRGGRGGYDRGGYRGRGGDRGGFRGGRGGGDRGGFGPGKMDSR,,GEHRQDRRERPY* 525 amino acids $=53.4 \mathrm{kDa}$ |  |  |
| FUS-210 | 78 .. 11,301 | $11,224 \mathrm{bp}$ ■ $\rightarrow$ | CDS |

( 15 segments $=1584 \mathrm{bp}$
Inote $\quad=$ coding sequence ENSP00000455282
 G,,GPRDQ GSRHDS,,AEQDNSDNNTIFVQ GLGENVTIESVADYFKQIGIIK,,TNKKTGQPMINLYTDRETGKLKGEATVSFDDPPSAKAAIDWFD,,GKEFSGNPIKVSFATRRADFNRGGGNGRGGRGRG,,GPMGRGGYGGGG SGGGGRGGFPSGGGGGGGQQRAGDWKCPNP,,TCENMNFSWRNECNQCKAPKPDGPGGGPGGSHM,,GGNYGDDRRGGRGGYDRGGYRGRGGDRGGFRGGRGGGDRGGFGPGKMDSR,,GEHRQDRRERPY* 527 amino acids $=53.5 \mathrm{kDa}$
$=$ gene ENSGO0000260304
IncRNA

| 2374 | . | 3148 | 775 bp | $\square$ | $\leftarrow$ | gene |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 2374 | .. | 3148 | 775 bp | $\square$ | * | prim_transcript |
| 3413 | .. | 4261 | 849 bp | $\square$ | $\rightarrow$ | prim_transcript |
| 4891 | . | 4957 | 67 bp | $\square$ | $\mapsto$ | misc_feature |
| 4906 | . | 4925 | 20 bp | $\square$ | $\mapsto$ | misc_feature |
| 4924 | .. | 4924 | 1 bp | $\square$ | $\mapsto$ | misc_feature |
| 4926 | .. | 4928 | 3 bp | $\square$ | $\mapsto$ | misc_feature |
| 4943 | .. | 7108 | 2166 bp | $\square$ | $\rightarrow$ | prim_transcript |
| 5362 | .. | 10,965 | 5604 bp | ㅁ | $\rightarrow$ | prim_transcript |
| 8015 | .. | 9983 | 1969 bp | $\square$ | $\rightarrow$ | prim_transcript |
| 8330 | .. | 9558 | 1229 bp | $\square$ | $\rightarrow$ | prim_transcript |
| 10,079 | .. | 11,468 | 1390 bp | $\square$ | $\rightarrow$ | prim_transcript |
| 10,236 | .. | 11,421 | 1186 bp | $\square$ | $\rightarrow$ | prim_transcript |

PCR Forward

| Donor Template WT -> SNV | 67-mer | $\bigcirc$ | 4891 .. 4957 | $\rightarrow$ | $86^{\circ} \mathrm{C}$ | 16, 2022 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |

/sequence $\quad=$ AGCGGTGGCTATGGACAGCAGGACCGTGGAGGCTGCGGCAGGGGTGGCAGTGGTGGCGGCGGCGGCG 75\% GC / 21,090.6 Da
gRNA Protospacer
$=$ CAGCAGGACCGTGGAGGCCG $75 \%$ GC / 6193.1 Da
sequence

PCR Reverse
/sequence
$=$ CCAGGAGTGGGATCATCGGT 60\% GC / 6198.1 Da
Sanger Sequencing Primer
/sequence $\quad=$ CCAGGAGTGGGATCATCGGT $60 \% \mathrm{GC} / 6198.1 \mathrm{Da}$

20-mer 4906 .. 4925 - $66^{\circ} \mathrm{C}$ Jun 16,2022

20-mer 5381 .. $5400-60^{\circ} \mathrm{C}$ Jun 16,2022

20-mer 5381 .. $5400-60^{\circ} \mathrm{C}$ Jun 16, 2022

