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4185

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GRN-201

45
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GRN-201

D K
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4725

GRN

GRN-201

GRN-201

Sanger Sequencing Primer

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PCR Forward

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4860

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120
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ENSE00002337665

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Donor Template WT -> SNV

Donor Template WT -> SNV

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Donor Template WT -> SNV

Protospacer Sequence

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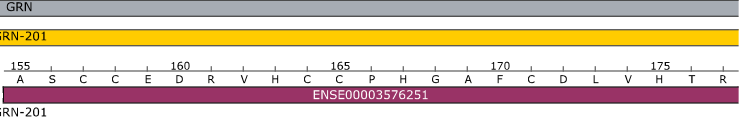
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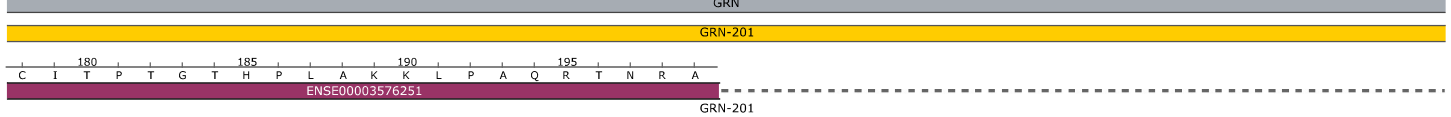
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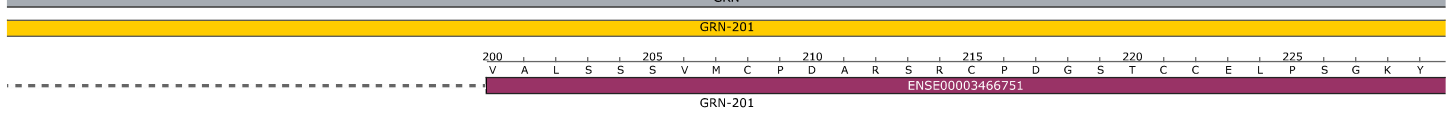
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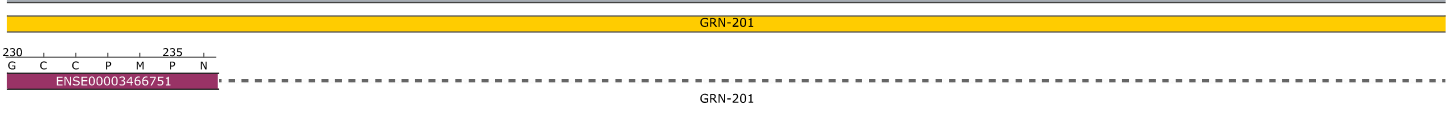
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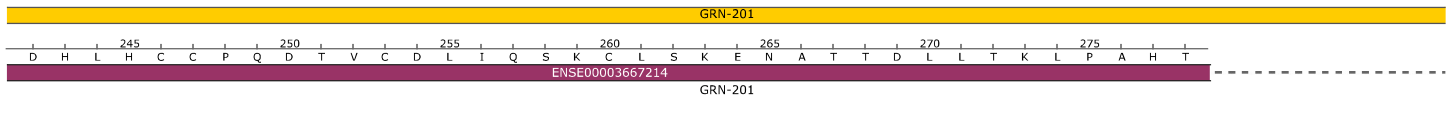
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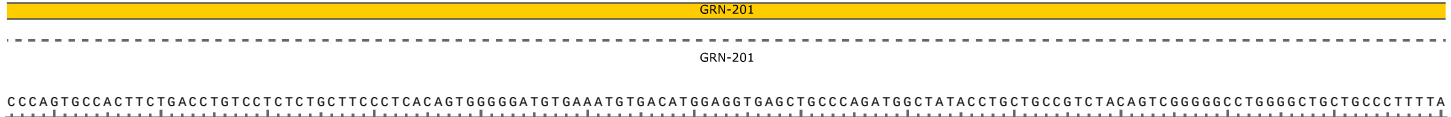
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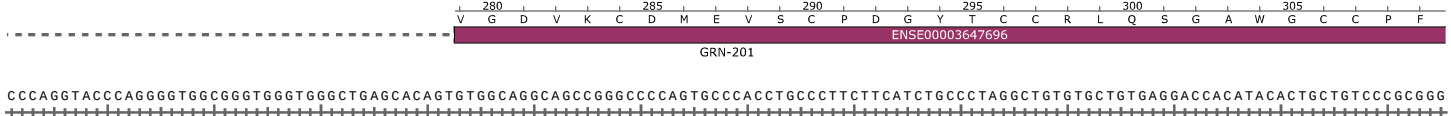
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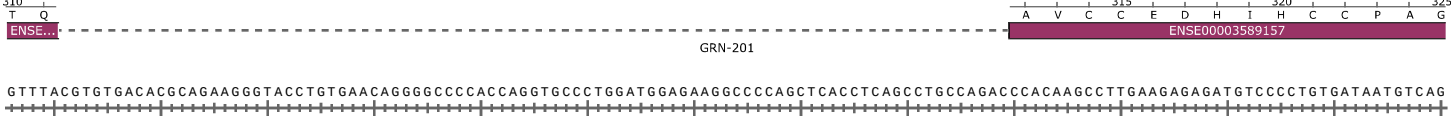
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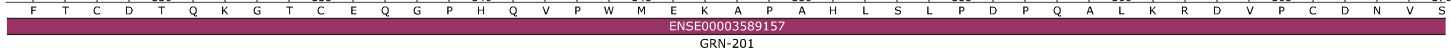
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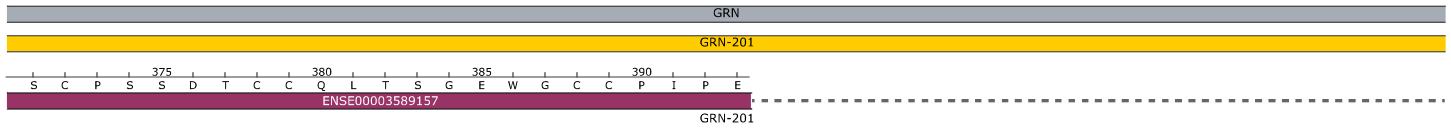
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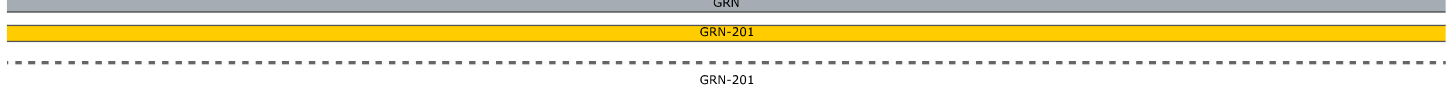
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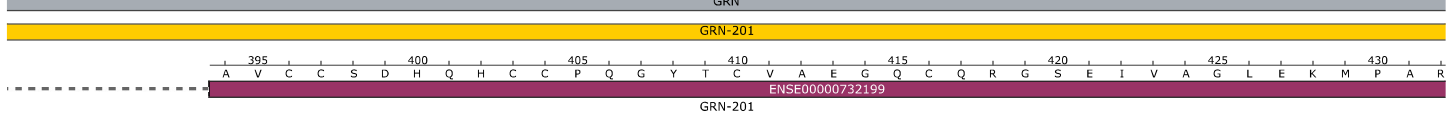
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6750



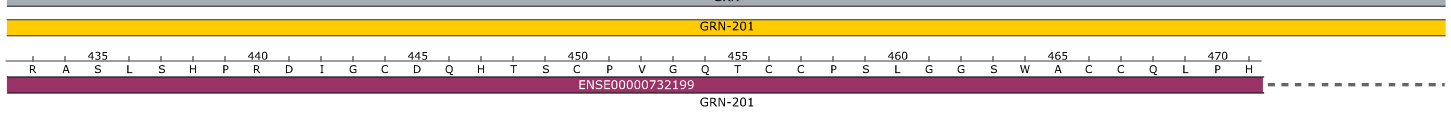
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6885



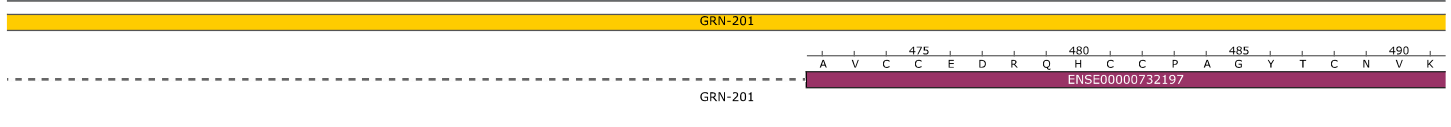
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7020



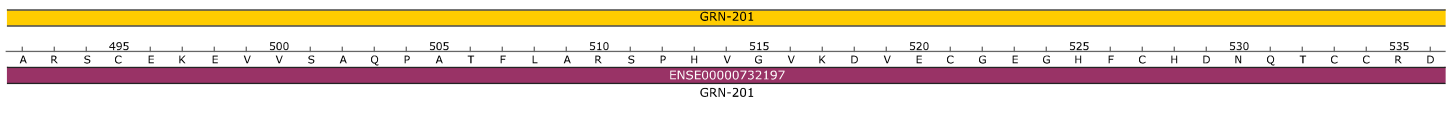
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7155



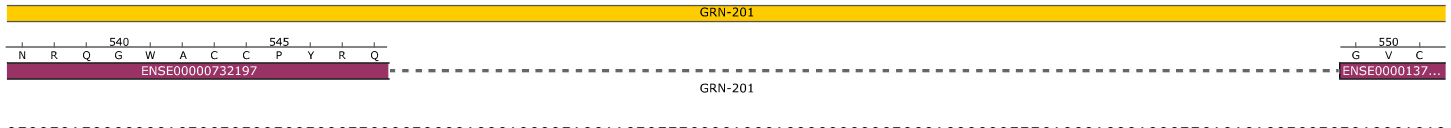
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7290



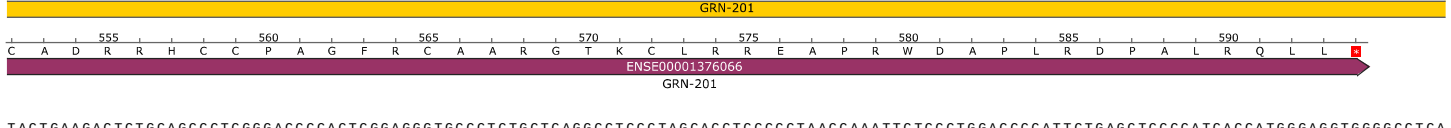
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7425



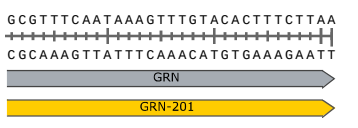
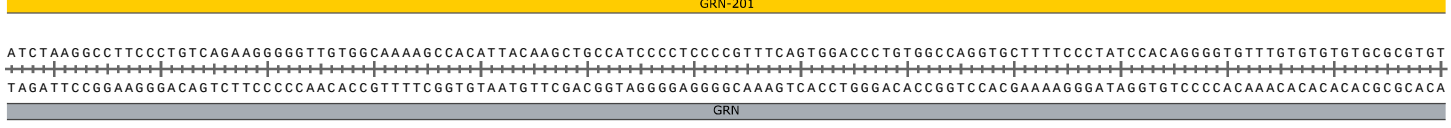
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7560



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7695



Feature	Location	Size	Type
GRN	1 .. 7861	7861 bp	gene
/note	= gene ENSG00000030582 Protein coding		
GRN-212	1 .. 4649	4649 bp	prim_transcript
/note	= primary transcript ENST00000588170 Retained intron		
GRN-215	17 .. 4491	4475 bp	prim_transcript
/note	= primary transcript ENST00000589536		
GRN-209	20 .. 5042	5023 bp	prim_transcript
/note	= primary transcript ENST00000587518		
GRN-207	20 .. 4229	4210 bp	prim_transcript
/note	= primary transcript ENST00000587109		
GRN-211	37 .. 5224	5188 bp	prim_transcript
/note	= primary transcript ENST00000588143		
GRN-221	53 .. 5080	5028 bp	prim_transcript
/note	= primary transcript ENST00000593167		
GRN-201	57 .. 7861	7805 bp	prim_transcript
/note	= primary transcript ENST00000053867		
GRN-213	57 .. 5799	5743 bp	prim_transcript
/note	= primary transcript ENST00000588237		
GRN-208	57 .. 5266	5210 bp	prim_transcript
/note	= primary transcript ENST00000587387		
GRN-220	57 .. 5059	5003 bp	prim_transcript
/note	= primary transcript ENST00000592783		
GRN-218	57 .. 5038	4982 bp	prim_transcript
/note	= primary transcript ENST00000591740		
GRN-219	57 .. 4544	4488 bp	prim_transcript
/note	= primary transcript ENST00000592323 Retained intron		
GRN-203	57 .. 4030	3974 bp	prim_transcript
/note	= primary transcript ENST00000585512		
GRN-214	60 .. 7837	7778 bp	prim_transcript
/note	= primary transcript ENST00000589265		
GRN-210	61 .. 4566	4506 bp	prim_transcript
/note	= primary transcript ENST00000587958 Retained intron		
GRN-206	69 .. 5918	5850 bp	prim_transcript
/note	= primary transcript ENST00000586782 Nonsense mediated decay		
GRN-201	3920 .. 7553	3634 bp	CDS
▶ 12 segments = 1782 bp			
/note	= coding sequence ENSP00000053867		
/translation	= MWTLVSWVALTAGLVA GTRCPDGGQFCPVACCLDPGGASYSCCRPLL,,DKWPTTLSRHLGGPCQVDAHCSAGHSCIFTVSGTSSCCPFPE,,AVACGDGHHCCPRGFHCSADGRSCFQRS,,GNNSVGAIQCPDSQFECPDFS TCCVMVDGSWGCCPMPQ,,ASCCEDRVHCCPHGAFCDLVHTRCITPTGTHPLAKKLPARTNRA,,VALSSSVMPDARSRCPDGSTCCCLPSGKYGCCPMPN,,ATCCSDHLHCCPQDVTVCDLIQSKLSKENATDDLTKL PAHT,,VGDVKCDMEVSCPDGYTCCRLQSGAWGCCPFTQ,,AVCCEDHIHCCPAGFTCDTQKGTCEQGPHQVPWMEKAPAHLSLPPDQALKRDVPCDNVSSCPSSDTCCQLTSGEWGCCPIPE,,AVCCSDHQHCCPQGY TCVAEGQCQRGSEIVAGLEKMPARRASLSHPRDIGCDQHTSCPVGQTCPPSLGGSWACCQLPH,,AVCCEDRQHCCPAGYTCNVKARSCEKEVVSQAQPATFLARSPHVGVKDVCEGEGHFCHDNQTCRRDRNQGAWACC BBAARRKCLREARWDDPLRDPALRQLL*		
GRN-214	3920 .. 7553	3634 bp	CDS
▶ 8 segments = 1311 bp			
/note	= coding sequence ENSP000000467616		
/translation	= MWTLVSWVALTAGLVA GTRCPDGGQFCPVACCLDPGGASYSCCRPLL,,DKWPTTLSRHLGGPCQVDAHCSAGHSCIFTVSGTSSCCPFPE,,AVACGDGHHCCPRGFHCSADGRSCFQRS,,GNNSVGAIQCPDSQFECPDFS TCCVMVDGSWGCCPMPQ,,AVCCEDRVHCCPHGAFCDLVHTRCITPTGTHPLAKKLPARTNRA,,VALSSSVMPDARSRCPDGSTCCCLPSGKYGCCPMPN,,ATCCSDHLHCCPQDVTVCDLIQSKLSKENATDDLTKL MPARRASLSHPRDIGCDQHTSCPVGQTCPPSLGGSWACCQLPH,,AVCCEDRQHCCPAGYTCNVKARSCEKEVVSQAQPATFLARSPHVGVKDVCEGEGHFCHDNQTCRRDRNQGAWACC BBAARRKCLREARWDDPLRDPALRQLL*		
GRN-222	3920 .. 7550	3631 bp	CDS
▶ 11 segments = 1314 bp			
/note	= coding sequence ENSP000000492014		
/translation	= MWTLVSWVALTAGLVA GTRCPDGGQFCPVACCLDPGGASYSCCRPLL,,DKWPTTLSRHLGGPCQVDAHCSAGHSCIFTVSGTSSCCPFPE,,AVACGDGHHCCPRGFHCSADGRSCFQRS,,GNNSVGAIQCPDSQFECPDFS TCCVMVDGSWGCCPMPQ,,ASCCEDRVHCCPHGAFCDLVHTRCITPTGTHPLAKKLPARTNRA,,VALSSSVMPDARSRCPDGSTCCCLPSGKYGCCPMPN,,ATCCSDHLHCCPQDVTVCDLIQSKLSKENATDDLTKL PAHT,,VGDVKCDMEVSCPDGYTCCRLQSGAWGCCPFTQ,,AVCCEDHIHCCPAGFTCDTQKGTCEQGPHQVPWMEKAPAHLSLPPDQALKRDVPCDNVSSCPSSDTCC,,RDRNQGAWCCPYRQ,,GVCCADRRHCCPAG BBAARRKCLREARWDDPLRDPALRQLL		
GRN-222	3920 .. 7550	3631 bp	prim_transcript
/note	= primary transcript ENST00000639447		
GRN-213	3920 .. 5799	1880 bp	CDS
▶ 5 segments = 518 bp			
/note	= coding sequence ENSP000000466611		
/translation	= MWTLVSWVALTAGLVA GTRCPDGGQFCPVACCLDPGGASYSCCRPLL,,DKWPTTLSRHLGGPCQVDAHCSAGHSCIFTVSGTSSCCPFPE,,ASCCEDRVHCCPHGAFCDLVHTRCITPTGTHPLAKKLPARTNRA,,VALSS SVMCPDARSRCPDGSTCCCLPSGKYGCCPMPN,,AT 172 amino acids = 18.1 kDa		
GRN-208	3920 .. 5266	1347 bp	CDS
▶ 5 segments = 574 bp			
/note	= coding sequence ENSP000000467431		
/translation	= MWTLVSWVALTAGLVA GTRCPDGGQFCPVACCLDPGGASYSCCRPLL,,DKWPTTLSRHLGGPCQVDAHCSAGHSCIFTVSGTSSCCPFPE,,IKGPCQCRFLCVPQAVACGDGHHCCPRGFHCSADGRSCFQRS,,GNNSVG AIQCPDSQFECPDFSTCCVMVDGSWGCCPMPQ,,ASCCEDRVHCCPHGAFCDLVHTR 191 amino acids = 20.3 kDa		

Feature	Location	Size	Start	End	Type
GRN-211	3920 .. 5224	1305 bp	■	→	CDS
▶ 5 segments = 490 bp					
/note	= coding sequence ENSP00000465375				
/translation	= MWTLVSWVALTAGLVA GTRCPDGGQFCPVACCLDPGGASYSCCRPLL,,DKWPTTLRHLGGPCQVDAHCSAGHSCIFTVSGTSSCCPFPE,,AVACGDGHHCCPRGFHCSADGRSCFQRS,,GNNSVGAIQCPDSQFECPDFS I6SVMMDGGSWGCSPMIDASCCEDRVH				
GRN-221	3920 .. 5080	1161 bp	■	→	CDS
▶ 4 segments = 447 bp					
/note	= coding sequence ENSP00000466405				
/translation	= MWTLVSWVALTAGLVA GTRCPDGGQFCPVACCLDPGGASYSCCRPLL,,DKWPTTLRHLGGPCQVDAHCSAGHSCIFTVSGTSSCCPFPE,,AVACGDGHHCCPRGFHCSADGRSCFQRS,,GNNSVGAIQCPDSQFECPDFS I6SVMMDGGSWGCSPMIDASCCEDRVH 15.6 kDa				
GRN-220	3920 .. 5059	1140 bp	■	→	CDS
▶ 4 segments = 426 bp					
/note	= coding sequence ENSP00000467870				
/translation	= MWTLVSWVALTAGLVA GTRCPDGGQFCPVACCLDPGGASYSCCRPLL,,DKWPTTLRHLGGPCQVDAHCSAGHSCIFTVSGTSSCCPFPE,,AVACGDGHHCCPRGFHCSADGRSCFQRS,,GNNSVGAIQCPDSQFECPDFS I6SVMMDGGSWGCSPMIDASCCEDRVH 14.9 kDa				
GRN-209	3920 .. 5042	1123 bp	■	→	CDS
▶ 4 segments = 409 bp					
/note	= coding sequence ENSP00000465518				
/translation	= MWTLVSWVALTAGLVA GTRCPDGGQFCPVACCLDPGGASYSCCRPLL,,DKWPTTLRHLGGPCQVDAHCSAGHSCIFTVSGTSSCCPFPE,,AVACGDGHHCCPRGFHCSADGRSCFQRS,,GNNSVGAIQCPDSQFECPDF 136 amino acids = 14.3 kDa				
GRN-218	3920 .. 5038	1119 bp	■	→	CDS
▶ 4 segments = 405 bp					
/note	= coding sequence ENSP00000467022				
/translation	= MWTLVSWVALTAGLVA GTRCPDGGQFCPVACCLDPGGASYSCCRPLL,,DKWPTTLRHLGGPCQVDAHCSAGHSCIFTVSGTSSCCPFPE,,AVACGDGHHCCPRGFHCSADGRSCFQRS,,GNNSVGAIQCPDSQFECPDF 135 amino acids = 14.1 kDa				
GRN-215	3920 .. 4491	572 bp	■	→	CDS
▶ 3 segments = 334 bp					
/note	= coding sequence ENSP00000466956				
/translation	= MWTLVSWVALTAGLVA GTRCPDGGQFCPVACCLDPGGASYSCCRPLL,,DKWPTTLRHLGGPCQVDAHCSAGHSCIFTVSGTSSCCPFPE,,AVACGDGHHCCPRGFHCSADGRS 111 amino acids = 11.5 kDa				
GRN-207	3920 .. 4229	310 bp	■	→	CDS
▶ 2 segments = 187 bp					
/note	= coding sequence ENSP00000466271				
/translation	= MWTLVSWVALTAGLVA GTRCPDGGQFCPVACCLDPGGASYSCCRPLL,,DKWPTTLRHLGGPCQ 62 amino acids = 6.6 kDa				
GRN-203	3920 .. 4030	111 bp	■	→	CDS
/note	= coding sequence ENSP00000467745				
/translation	= MWTLVSWVALTAGLVA GTRCPDGGQFCPVACCLDPGGA 37 amino acids = 3.8 kDa				
Donor Template WT -> SNV	4971 .. 5041	71 bp	■	↔	misc_feature
PAM	5005 .. 5007	3 bp	■	↔	misc_feature
SNV	5006 .. 5006	1 bp	■	↔	misc_feature
/note	= WT = C SNV = T				
Protospacer Sequence	5008 .. 5027	20 bp	■	↔	misc_feature
GRN-217	5044 .. 6055	1012 bp	■	→	prim_transcript
/note	= primary transcript ENST00000590984 Retained intron				
GRN-205	5294 .. 7798	2505 bp	■	→	prim_transcript
/note	= primary transcript ENST00000586443				
GRN-205	5294 .. 7407	2114 bp	■	→	CDS
▶ 7 segments = 1166 bp					
/note	= coding sequence ENSP00000465673				
/translation	= WQRSSLRGLTGQ,,WPCPARSCVIRTHGPGALMVLPAVSCPVGSMASAAQCPT,,PPAAPITCTAAPKTLCVT*SRVSA SPRRTLPRTSLSLCLRTQ,,WGM*NVTWR*AAQMAIPA AVYSRGPAA ALLPR,,LC AVRTTYTAVP RGLRVTRRRV/PVNRGPTRC PGWRRPQLTSACQTHKP*REMSPVMSAAVPPIPA ANSRLGSGAAVQSQR,,LSAARTTSTAAPRATRV*LRGSVSEEARSWLDWRRC LAPGLPYPTPETS AVTSTPAARWGRPAARAWVGA GPAASC PM,,LCA ARIASTAARLATPAT*RLDPARRKWSLPLPPSWPVALTWV*RTWSVGKDTSAMITRPA AETTD RAGPAVPTARSVP TPI LGLGMARDQVPPRPTLSPSS 388 codons (7 internal stop codons)				
GRN-216	5527 .. 6550	1024 bp	■	→	prim_transcript
/note	= primary transcript ENST00000589923				
GRN-202	5666 .. 6354	689 bp	■	→	prim_transcript
/note	= primary transcript ENST00000585348 Retained intron				
GRN-204	6957 .. 7857	901 bp	■	→	prim_transcript
/note	= primary transcript ENST00000586242				
GRN-204	6957 .. 7524	568 bp	■	→	CDS
▶ 3 segments = 332 bp					
/note	= coding sequence ENSP00000467837				
/translation	= AARA WVGAPAA SC PM,,LCA ARIASTAARLATPAT*RLDPARRKWSLPLPPSWPVALTWV*RTWSVGKDTSAMITRPA AETTD RAGPAVPTAS,,QGYQVFAQGGPALGRPF 110 codons (2 internal stop codons)				

Primer	Length	Binding Sites	Tm	Date Added
✓ PCR Forward	20-mer	4738 .. 4757 →	59°C	Jun 17, 2022
/sequence = GAGCTGGGCCGGTCTAATAC 60% GC / 6158.1 Da				
✓ Sanger Sequencing Primer	20-mer	4738 .. 4757 →	59°C	Jun 17, 2022
/sequence = GAGCTGGGCCGGTCTAATAC 60% GC / 6158.1 Da				
✓ Donor Template WT -> SNV	71-mer	4971 .. 5041 →	76°C	Jun 17, 2022
/sequence = TCTTGTCACAGGTAACAACCTCCGTGGGTGCCATCTAGTGCCCTGATAGTCAGTTCGAATGCCCGGACTTC 54% GC / 21,772.1 Da				
✓ gRNA Protospacer	20-mer	5008 .. 5027 ←	58°C	Jun 17, 2022
/sequence = CGAACTGACTATCAGGGCAC 55% GC / 6111.0 Da				
✓ PCR Reverse	20-mer	5166 .. 5185 ←	57°C	Jun 17, 2022
/sequence = TGGTCCTGACTCCGTCTTCA 55% GC / 6035.0 Da				