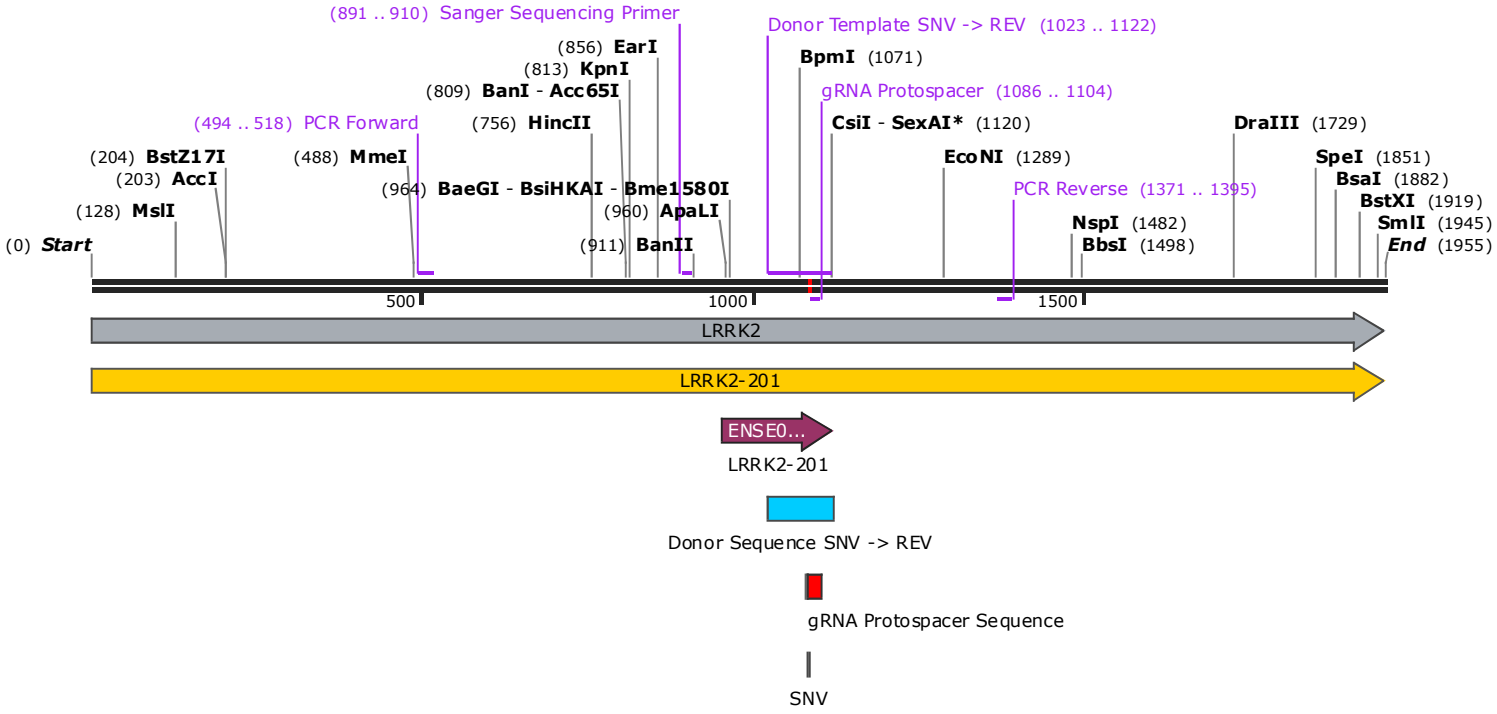
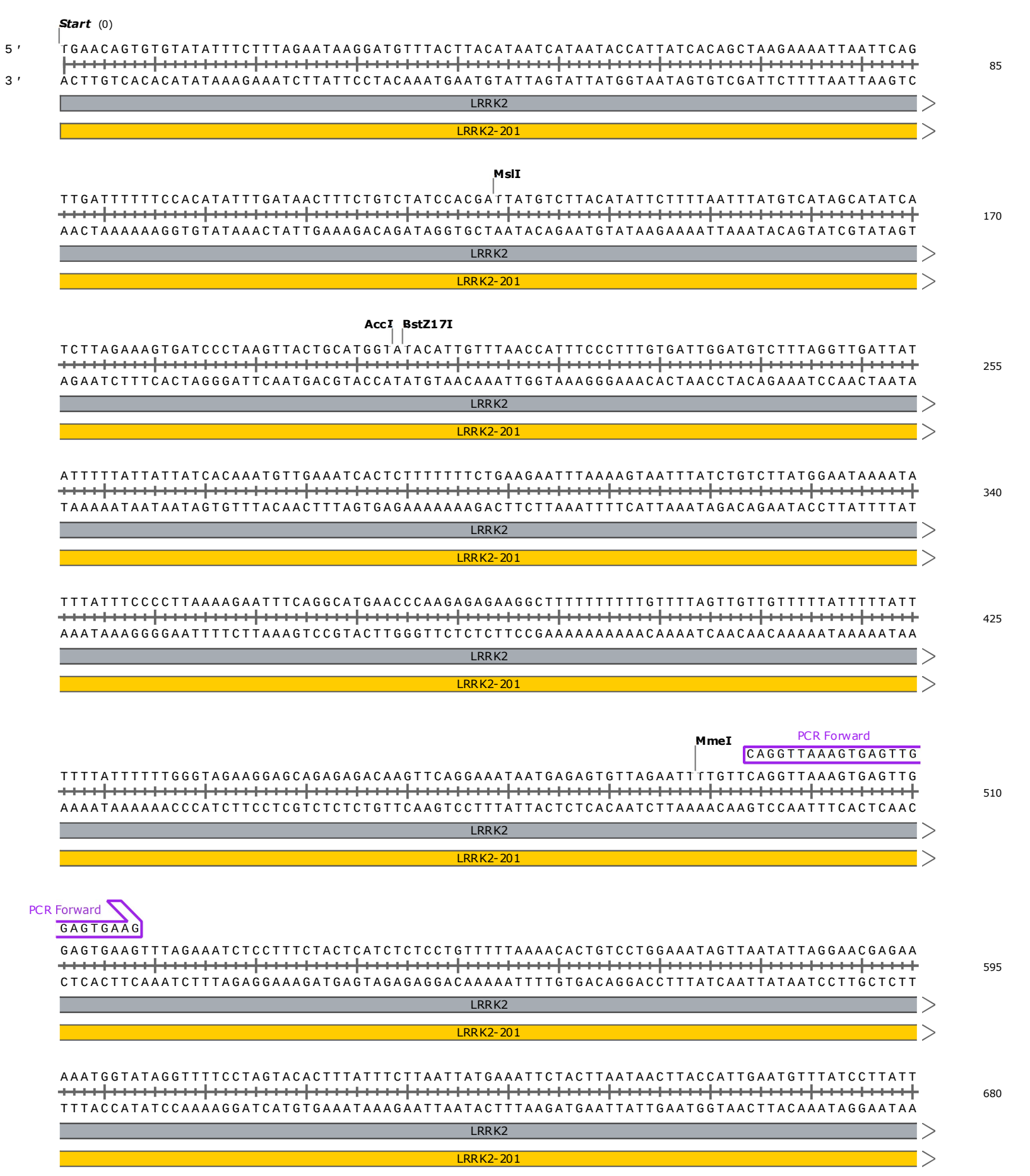
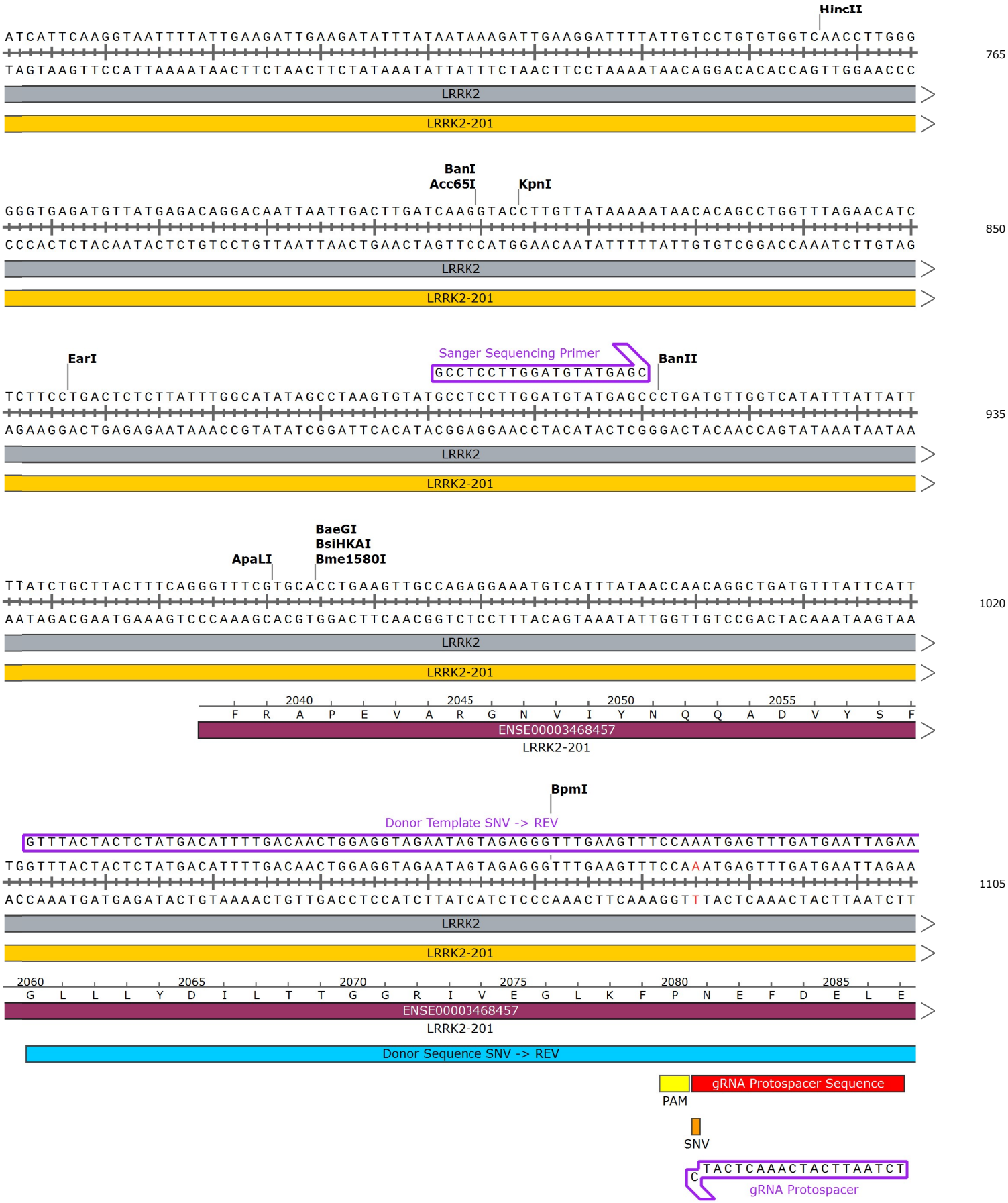
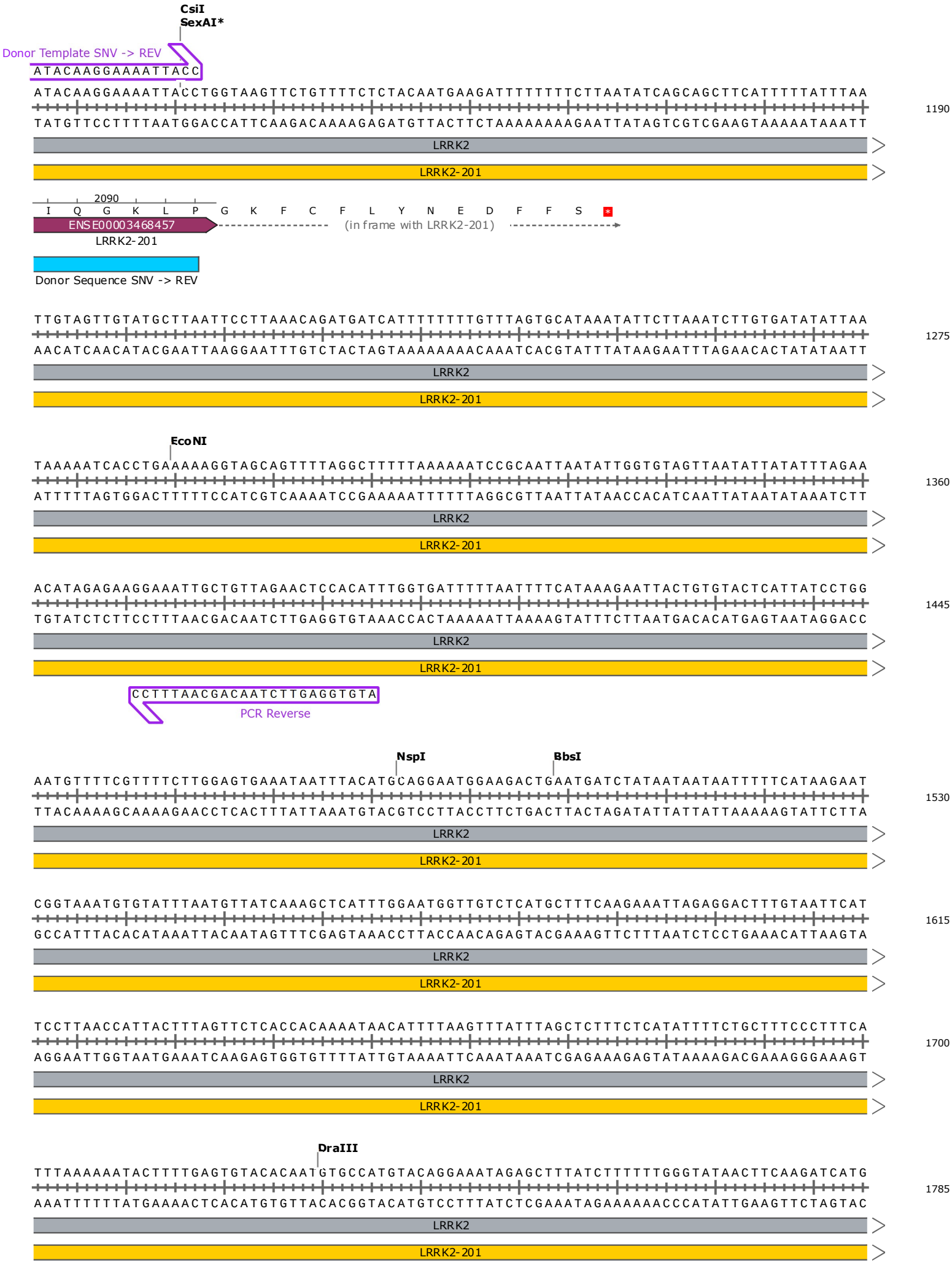


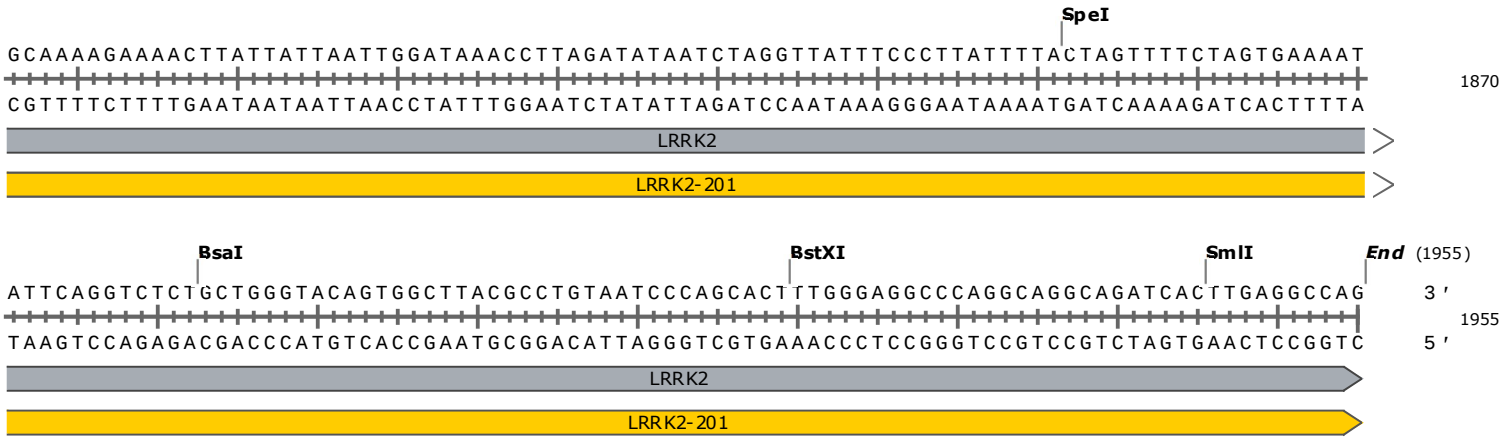
ASK2J00180R_LRRK2_N2081D_C02_AA

1955 bp


Start (0)
5 ' TGAACAGTGTGTATATTTCTTTAGAATAAGGATGTTTACTTACATAATCATAATACCATTATCACAGCTAAGAAAATTAATTCAG
3 ' ACTTGTCACACATATAAAGAAATCTTATTCCTACAAATGAATGTATTAGTATTATGGTAATAGTGTCGATTCTTTTAATTAAGTC
85
LRRK2
LRRK2-201
MslI
TTGATTTTTTCCACATATTTGATAACTTTCTGTCTATCCACGATTATGTCTTACATATTCTTTTAATTTATGTCATAGCATATCA
AACTAAAAAAGGTGTATAAACTATTGAAAGACAGATAGGTGCTAATACAGAATGTATAAGAAAATTAAATACAGTATCGTATAGT
170
LRRK2
LRRK2-201
AccI
BstZ17I
TCTTAGAAAGTGATCCCTAAGTTACTGCATGGTATACATTGTTTAACCATTTCCCTTTGTGATTGGATGTCTTTAGGTTGATTAT
AGAATCTTTCACTAGGGATTCAATGACGTACCATATGTAACAAATTGGTAAAGGGAAACACTAACCTACAGAAATCCAACTAATA
255
LRRK2
LRRK2-201
ATTTTTATTATTATCACAAATGTTGAAATCACTCTTTTTTTCTGAAGAATTTAAAAGTAATTTATCTGTCTTATGGAATAAAATA
TAAAAATAATAATAGTGTTTACAACTTTAGTGAGAAAAAAAGACTTCTTAAATTTTCATTAAATAGACAGAATACCTTATTTTAT
340
LRRK2
LRRK2-201
TTTATTTCCCCTTAAAAGAATTTCAGGCATGAACCCAAGAGAGAAGGCTTTTTTTTTTGTTTTAGTTGTTGTTTTTATTTTTATT
AAATAAAGGGGAATTTTCTTAAAGTCCGTACTTGGGTTCTCTCTTCCGAAAAAAAAAACAAAATCAACAACAAAAATAAAAATAA
425
LRRK2
LRRK2-201
MmeI
PCR Forward
CAGGTTAAAGTGAGTTG
TTTTATTTTTTGGGTAGAAGGAGCAGAGAGACAAGTTCAGGAAATAATGAGAGTGTTAGAATTTTGTTCAGGTTAAAGTGAGTTG
AAAATAAAAAACCCATCTTCCTCGTCTCTCTGTTCAAGTCCTTTATTACTCTCACAATCTTAAAACAAGTCCAATTTCACTCAAC
510
LRRK2
LRRK2-201
PCR Forward
GAGTGAAG
GAGTGAAGTTTAGAAATCTCCTTTCTACTCATCTCTCCTGTTTTTAAAACACTGTCCTGGAAATAGTTAATATTAGGAACGAGAA
CTCACTTCAAATCTTTAGAGGAAAGATGAGTAGAGAGGACAAAAATTTTGTGACAGGACCTTTATCAATTATAATCCTTGCTCTT
595
LRRK2
LRRK2-201
AAATGGTATAGGTTTTCCTAGTACACTTTATTTCTTAATTATGAAATTCTACTTAATAACTTACCATTGAATGTTTATCCTTATT
TTTACCATATCCAAAAGGATCATGTGAAATAAAGAATTAATACTTTAAGATGAATTATTGAATGGTAACTTACAAATAGGAATAA
680
LRRK2
LRRK2-201


HincII
ATCATTCAAGGTAATTTTATTGAAGATTGAAGATATTTATAATAAAGATTGAAGGATTTTATTGTCCTGTGTGGTCAACCTTGGG
765
TAGTAAGTTCCATTAAAATAACTTCTAACTTCTATAAATATTATTTCTAACTTCCTAAAATAACAGGACACACCAGTTGGAACCC
LRRK2
LRRK2-201
BanI
Acc65I
KpnI
GGGTGAGATGTTATGAGACAGGACAATTAATTGACTTGATCAAGGTACCTTGTTATAAAAATAACACAGCCTGGTTTAGAACATC
850
CCCACTCTACAATACTCTGTCCTGTTAATTAACTGAACTAGTTCCATGGAACAATATTTTTATTGTGTCGGACCAAATCTTGTAG
LRRK2
LRRK2-201
EarI
Sanger Sequencing Primer
BanII
TCTTCCTGACTCTCTTATTTGGCATATAGCCTAAGTGTATGCCTCCTTGGATGTATGAGCCCTGATGTTGGTCATATTTATTATT
GCCTCCTTGGATGTATGAGC
935
AGAAGGACTGAGAGAATAAACCGTATATCGGATTCACATACGGAGGAACCTACATACTCGGGACTACAACCAGTATAAATAATAA
LRRK2
LRRK2-201
BaeGI
BsiHKAI
Bme1580I
ApaLI
TTATCTGCTTACTTTCAGGGTTTCGTGCACCTGAAGTTGCCAGAGGAAATGTCATTTATAACCAACAGGCTGATGTTTATTCATT
1020
AATAGACGAATGAAAGTCCCAAAGCACGTGGACTTCAACGGTCTCCTTTACAGTAAATATTGGTTGTCCGACTACAAATAAGTAA
LRRK2
LRRK2-201
2040
2045
2050
2055
F R A P E V A R G N V I Y N Q Q A D V Y S F
ENSE00003468457
LRRK2-201
BpmI
Donor Template SNV -> REV
GTTTACTACTCTATGACATTTTGACAACTGGAGGTAGAATAGTAGAGGGTTTGAAGTTTCCAAATGAGTTTGATGAATTAGAA
TGGTTTACTACTCTATGACATTTTGACAACTGGAGGTAGAATAGTAGAGGGTTTGAAGTTTCCAAATGAGTTTGATGAATTAGAA
1105
ACCAAATGATGAGATACTGTAAAACTGTTGACCTCCATCTTATCATCTCCCAAACTTCAAAGGTTTACTCAAACTACTTAATCTT
LRRK2
LRRK2-201
2060
2065
2070
2075
2080
2085
G L L L Y D I L T T G G R I V E G L K F P N E F D E L E
ENSE00003468457
LRRK2-201
Donor Sequence SNV -> REV
gRNA Protospacer Sequence
PAM
SNV
C TACTCAAACTACTTAATCT
gRNA Protospacer


CsiI
SexAI*
Donor Template SNV -> REV
ATACAAGGAAAATTACC
ATACAAGGAAAATTACCTGGTAAGTTCTGTTTTCTCTACAATGAAGATTTTTTTTCTTAATATCAGCAGCTTCATTTTTATTTAA
TATGTTCCTTTTAATGGACCATTCAAGACAAAAGAGATGTTACTTCTAAAAAAAAGAATTATAGTCGTCGAAGTAAAAATAAATT
1190
LRRK2
LRRK2-201
2090
I Q G K L P G K F C F L Y N E D F F S *
ENSE00003468457
LRRK2-201
(in frame with LRRK2-201)
Donor Sequence SNV -> REV
TTGTAGTTGTATGCTTAATTCCTTAAACAGATGATCATTTTTTTTGTTTAGTGCATAAATATTCTTAAATCTTGTGATATATTAA
AACATCAACATACGAATTAAGGAATTTGTCTACTAGTAAAAAAAACAAATCACGTATTTATAAGAATTTAGAACACTATATAATT
1275
LRRK2
LRRK2-201
EcoNI
TAAAAATCACCTGAAAAAGGTAGCAGTTTTAGGCTTTTTAAAAAATCCGCAATTAATATTGGTGTAGTTAATATTATATTTAGAA
ATTTTTAGTGGACTTTTTCCATCGTCAAAATCCGAAAAATTTTTTAGGCGTTAATTATAACCACATCAATTATAATATAAATCTT
1360
LRRK2
LRRK2-201
ACATAGAGAAGGAAATTGCTGTTAGAACTCCACATTTGGTGATTTTTAATTTTCATAAAGAATTACTGTGTACTCATTATCCTGG
TGTATCTCTTCCTTTAACGACAATCTTGAGGTGTAAACCACTAAAAATTAAAAGTATTTCTTAATGACACATGAGTAATAGGACC
1445
LRRK2
LRRK2-201
CCTTTAACGACAATCTTGAGGTGTA
PCR Reverse
NspI
BbsI
AATGTTTTCGTTTTCTTGGAGTGAAATAATTTACATGCAGGAATGGAAGACTGAATGATCTATAATAATAATTTTTCATAAGAAT
TTACAAAAGCAAAAGAACCTCACTTTATTAAATGTACGTCCTTACCTTCTGACTTACTAGATATTATTATTAAAAAGTATTCTTA
1530
LRRK2
LRRK2-201
CGGTAAATGTGTATTTAATGTTATCAAAGCTCATTTGGAATGGTTGTCTCATGCTTTCAAGAAATTAGAGGACTTTGTAATTCAT
GCCATTTACACATAAATTACAATAGTTTCGAGTAAACCTTACCAACAGAGTACGAAAGTTCTTTAATCTCCTGAAACATTAAGTA
1615
LRRK2
LRRK2-201
TCCTTAACCATTACTTTAGTTCTCACCACAAAATAACATTTTAAGTTTATTTAGCTCTTTCTCATATTTTCTGCTTTCCCTTTCA
AGGAATTGGTAATGAAATCAAGAGTGGTGTTTTATTGTAAAATTCAAATAAATCGAGAAAGAGTATAAAAGACGAAAGGGAAAGT
1700
LRRK2
LRRK2-201
DraIII
TTTAAAAAATACTTTTGAGTGTACACAATGTGCCATGTACAGGAAATAGAGCTTTATCTTTTTTGGGTATAACTTCAAGATCATG
AAATTTTTTATGAAAACTCACATGTGTTACACGGTACATGTCCTTTATCTCGAAATAGAAAAAACCCATATTGAAGTTCTAGTAC
1785
LRRK2
LRRK2-201


SpeI
GCAAAAGAAAACTTATTATTAATTGGATAAACCTTAGATATAATCTAGGTTATTTCCCTTATTTTACTAGTTTTCTAGTGAAAAT
1870
CGTTTTCTTTTGAATAATAATTAACCTATTTGGAATCTATATTAGATCCAATAAAGGGAATAAAATGATCAAAAGATCACTTTTA
LRRK2
LRRK2-201

BsaI
BstXI
SmlI
End (1955)
ATTCAGGTCTCTGCTGGGTACAGTGGCTTACGCCTGTAATCCCAGCACTTTGGGAGGCCCAGGCAGGCAGATCACTTGAGGCCAG 3'
1955
TAAGTCCAGAGACGACCCATGTCACCGAATGCGGACATTAGGGTCGTGAAACCCTCCGGGTCCGTCCGTCTAGTGAACTCCGGTC 5'
LRRK2
LRRK2-201

	Feature			Location		Size		⇄	Type
	LINC02471			1 .. 1955		1955 bp		→	gene
	/note	=	gene ENSG00000223914 lncRNA						
✓	**LRRK2**			1 .. 1955		1955 bp		→	gene
	/note	=	gene ENSG00000188906 Protein coding						
	LINC02471-202			1 .. 1955		1955 bp		→	prim_transcript
	/note	=	primary transcript ENST00000641941 lncRNA						
✓	**LRRK2-201**			1 .. 1955		1955 bp		→	prim_transcript
	/note	=	primary transcript ENST00000298910						
	LRRK2-204			1 .. 1955		1955 bp		→	prim_transcript
	/note	=	primary transcript ENST00000430804 Nonsense mediated decay						
	LRRK2-206			1 .. 1955		1955 bp		→	prim_transcript
	/note	=	primary transcript ENST00000479187 Retained intron						
	LRRK2-210			1 .. 1955		1955 bp		→	prim_transcript
	/note	=	primary transcript ENST00000679360 Nonsense mediated decay						
	LRRK2-211			1 .. 1955		1955 bp		→	prim_transcript
	/note	=	primary transcript ENST00000679532 Nonsense mediated decay						
	LRRK2-213			1 .. 1955		1955 bp		→	prim_transcript
	/note	=	primary transcript ENST00000680018 Nonsense mediated decay						
	LRRK2-215			1 .. 1955		1955 bp		→	prim_transcript
	/note	=	primary transcript ENST00000680422 Nonsense mediated decay						
	LRRK2-216			1 .. 1955		1955 bp		→	prim_transcript
	/note	=	primary transcript ENST00000680425 Nonsense mediated decay						
	LRRK2-217			1 .. 1955		1955 bp		→	prim_transcript
	/note	=	primary transcript ENST00000680453 Nonsense mediated decay						
	LRRK2-218			1 .. 1955		1955 bp		→	prim_transcript
	/note	=	primary transcript ENST00000680790						
	LRRK2-219			1 .. 1955		1955 bp		→	prim_transcript
	/note	=	primary transcript ENST00000681136 protein_coding_CDS_not_defined						
	LRRK2-220			1 .. 1955		1955 bp		→	prim_transcript
	/note	=	primary transcript ENST00000681696						
	LRRK2-212			954 .. 1955		1002 bp		→	prim_transcript
	/note	=	primary transcript ENST00000679683 Nonsense mediated decay						
✓	**LRRK2-201**			954 .. 1124		171 bp		→	CDS
	/codon_start	=	1						
	/note	=	coding sequence ENSP00000298910						
	/translation	=	FRAPEVARGNVIYNQQADVYSFGLLLYDILTTGGRIVEGLKFPNEFDELEIQGKLP 57 amino acids = 6.3 kDa						
	LRRK2-218			954 .. 1124		171 bp		→	CDS
	/codon_start	=	1						
	/note	=	coding sequence ENSP00000505335						
	/translation	=	FRAPEVARGNVIYNQQADVYSFGLLLYDILTTGGRIVEGLKFPNEFDELEIQGKLP 57 amino acids = 6.3 kDa						

	Feature			Location			Size			Type
	LRRK2-220			954	..	1124	171 bp			CDS
	/codon_start	=	1							
	/note	=	coding sequence ENSP00000505871							
	/translation	=	FRAPEVARGNVIYNQQADVYSFGLLLYDILTTGGRIVEGLKFPNEFDELEIQGKLP 57 amino acids = 6.3 kDa							
✓	**Donor Sequence SNV -> REV**			1023	..	1122	100 bp			misc_feature
	LRRK2-208			1048	..	1955	908 bp			prim_transcript
	/note	=	primary transcript ENST00000636518							
	LRRK2-208			1048	..	1124	77 bp			CDS
	/note	=	coding sequence ENSP00000490200							
	/translation	=	NWR*NSRGFEVSK*V**IRNTRKIT 25 codons (4 internal stop codons)							
✓	**PAM**			1082	..	1084	3 bp			misc_feature
✓	**gRNA Protospacer Sequence**			1085	..	1104	20 bp			misc_feature
✓	**SNV**			1085	..	1085	1 bp			misc_feature
	/note	=	SNV = G REV = A							

	Primer	Length	Binding Sites		Tm	Date Added
✓	**PCR Forward**	25-mer	494 .. 518	→	59°C	Aug 18, 2023
	/sequence = CAGGTTAAAGTGAGTTGGAGTGAAG 44% GC / 7850.2 Da					
✓	**Sanger Sequencing Primer**	20-mer	891 .. 910	→	58°C	Aug 18, 2023
	/sequence = GCCTCCTTGGATGTATGAGC 55% GC / 6124.0 Da					
✓	**Donor Template SNV -> REV**	100-mer	1023 .. 1122	→	69°C	Aug 18, 2023
	/sequence = GTTTACTACTCTATGACATTTTGACAACTGGAGGTAGAATAGTAGAGGGTTTGAAGTTTCCAAATGAGTTTGATGAATTAGAAATACAAGGAAAATTACC 33% GC / 31,067.4 Da					
✓	**gRNA Protospacer**	20-mer	1086 .. 1104	←	46°C	Aug 18, 2023
	/sequence = TCTAATTCATCAAACTCATC 30% GC / 5995.0 Da					
✓	**PCR Reverse**	25-mer	1371 .. 1395	←	58°C	Aug 18, 2023
	/sequence = ATGTGGAGTTCTAACAGCAATTTCC 40% GC / 7656.1 Da					