

ENSE00...

LRRK2-201

Donor Sequence WT -> SNV

gRNA Protospacer Sequence

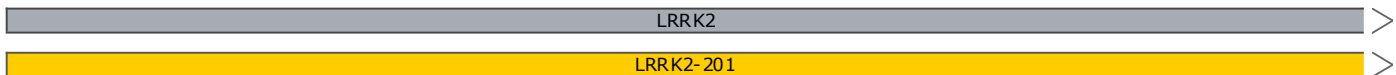
SNV

**ASK2J00180\_LRRK2\_N2081D\_E12\_BB**  
1940 bp

5'  
3'

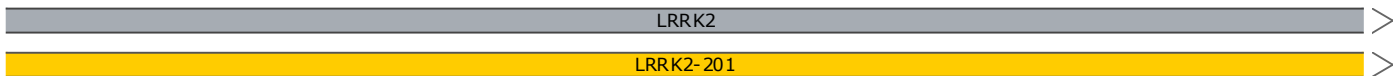
TCATTGATTATTTACTTTTTGCCCTACTTGTCTTTCTTGTCTCTTTTGCATGAATGAATCATTGAAATTAAGTTGTAGACAT  
AGTAACTAATAAATGAAAAACGGGATGAACAAGAAAGAACGAGAGAAACGCATACTTACTTAGTAACTTTAATTC AACATCTGTA

85



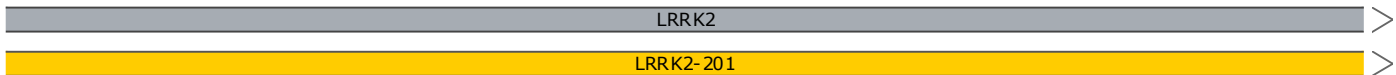
CATGCCATATCACCTCTGAACAGTGTGTATATTTCTTTAGAATAAGGATGTTTACTTACATAATCATAATACCATTATCACAGCT  
GTACGGTATAGTGGAGACTTGTCCACACATATAAAGAAATCTTATTCCTACAAATGAATGTATTAGTATTATGGTAATAGTGTCTGA

170



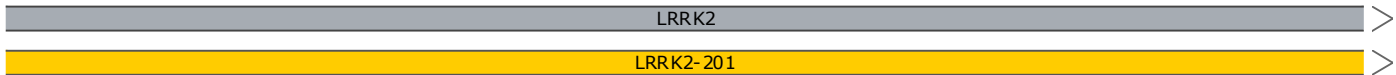
AAGAAAATTAATTCAGTTGATTTTTCCACATATTTGATAACTTTCTGTCTATCCACGATTATGTCTTACATATTCTTTAATTT  
TTCTTTTAATTAAGTCAACTAAAAAAGGTGTATAAACTATTGAAAGACAGATAGGTGCTAATACAGAATGTATAAGAAAATTA

255



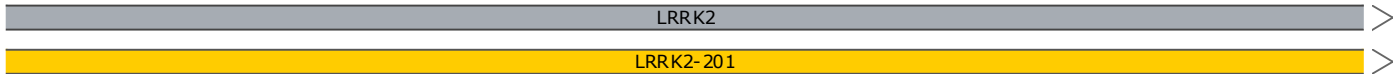
ATGTCATAGCATATCATCTTAGAAAGTGATCCCTAAGTTACTGCATGGTATACATTGTTTAACCATTTCCCTTTGTGATTGGATG  
TACAGTATCGTATAGTAGAATCTTTCACTAGGGATTCAATGACGTACCATATGTAACAAATTGGTAAAGGGAAACACTAACCTAC

340



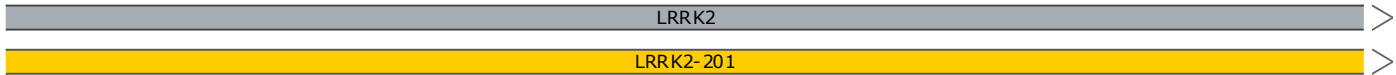
TCTTTAGGTTGATTATATTTTTATTATTATCACAAATGTTGAAATCACTCTTTTTTTCTGAAGAATTTAAAAGTAATTTATCTGT  
AGAAATCCAACATAATAAAAAATAAATAGTGTTTACAACCTTAGTGAGAAAAAAGACTTCTTAAATTTTCATTAATAGACA

425



CTTATGGAATAAAATATTTATTTCCCTTAAAAGAATTTCAAGGCATGAACCCAAGAGAGAAGGCTTTTTTTTTGTTTTAGTTGT  
GAATACCTTATTTTATAAATAAAGGGGAATTTCTTAAAGTCCGTACTTGGGTTCTCTCTTCCGAAAAAAAAAACAAAATCAACA

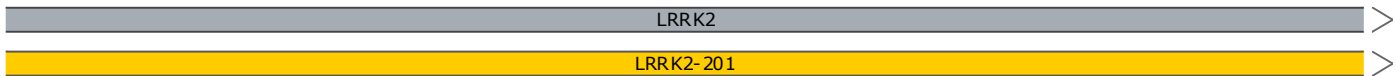
510



PCR Forward  
C

TGTTTTATTTTTATTTTTATTTTTGGGTAGAAGGAGCAGAGAGACAAGTTCAGGAAATAATGAGAGTGTAGAAATTTGTTT  
ACAAAAATAAAAAATAAAAAATAAAAAACCCATCTTCTCGTCTCTCTGTTCAAGTCTTTATTACTCTCACAATCTTAAAAACAAG

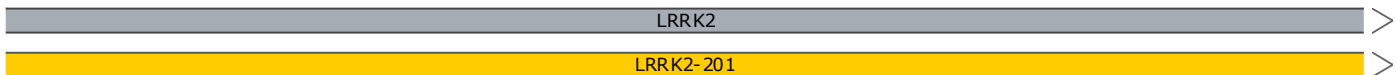
595



PCR Forward  
AGGTTAAAGTGAGTTGGAGTGAAG

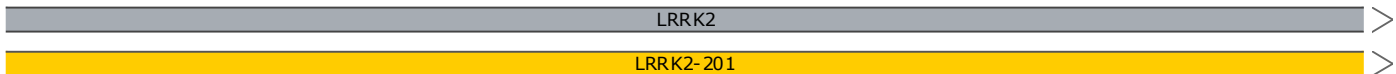
AGGTTAAAGTGAGTTGGAGTGAAGTTTAGAAATCTCTTTCTACTCATCTCTCTGTTTTTAAAACACTGTCCTGGAAATAGTTA  
TCCAATTTCACTCAACCTCACTTCAAATCTTTAGAGGAAAGATGAGTAGAGAGGACAAAAATTTGTGACAGGACCTTTATCAAT

680



ATATTAGGAACGAGAAAAATGGTATAGGTTTTCTAGTACACTTTATTTCTTAATTATGAAATTTCTACTTAATAACTTACCATTG  
TATAATCCTTGCTCTTTTTACCATATCCAAAAGGATCATGTGAAATAAAGAATTAATACTTTAAGATGAATTATTGAATGGTAAC

765



AATGTTTATCCTTATTATCATTCAAGGTAATTTTATTGAAGATTGAAGATATTTATAATAAAGATTGAAGGATTTTATTGTCCTG  
TTACAAATAGGAATAATAGTAAGTTCATTAAAATAACTTCTAACTTCTATAAATATTATTTCTAACTTCTAAAATAACAGGAC

850

LRRK2

LRRK2-201

TGTGGTCAACCTTGGGGGGTGAGATGTTATGAGACAGGACAATTAATTGACTTGATCAAGGTACCTTGTATAAAAATAACACAG  
ACACCAGTTGGAACCCCCCACTCTACAATACTCTGTCCTGTTAATTAACTGAACTAGTTCATGGAACAATATTTTTATTGTGTC

935

LRRK2

LRRK2-201

Sanger Sequencing Primer

GCCTCCTTGGATGTATGAGC

CCTGGTTTGAACATCTCTTCTGACTCTCTTATTTGGCATATAGCCTAAGTGTATGCCTCCTTGGATGTATGAGCCCTGATGT  
GGACCAAATCTTGTAGAGAAGGACTGAGAGAATAAACCGTATATCGGATTCACATACGGAGGAACCTACATACTCGGGACTACAA

1020

LRRK2

LRRK2-201

GGTCATATTTATTATTTTATCTGCTTACTTTTCAGGGTTTCGTGCACCTGAAGTTGCCAGAGGAAATGTCATTTATAACCAACAGG  
CCAGTATAAATAAATAAATAGACGAATGAAAGTCCCAAAGCACGTGGACTTCAACGGTCTCCTTTACAGTAAATATTGGTTGTCC

1105

LRRK2

LRRK2-201

F R A P E V A R G N V I Y N Q Q

ENS E00003468457

LRRK2-201

Donor Template WT -> SNV

GTTTACTACTCTATGACATTTTGCACACTGGAGGTAGAATAGTAGAGGGTTTGAAGTTTCCAATGA

CTGATGTTTATTCATTTGGTTTACTACTCTATGACATTTTGCACACTGGAGGTAGAATAGTAGAGGGTTTGAAGTTTCCAATGA  
GACTACAAATAAGTAAACCAAATGATGAGATACTGTAAACTGTTGACCTCCATCTTATCATCTCCCAAACCTCAAAGGTTACT

1190

LRRK2

LRRK2-201

A D V Y S F G L L L Y D I L T T G G R I V E G L K F P N E

ENS E00003468457

LRRK2-201

Donor Sequence WT -> SNV

PAM gRN...

SNV

TTACT

gRNA Protospacer

Donor Template WT -> SNV

GTTTGATGAATTAGAAATACAAGGAAAATTACC

GTTTGATGAATTAGAAATACAAGGAAAATTACCTGGTAAGTTCTGTTTTCTCTACAATGAAGATTTTTTTTCTTAATATCAGCAG  
CAAACACTTAACTTTATGTTCCTTTTAATGGACCATTCAAGACAAAAGAGATGTTACTTCTAAAAAAAAGAATTATAGTCGTC

1275

LRRK2

LRRK2-201

2085 2090

F D E L E I Q G K L P G K F C F L Y N E D F F S \*

ENS E0003468457

(in frame with LRRK2-201)

LRRK2-201

Donor Sequence WT -> SNV

gRNA Protospacer Sequence

CAAACACTTAACTCT

gRNA Protospacer

CTTCATTTTTATTTAATTGTAGTTGTATGCTTAATTCCTTAAACAGATGATCATTTTTTTTGTTTAGTGCATAAATATTCTTAA  
GAAGTAAAAATAAATTAACATCAACATACGAATTAAGGAATTTGCTACTAGTAAAAAAAACAAATCACGTATTTATAAGAATTT

1360

LRRK2

LRRK2-201

TCTTGTGATATATTAATAAAAAATCACCTGAAAAAGGTAGCAGTTTTAGGCTTTTTAAAAAATCCGCAATTAATATTGGTGTAGTT  
AGAACACTATATAATTATTTTTAGTGGACTTTTTCCATCGTCAAATCCGAAAAATTTTTAGGCGTTAATTATAACCATCA

1445

LRRK2

LRRK2-201

AATATTATATTTAGAAACATAGAGAAGGAAATTGCTGTTAGAACTCCACATTTGGTGATTTTTAATTTTCATAAAGAATTACTGT  
TTATAATATAAATCTTTGTATCTCTTCTTTAACGACAATCTTGAGGTGTAACCCTAAAAATTTAAAAGTATTTCTTAATGACA

1530

LRRK2

LRRK2-201

CCTTTAACGACAATCTTGAGGTGTA

PCR Reverse

GTACTCATTATCCTGGAATGTTTTCGTTTTCTTGGAGTGAATAATTTACATGCAGGAATGGAAGACTGAATGATCTATAATAAT  
CATGAGTAATAGGACCTTACAAAAGCAAAGAACCCTCACTTTATTAATGTACGTCCTTACCTTCTGACTTACTAGATATTATTA

1615

LRRK2

LRRK2-201

AATTTTTATAAAGAATCGGTAATGTGTATTTAATGTTATCAAAGCTCATTGGAATGGTTGTCTCATGCTTTCAAGAAATTAGA  
TTAAAAGTATTTCTTAGCCATTTACACATAAATTACAATAGTTTTGAGTAAACCTTACCAACAGAGTACGAAAGTTCTTTAATCT

1700

LRRK2

LRRK2-201

GGACTTTGTAATTCATTCTTAACCATTACTTTAGTTCTCACCACAAAATAACATTTTAAGTTTTATTTAGCTCTTTCTCATATTT  
CCTGAAACATTAAGTAAGGAATTGGTAATGAAATCAAGAGTGGTGTTTTATTGTAATAATCAAATAAATCGAGAAAGAGTATAAA

1785

LRRK2

LRRK2-201

TCTGCTTTCCCTTTCAATTTAAAAAATACTTTTGGAGTGTACACAATGTGCCATGTACAGGAAATAGAGCTTTATCTTTTTGGGTA  
AGACGAAAGGGAAAGTAAATTTTTTATGAAAACCTCACATGTGTTACACGGTACATGTCCTTTATCTCGAAATAGAAAAACCCAT

1870

LRRK2

LRRK2-201

TAACTTCAAGATCATGGCAAAAGAAAACCTTATTATTAATTGGATAAACCTTAGATATAATCTAGGTTATT  
ATTGAAGTTCTAGTACCGTTTTCTTTTGAATAATAATTAACCTATTTGGAATCTATATTAGATCCAATAA

3 '  
1940  
5 '



Feature	Location	Size	Start	End	Type
<b>LINC02471</b>	1 .. 1940	1940 bp	■	➔	gene
/note = gene <a href="#">ENSG00000223914</a> lncRNA					
✓ <b>LRRK2</b>	1 .. 1940	1940 bp	■	➔	gene
/note = gene <a href="#">ENSG00000188906</a> Protein coding					
<b>LINC02471-202</b>	1 .. 1940	1940 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000641941</a> lncRNA					
✓ <b>LRRK2-201</b>	1 .. 1940	1940 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000298910</a>					
<b>LRRK2-204</b>	1 .. 1940	1940 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000430804</a> Nonsense mediated decay					
<b>LRRK2-206</b>	1 .. 1940	1940 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000479187</a> Retained intron					
<b>LRRK2-210</b>	1 .. 1940	1940 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000679360</a> Nonsense mediated decay					
<b>LRRK2-211</b>	1 .. 1940	1940 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000679532</a> Nonsense mediated decay					
<b>LRRK2-213</b>	1 .. 1940	1940 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000680018</a> Nonsense mediated decay					
<b>LRRK2-215</b>	1 .. 1940	1940 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000680422</a> Nonsense mediated decay					
<b>LRRK2-216</b>	1 .. 1940	1940 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000680425</a> Nonsense mediated decay					
<b>LRRK2-217</b>	1 .. 1940	1940 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000680453</a> Nonsense mediated decay					
<b>LRRK2-218</b>	1 .. 1940	1940 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000680790</a>					
<b>LRRK2-219</b>	1 .. 1940	1940 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000681136</a> protein_coding_CDS_not_defined					
<b>LRRK2-220</b>	1 .. 1940	1940 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000681696</a>					
<b>LRRK2-212</b>	1055 .. 1940	886 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000679683</a> Nonsense mediated decay					
✓ <b>LRRK2-201</b>	1055 .. 1225	171 bp	■	➔	CDS
/codon_start = 1					
/note = coding sequence <a href="#">ENSP00000298910</a>					
/translation = FRAPEVARGNVIYNQQADVYSFGLLLYDILTTGGRIVEGLKFPNEFDELEIQGKLP 57 amino acids = 6.3 kDa					
<b>LRRK2-218</b>	1055 .. 1225	171 bp	■	➔	CDS
/codon_start = 1					
/note = coding sequence <a href="#">ENSP00000505335</a>					
/translation = FRAPEVARGNVIYNQQADVYSFGLLLYDILTTGGRIVEGLKFPNEFDELEIQGKLP 57 amino acids = 6.3 kDa					

Feature	Location	Size	Start	End	Type
<b>LRRK2-220</b>	1055 .. 1225	171 bp	■	→	CDS
/codon_start = 1					
/note = coding sequence <a href="#">ENSP00000505871</a>					
/translation = FRAPEVARGNVIYNQQADVYSFGLLLYDILTTGGRIVEGLKFPNEFDELEIQGKLP 57 amino acids = 6.3 kDa					
✓ <b>Donor Sequence WT -&gt; SNV</b>	1124 .. 1223	100 bp	■		misc_feature
<b>LRRK2-208</b>	1149 .. 1940	792 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000636518</a>					
<b>LRRK2-208</b>	1149 .. 1225	77 bp	■	→	CDS
/note = coding sequence <a href="#">ENSP00000490200</a>					
/translation = NWR*NSRGFEVSK*V**IRNTRKIT 25 codons (4 internal stop codons)					
✓ <b>PAM</b>	1183 .. 1185	3 bp	■		misc_feature
✓ <b>gRNA Protospacer Sequence</b>	1186 .. 1205	20 bp	■		misc_feature
✓ <b>SNV</b>	1186 .. 1186	1 bp	■		misc_feature
/note = WT = A SNV = G					

Primer	Length	Binding Sites	Tm	Date Added
✓ <b>PCR Forward</b> /sequence = CAGGTTAAAGTGAGTTGGAGTGAAG 44% GC / 7850.2 Da	25-mer	595 .. 619 →	59°C	Aug 18, 2023
✓ <b>Sanger Sequencing Primer</b> /sequence = GCCTCCTTGGATGTATGAGC 55% GC / 6124.0 Da	20-mer	992 .. 1011 →	58°C	Aug 18, 2023
✓ <b>Donor Template WT -&gt; SNV</b> /sequence = GTTTACTACTCTATGACATTTTGACAACCTGGAGGTAGAATAGTAGAGGGTTTGAAGTTTCCAGATGAGTTTGATGAATTAGAAATACAAGGAAAATTACC 34% GC / 31,083.4 Da	100-mer	1124 .. 1223 →	68°C	Aug 18, 2023
✓ <b>gRNA Protospacer</b> /sequence = TCTAATTCATCAAACCTCATT 25% GC / 6010.0 Da	20-mer	1186 .. 1205 ←	47°C	Aug 18, 2023
✓ <b>PCR Reverse</b> /sequence = ATGTGGAGTTCTAACAGCAATTTCC 40% GC / 7656.1 Da	25-mer	1472 .. 1496 ←	58°C	Aug 18, 2023