

LRRK2-201



Donor Sequence WT -> SNV

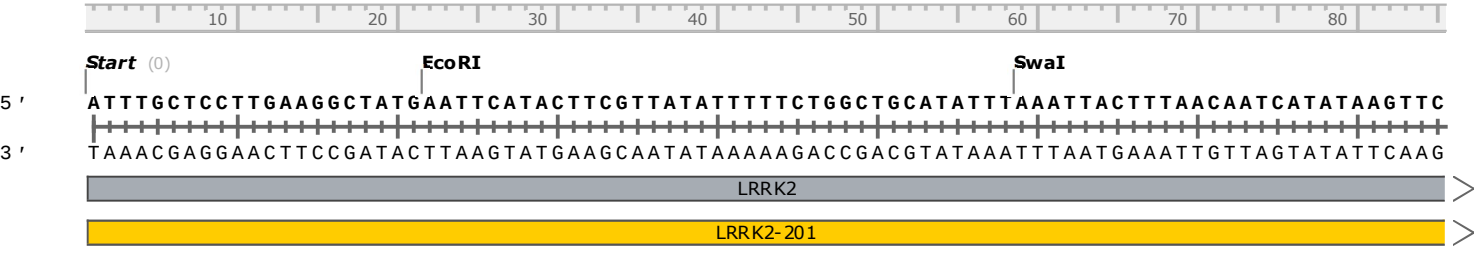


gRNA Protospacer Sequence

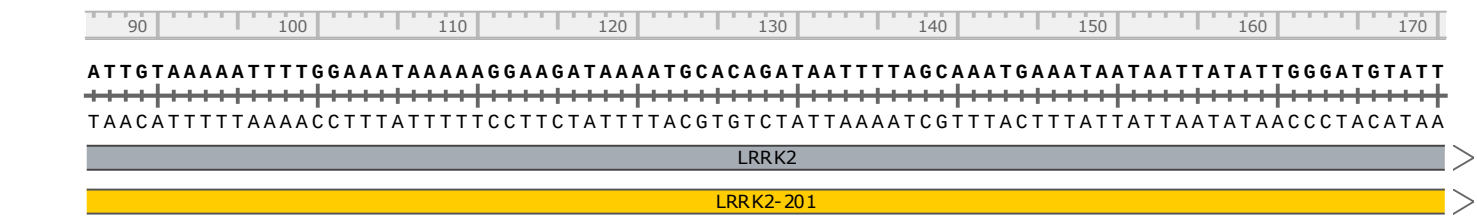


SNV

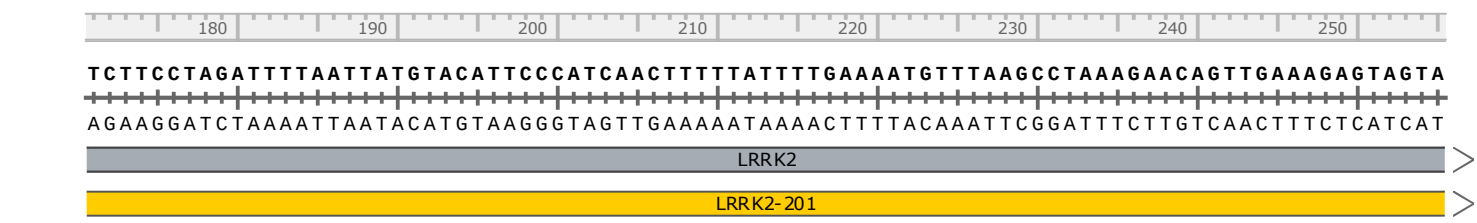
**ASK2J00180\_LRRK2\_N2081D\_E03\_AB**  
3102 bp



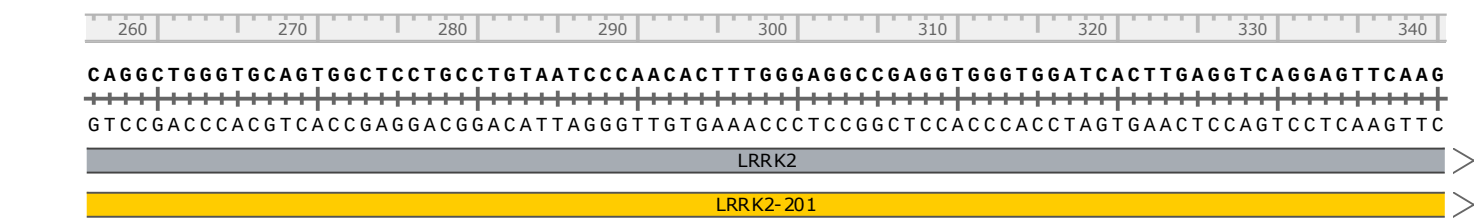
ATTTGCTCCTTGAAGGCTATGAATTCATACTTCGTTATATTTTTCTGGCTGCATATTTAAATTACTTTAACAATCATATAAGTTC



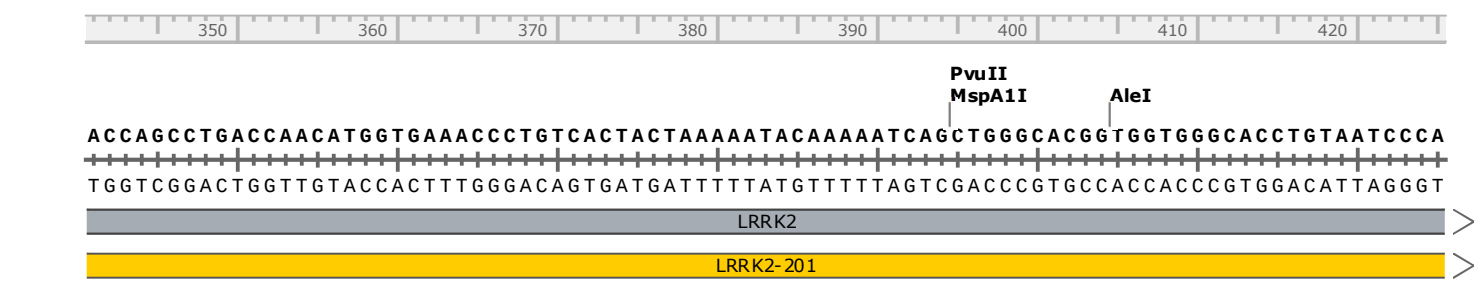
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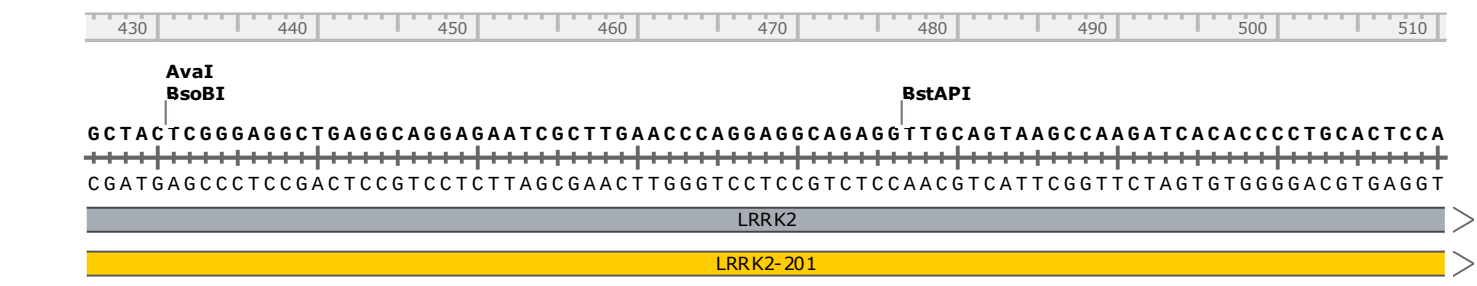
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CAGGCTGGGTGCAGTGGCTCCTGCCTGTAATCCCAACACTTTGGGAGGCCGAGGTGGGTGGATCACTTGAGGTCAGGAGTTCAAG



ACCAGCCTGACCAACATGGTGA AACCTGTCACTACTAAAAATACAAAAATCAGCTGGGCACGGTGGTGGGCACCTGTAATCCCA



GCTACTCGGGAGGCTGAGGCAGGAGAATCGCTTGAACCCAGGAGGCAGAGGTTGCAGTAAGCCAAGATCACACCCCTGCACTCCA

520 530 540 550 560 570 580 590  
GCCTGGGCAAGAGAGTGAGACTCCATCTCAAAAAAAAAAAAAAAAAAAAAAAAAAGAAAGAAAGAGTAGTACAATATACATTCACT  
CGGACCCGTTCTCTCACTCTGAGGTAGAGTTTTTTTTTTTTTTTTTTTTTTTTTTCTTTCTTCTCATCATGTTATATGTAAGTATGA  
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GCCTGGGCAAGAGAGTGAGACTCCATCTCAAAAAAAAAAAAAAAAAAAAAAAAAAGAAAGAAAGAGTAGTACAATATACATTCACT  
600 610 620 630 640 650 660 670 680  
CATATACCCGACGCATAAATTCATTGATTATTTACTTTTTGCCCTACTTGTCTTTCTTGTCTCTTTGCGTATGAATGAATCAT  
GTATATGGGCTGCGTATTTAAGTAACTAATAAATGAAAAACGGGATGAACAAGAAAGAACGAGAGAAACGCATACTTACTTAGTA  
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CATATACCCGACGCATAAATTCATTGATTATTTACTTTTTGCCCTACTTGTCTTTCTTGTCTCTTTGCGTATGAATGAATCAT  
690 700 710 720 730 740 750 760  
TGAATTAAGTTGTAGACATCATGCCATATCACCTCTGAACAGTGTGTATATTTCTTTAGAATAAGGATGTTTACTTACATAATC  
ACTTTAATTCACATCTGTAGTACGGTATAGTGGAGACTTGTACACATATAAAGAAATCTTATTCCTACAAATGAATGTATTAG  
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TGAATTAAGTTGTAGACATCATGCCATATCACCTCTGAACAGTGTGTATATTTCTTTAGAATAAGGATGTTTACTTACATAATC  
770 780 790 800 810 820 830 840 850  
ATAATACCATTATCACAGCTAAGAAAATTAATTCAGTTGATTTTTCCACATATTTGATAACTTTCTGTCTATCCACGATTATGT  
TATTATGGTAATAGTGTGATTCTTTAATTAAGTCAACTAAAAAGGTGTATAAATCTTGAAGACAGATAGGTGCTAATACA  
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ATAATACCATTATCACAGCTAAGAAAATTAATTCAGTTGATTTTTCCACATATTTGATAACTTTCTGTCTATCCACGATTATGT  
860 870 880 890 900 910 920 930  
CTTACATATTTCTTTAATTTATGTCATAGCATATCATCTTAGAAAGTGATCCCTAAGTTACTGCATGGTATACATTGTTTAACCA  
GAATGTATAAGAAAATTAATACAGTATCGTATAGTAGAATCTTTCACTAGGGATTCAATGACGTACCATATGTAACAAATTGGT  
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CTTACATATTTCTTTAATTTATGTCATAGCATATCATCTTAGAAAGTGATCCCTAAGTTACTGCATGGTATACATTGTTTAACCA  
940 950 960 970 980 990 1000 1010 1020  
TTTCCCTTTGTGATTGGATGTCTTTAGGTTGATTATATTTTTATTATTATCACAAATGTTGAAATCACTCTTTTTTTCTGAAGAA  
AAAGGGAAACACTAACCTACAGAAATCCAATAATAAAAAATAAATAGTGTTTACAACCTTGTAGGAGAAAAAAGACTTCTT  
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TTTCCCTTTGTGATTGGATGTCTTTAGGTTGATTATATTTTTATTATTATCACAAATGTTGAAATCACTCTTTTTTTCTGAAGAA

1030 1040 1050 1060 1070 1080 1090 1100

TTTAAAAGTAATTTATCTGTCTTATGGAATAAAATATTTATTTCCCTTAAAAGAATTTTCAGGCATGAACCCAAGAGAGAAGGCT  
AAATTTTCATTAATAGACAGAATACCTTATTTTATAAATAAAGGGGAATTTCTTAAAGTCCGTACTIONTGGGTTCTCTCTCCGA

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TTTAAAAGTAATTTATCTGTCTTATGGAATAAAATATTTATTTCCCTTAAAAGAATTTTCAGGCATGAACCCAAGAGAGAAGGCT

1110 1120 1130 1140 1150 1160 1170 1180 1190

TTTTTTTTGTTTTAGTTGTTGTTTTATTTTTATTTTTATTTTTGGGTAGAAGGAGCAGAGAGACAAGTTCAGGAAATAATG  
AAAAAAAAACAAAATCAACAACAAAAATAAAAAATAAAAAACCCATCTTCTCTGTTCAAGTCTTTATTAC

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TTTTTTTTGTTTTAGTTGTTGTTTTATTTTTATTTTTATTTTTGGGTAGAAGGAGCAGAGAGACAAGTTCAGGAAATAATG

1200 1210 1220 1230 1240 1250 1260 1270



AGAGTGTTAGAATTTGTTTCAGGTTAAAGTGAGTTGGAGTGAAGTTTAGAAATCTCCTTTCTACTCATCTCTCTGTTTTTAAAA  
TCTCACAATCTTAAAACAAGTCCAATTTCACTCAACCTCACTTCAAATCTTTAGAGGAAAGATGAGTAGAGAGGACAAAAATTTT

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AGAGTGTTAGAATTTGTTTCAGGTTAAAGTGAGTTGGAGTGAAGTTTAGAAATCTCCTTTCTACTCATCTCTCTGTTTTTAAAA

1280 1290 1300 1310 1320 1330 1340 1350 1360

CACTGTCCTGGAAATAGTTAATATTAGGAACGAGAAAAATGGTATAGGTTTTCTAGTACACTTTATTTCTTAATTATGAAATTC  
GTGACAGGACCTTTATCAATTATAATCCTTGCTCTTTTTACCATATCCAAAAGGATCATGTGAAATAAAGAATTAATACTTTAAG

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CACTGTCCTGGAAATAGTTAATATTAGGAACGAGAAAAATGGTATAGGTTTTCTAGTACACTTTATTTCTTAATTATGAAATTC

1370 1380 1390 1400 1410 1420 1430 1440

TACTTAATAACTTACCATTGAATGTTTATCCTTATTATCATTCAAGGTAATTTTATTGAAGATTGAAGATATTTATAATAAAGAT  
ATGAATTATTGAATGGTAACCTTACAAATAGGAATAATAGTAAGTTCATTAAAATAACTTCTAACTTCTATAAATATTATTCTA

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TACTTAATAACTTACCATTGAATGTTTATCCTTATTATCATTCAAGGTAATTTTATTGAAGATTGAAGATATTTATAATAAAGAT

1450 1460 1470 1480 1490 1500 1510 1520 1530

HincII

Acc65I

KpnI

TGAAGGATTTTATTGTCCTGTGTGGTCAACCTTGGGGGGTGAGATGTTATGAGACAGGACAATTAATTGACTTGATCAAGGTACC  
ACTTCTAAAATAACAGGACACACCAGTTGGAACCCCACTCTACAATACTCTGTCCTGTTAATTAAGTGAAGTACTGATTTCCATGG

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TGAAGGATTTTATTGTCCTGTGTGGTCAACCTTGGGGGGTGAGATGTTATGAGACAGGACAATTAATTGACTTGATCAAGGTACC

1540 1550 1560 1570 1580 1590 1600 1610

EarI

Sanger Sequencing Primer

GCCTCCTTG

TTGTTATAAAAATAACACAGCCTGGTTTAGAACATCTCTTCCTGACTCTCTTATTTGGCATATAGCCTAAGTGTATGCCTCCTTG  
AACAATATTTTTATTGTGTCGGACCAAATCTTGTAGAGAAGGACTGAGAGAATAAACCGTATATCGGATTCACATACGGAGGAAC

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TTGTTATAAAAATAACACAGCCTGGTTTAGAACATCTCTTCCTGACTCTCTTATTTGGCATATAGCCTAAGTGTATGCCTCCTTG

1620 1630 1640 1650 1660 1670 1680 1690 1700

Sanger Sequencing Primer

GATGTATGAGC

BanII

GATGTATGAGCCCTGATGTTGGTCATATTTATTATTTTATCTGCTTACTTTTCAGGGTTTCGTGCACCTGAAGTTGCCAGAGGAAA  
CTACATACTCGGGACTACAACCAGTATAAATAATAAAAATAGACGAATGAAAGTCCCAAAGCACGTGGACTTCAACGGTCTCCTTT

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F R A P E V A R G N  
2040 2045  
ENS E00003468457  
LRRK2-201

GATGTATGAGCCCTGATGTTGGTCATATTTATTATTTTATCTGCTTACTTTTCAGGGTTTCGTGCACCTGAAGTTGCCAGAGGAAA

1710 1720 1730 1740 1750 1760 1770 1780

Donor Template WT -> SNV

GTTTACTACTCTATGACATTTTGACAACCTGGAGGTAGAATAGTAGAG

TGTCATTTATAACCAACAGGCTGATGTTTATTCATTTGGTTTACTACTCTATGACATTTTGACAACCTGGAGGTAGAATAGTAGAG  
ACAGTAAATATTGGTTGTCCGACTACAAATAAGTAAACCAAATGATGAGATACTGTAAAACCTGTTGACCTCCATCTTATCATCTC

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V I Y N Q Q A D V Y S F G L L L Y D I L T T G G R I V E  
2050 2055 2060 2065 2070 2075  
ENS E00003468457  
LRRK2-201

Donor Sequence WT -> SNV

TGTCATTTATAACCAACAGGCTGATGTTTATTCATTTGGTTTACTACTCTATGACATTTTGACAACCTGGAGGTAGAATAGTAGAG

1790 1800 1810 1820 1830 1840 1850 1860 1870

**CsiI**  
**SexAI\***

Donor Template WT -> SNV

GGTTTGAAGTTTCCA **G** ATGAGTTTGTATGAATTAGAAATACAAGGAAAATTACC

GGTTTGAAGTTTCCA **A** ATGAGTTTGTATGAATTAGAAATACAAGGAAAATTACCTGGTAAGTTCTGTTTTCTCTACAATGAAGATT

CCAAACCTTCAAAGGT **T** TACTCAAAC TACTTAATCTTTATGTTCTTTTAATGGACCATTCAAGACAAAAGAGATGTTACTTCTAA



2080 2085 2090

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

ENSE00003468457

LRRK2-201

(in frame with LRRK2-201)



TTACTCAAAC TACTTAATCT

gRNA Protospacer

GGTTTGAAGTTTCCAAATGAGTTTGTATGAATTAGAAATACAAGGAAAATTACCTGGTAAGTTCTGTTTTCTCTACAATGAAGATT

1880 1890 1900 1910 1920 1930 1940 1950

TTTTTTCTTAATATCAGCAGCTTCATTTTTATTTAATTGTAGTTGTATGCTTAATTCCTTAAACAGATGATCATTTTTTTTGTTT

AAAAAAGAATTATAGTCGTCGAAGTAAAAATAAATTAACATCAACATACGAATTAAGGAATTTGTCTACTAGTAAAAAAAACAAA



F F S \*

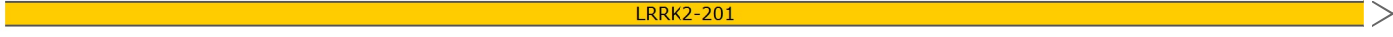
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1960 1970 1980 1990 2000 2010 2020 2030 2040

**EcoNI**

AGTGCATAAATATTCTTAAATCTTGTGATATATTAATAAAAAATCACCTGAAAAAGGTAGCAGTTTTAGGCTTTTTAAAAAATCCG

TCACGTATTTATAAGAATTTAGAACACTATATAATTATTTTTAGTGGACTTTTTCCATCGTCAAATCCGAAAAATTTTTTAGGC

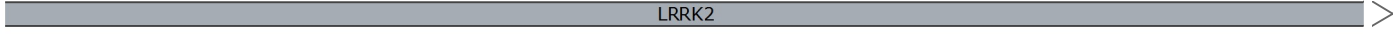


AGTGCATAAATATTCTTAAATCTTGTGATATATTAATAAAAAATCACCTGAAAAAGGTAGCAGTTTTAGGCTTTTTAAAAAATCCG

2050 2060 2070 2080 2090 2100 2110 2120

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GTTAATTATAACCACATCAATTATAATATAAATCTTTGTATCTCTTCTTTAACGACAATCTTGAGGTGTAACCCTAAAAATT



CCTTTAACGACAATCTTGAGGTGTA

PCR Reverse

CAATTAATATTGGTGTAGTTAATATTATATTTAGAAACATAGAGAAGGAAATTGCTGTTAGAACTCCACATTTGGTGATTTTTAA

2130 2140 2150 2160 2170 2180 2190 2200 2210

TTTTATAAAGAATTACTGTGTACTCATTATCCTGGAATGTTTTCGTTTTCTTGGAGTGAATAATTTACATGCAGGAATGGAAG  
AAAAGTATTTCTTAATGACACATGAGTAATAGGACCTTACAAAAGCAAAGAACCTCACTTTATTAATGTACGTCCTTACCTTC

LRRK2

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TTTTATAAAGAATTACTGTGTACTCATTATCCTGGAATGTTTTCGTTTTCTTGGAGTGAATAATTTACATGCAGGAATGGAAG

2220 2230 2240 2250 2260 2270 2280 2290

BbsI

ACTGAATGATCTATAATAATAATTTTTATAAAGAATCGGTAATGTGTATTTAATGTTATCAAAGCTCATTGGAAATGGTTGCT  
TGACTTACTAGATATTATTATTAATAAAGTATTCTTAGCCATTTACACATAAATTACAATAGTTTCGAGTAAACCTTACCAACAGA

LRRK2

LRRK2-201

ACTGAATGATCTATAATAATAATTTTTATAAAGAATCGGTAATGTGTATTTAATGTTATCAAAGCTCATTGGAAATGGTTGCT

2300 2310 2320 2330 2340 2350 2360 2370 2380

CATGCTTTCAAGAAATTAGAGGACTTTGTAATTCATTCCTTAACCATTACTTTAGTTCTCACCACAAAATAACATTTTAAGTTTA  
GTACGAAAGTTCTTTAATCTCCTGAAACATTAAGTAAGGAATTGGTAATGAAATCAAGAGTGGTGTTTTATTGTAATAATCAAAAT

LRRK2

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CATGCTTTCAAGAAATTAGAGGACTTTGTAATTCATTCCTTAACCATTACTTTAGTTCTCACCACAAAATAACATTTTAAGTTTA

2390 2400 2410 2420 2430 2440 2450 2460

DraIII

TTTAGCTCTTTCTCATATTTCTGCTTTCCCTTTCAATTAATAAATACTTTTGAGTGTACACAATGTGCCATGTACAGGAAATAG  
AAATCGAGAAAGAGTATAAAAAGACGAAAGGGAAAGTAAATTTTTATGAAAACACATGTGTTACACGGTACATGTCCTTTATC

LRRK2

LRRK2-201

TTTAGCTCTTTCTCATATTTCTGCTTTCCCTTTCAATTAATAAATACTTTTGAGTGTACACAATGTGCCATGTACAGGAAATAG

2470 2480 2490 2500 2510 2520 2530 2540 2550

AGCTTTATCTTTTTGGGTATAACTTCAAGATCATGGCAAAGAAAACCTTATTATTAATTGGATAAACCTTAGATATAATCTAGG  
TCGAAATAGAAAAACCCATATTGAAGTTCTAGTACCGTTTTCTTTGAATAATAATTAACCTATTTGGAATCTATATTAGATCC

LRRK2

LRRK2-201

AGCTTTATCTTTTTGGGTATAACTTCAAGATCATGGCAAAGAAAACCTTATTATTAATTGGATAAACCTTAGATATAATCTAGG

2560 2570 2580 2590 2600 2610 2620 2630

SpeI

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LRRK2

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TTATTTCCCTTATTTTACTAGTTTTCTAGTGAAAATATTCAGGTCTCTGCTGGGTACAGTGGCTTACGCCTGTAATCCCAGCACT

2640 2650 2660 2670 2680 2690 2700 2710 2720

PfMI\*  
AlwNI\* EaeI\* MscI\*

TTGGGAGGCCAGGCAGGCAGATCACTTGAGGCCAGGAGCTGGAGACCAGCCTGGCCAACATGGTGAAACCCTGTCTCTACTAAA  
AACCCTCCGGGTCCGTCCGTCTAGTGAACCTCCGGTCCTCGACCTCTGGTCGGACCGGTTGTACCACTTTGGGACAGAGATGATT

LRRK2

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TTGGGAGGCCAGGCAGGCAGATCACTTGAGGCCAGGAGCTGGAGACCAGCCTGGCCAACATGGTGAAACCCTGTCTCTACTAAA

2730 2740 2750 2760 2770 2780 2790 2800

BssSI  
BssSaI

AATACAAAAAGTAGCTGGGCATGGTGGCATGTGCGTGTAGTCCCAGCTACCAAGGAGGCTGAGGCACGAGAATAGCTTGAACCTG  
TTATGTTTTTCATCGACCCGTACCACCGTACACGCACATCAGGGTCGATGGTTCCTCCGACTCCGTGCTCTTATCGAACTTGGAC

LRRK2

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AATACAAAAAGTAGCTGGGCATGGTGGCATGTGCGTGTAGTCCCAGCTACCAAGGAGGCTGAGGCACGAGAATAGCTTGAACCTG

2810 2820 2830 2840 2850 2860 2870 2880 2890

GGAGGTTGCAGTGAGCCGAGATTGCGCCACTGCACTCCAGCCTAAGCAACAAAGTGAGACTCCATCTTAAAAAAAAAAATTCAGT  
CCTCCAACGTCACCTCGGCTCTAACGCGGTGACGTGAGGTCGGATTCGTTGTTTCACTCTGAGGTAGAATTTTTTTTTTAAAGTCA

LRRK2

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GGAGGTTGCAGTGAGCCGAGATTGCGCCACTGCACTCCAGCCTAAGCAACAAAGTGAGACTCCATCTTAAAAAAAAAAATTCAGT

2900 2910 2920 2930 2940 2950 2960 2970

TCTGTGTTCTGCATCAACCAGAATAAGCTACGCCTCTTATAAAAAACAAATGTGCACAAACCATCTGTGAGGACATAAGGATTA  
AGACACAAGACGTAGTTGGTCTTATTCGATGCGGAGAATATTTTTGTTTACACGTGTTTGGTAGACACTCCTGTATTCCTAATT

LRRK2

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TCTGTGTTCTGCATCAACCAGAATAAGCTACGCCTCTTATAAAAAACAAATGTGCACAAACCATCTGTGAGGACATAAGGATTA

2980 2990 3000 3010 3020 3030 3040 3050 3060

HindIII BfuAI  
BspMI

ATGCTTGCTTACTTTGAGTATTAATAAAAAAGTAGAAGCTTTATTATATGAGTAAAAGTGTTTCCAAAGTCTATTTGAAATGCA  
TACGAACGAATGAAACTCATAATTTTATTTTCATCTTCGAAATAATATACTCATTTTCACAAAGGTTTCAGATAAACTTTACGT

LRRK2

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ATGCTTGCTTACTTTGAGTATTAATAAAAAAGTAGAAGCTTTATTATATGAGTAAAAGTGTTTCCAAAGTCTATTTGAAATGCA



3070

3080

3090

3100

**End** (3102)

GGTACAGAATGAAAATCTGTTATTTTATTAAATCGTTATTTG

3'

CCATGTCTTACTTTTAGACAATAAAATAATTTAGCAATAAAC

5'

LRRK2

LRRK2-201

GGTACAGAATGAAAATCTGTTATTTTATTAAATCGTTATTTG

Original Sequence: ASK2J00180\_LRRK2\_N2081D\_E03\_AB.dna

Feature	Location	Size	Start	End	Type
<b>LINC02471</b>	1 .. 3102	3102 bp	■	→	gene
/note = gene <a href="#">ENSG00000223914</a> lncRNA					
✓ <b>LRRK2</b>	1 .. 3102	3102 bp	■	→	gene
/note = gene <a href="#">ENSG00000188906</a> Protein coding					
<b>LINC02471-202</b>	1 .. 3102	3102 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000641941</a> lncRNA					
✓ <b>LRRK2-201</b>	1 .. 3102	3102 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000298910</a>					
<b>LRRK2-204</b>	1 .. 3102	3102 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000430804</a> Nonsense mediated decay					
<b>LRRK2-206</b>	1 .. 3102	3102 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000479187</a> Retained intron					
<b>LRRK2-210</b>	1 .. 3102	3102 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000679360</a> Nonsense mediated decay					
<b>LRRK2-211</b>	1 .. 3102	3102 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000679532</a> Nonsense mediated decay					
<b>LRRK2-213</b>	1 .. 3102	3102 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000680018</a> Nonsense mediated decay					
<b>LRRK2-215</b>	1 .. 3102	3102 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000680422</a> Nonsense mediated decay					
<b>LRRK2-216</b>	1 .. 3102	3102 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000680425</a> Nonsense mediated decay					
<b>LRRK2-217</b>	1 .. 3102	3102 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000680453</a> Nonsense mediated decay					
<b>LRRK2-218</b>	1 .. 3102	3102 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000680790</a>					
<b>LRRK2-219</b>	1 .. 3102	3102 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000681136</a> protein_coding_CDS_not_defined					
<b>LRRK2-220</b>	1 .. 3102	3102 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000681696</a>					
<b>LRRK2-212</b>	1670 .. 3102	1433 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000679683</a> Nonsense mediated decay					
✓ <b>LRRK2-201</b>	1670 .. 1840	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>	1670 .. 1840	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>	1670 .. 1840	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>	1739 .. 1838	100 bp	■		misc_feature
<b>LRRK2-208</b>	1764 .. 3102	1339 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000636518</a>					
<b>LRRK2-208</b>	1764 .. 1840	77 bp	■	→	CDS
/note = coding sequence <a href="#">ENSP00000490200</a>					
/translation = NWR*NSRGFEVSK*V**IRNTRKIT 25 codons (4 internal stop codons)					
✓ <b>PAM</b>	1798 .. 1800	3 bp	■		misc_feature
✓ <b>gRNA Protospacer Sequence</b>	1801 .. 1820	20 bp	■		misc_feature
✓ <b>SNV</b>	1801 .. 1801	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	1210 .. 1234 →	59°C	Aug 18, 2023
✓ <b>Sanger Sequencing Primer</b>  /sequence = GCCTCCTTGGATGTATGAGC 55% GC / 6124.0 Da	20-mer	1607 .. 1626 →	58°C	Aug 18, 2023
✓ <b>Donor Template WT -&gt; SNV</b>  /sequence = GTTTACTACTCTATGACATTTTGACAACCTGGAGGTAGAATAGTAGAGGGTTTGAAGTTTCCAGATGAGTTTGATGAATTAGAAATACAAGGAAAATTACC 34% GC / 31,083.4 Da	100-mer	1739 .. 1838 →	68°C	Aug 18, 2023
✓ <b>gRNA Protospacer</b>  /sequence = TCTAATTCATCAAATCATT 25% GC / 6010.0 Da	20-mer	1801 .. 1820 ←	47°C	Aug 18, 2023
✓ <b>PCR Reverse</b>  /sequence = ATGTGGAGTTCTAACAGCAATTTCC 40% GC / 7656.1 Da	25-mer	2087 .. 2111 ←	58°C	Aug 18, 2023