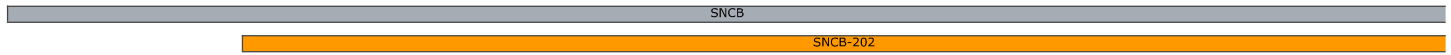


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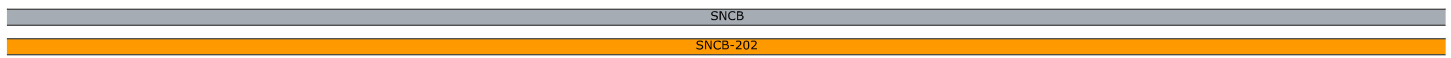
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135



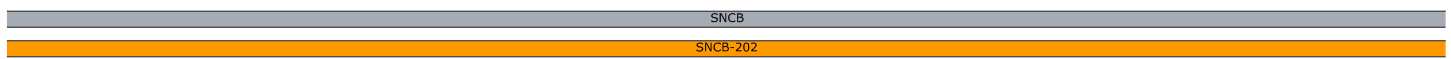
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270



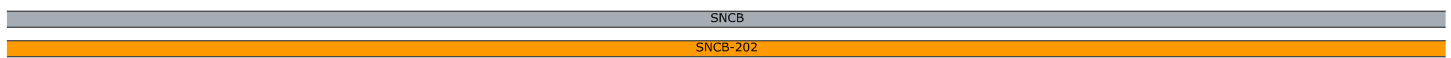
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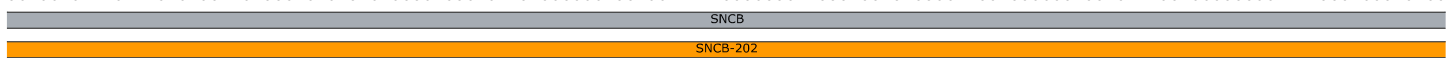
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540



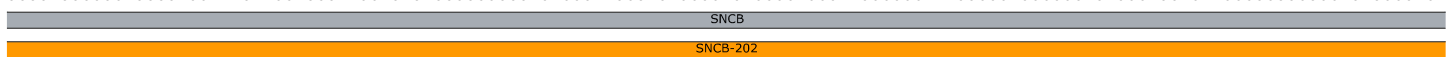
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675



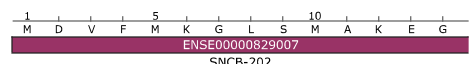
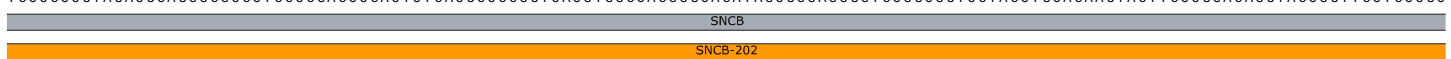
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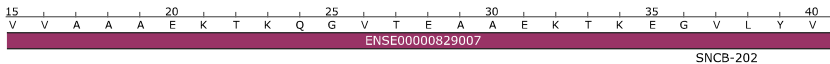
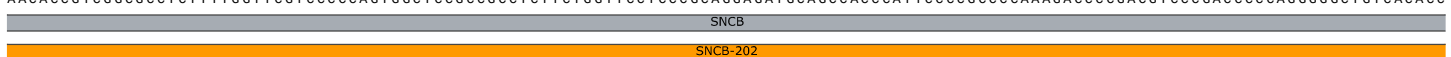
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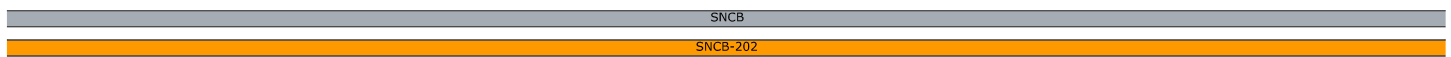
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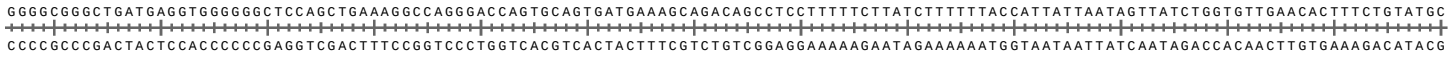
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1215



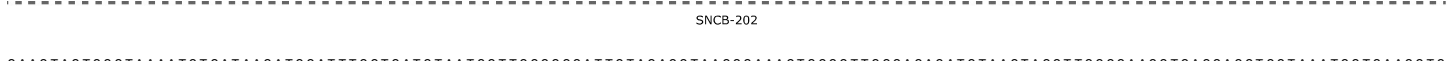
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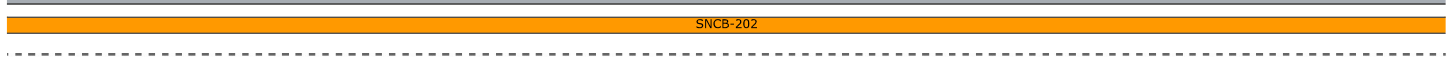
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1485

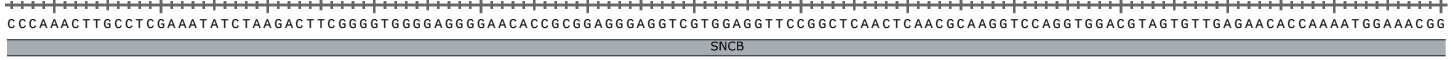


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1755

SNCB

SNCB-202

SNCB-202

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1890

SNCB

SNCB-202

SNCB-202

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2025

SNCB

SNCB-202

SNCB-202

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2160

SNCB

SNCB-202

SNCB-202

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2295

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SNCB-202

SNCB-202

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2430

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SNCB-202

SNCB-202

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2565

SNCB

SNCB-202

SNCB-202

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2700

SNCB

SNCB-202

SNCB-202

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2835

SNCB

SNCB-202

SNCB-202

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2970

SNCB

SNCB-202

SNCB-202

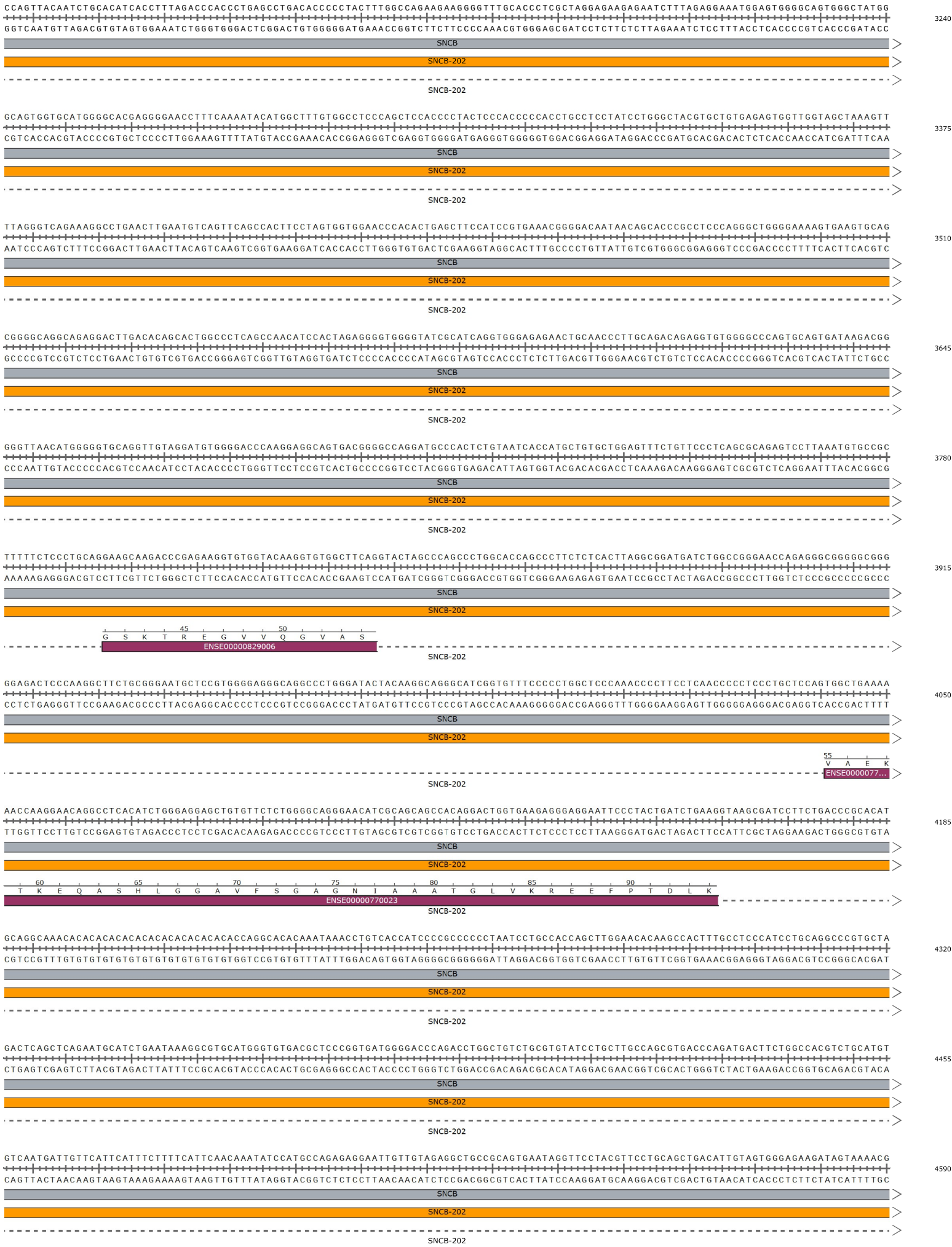
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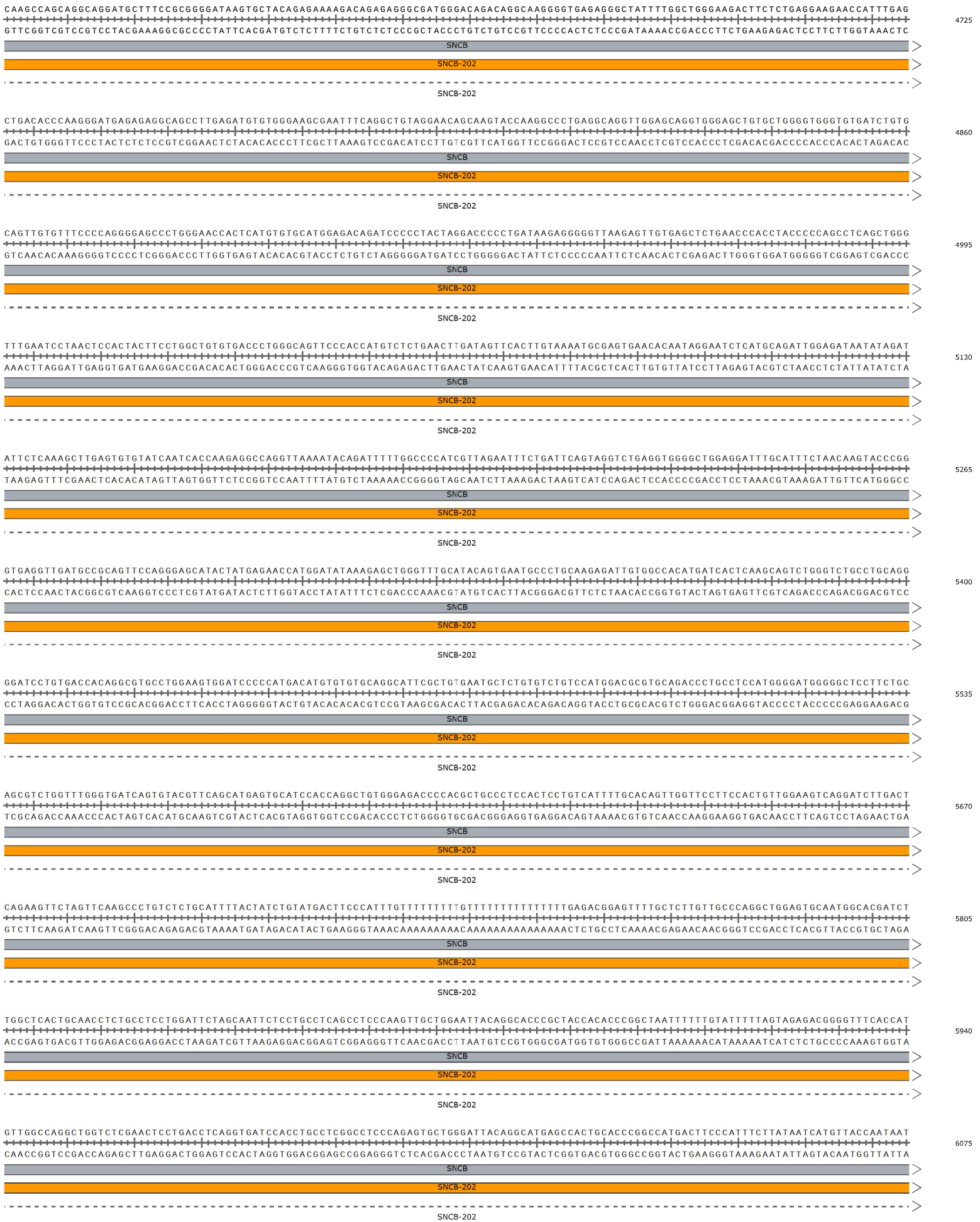
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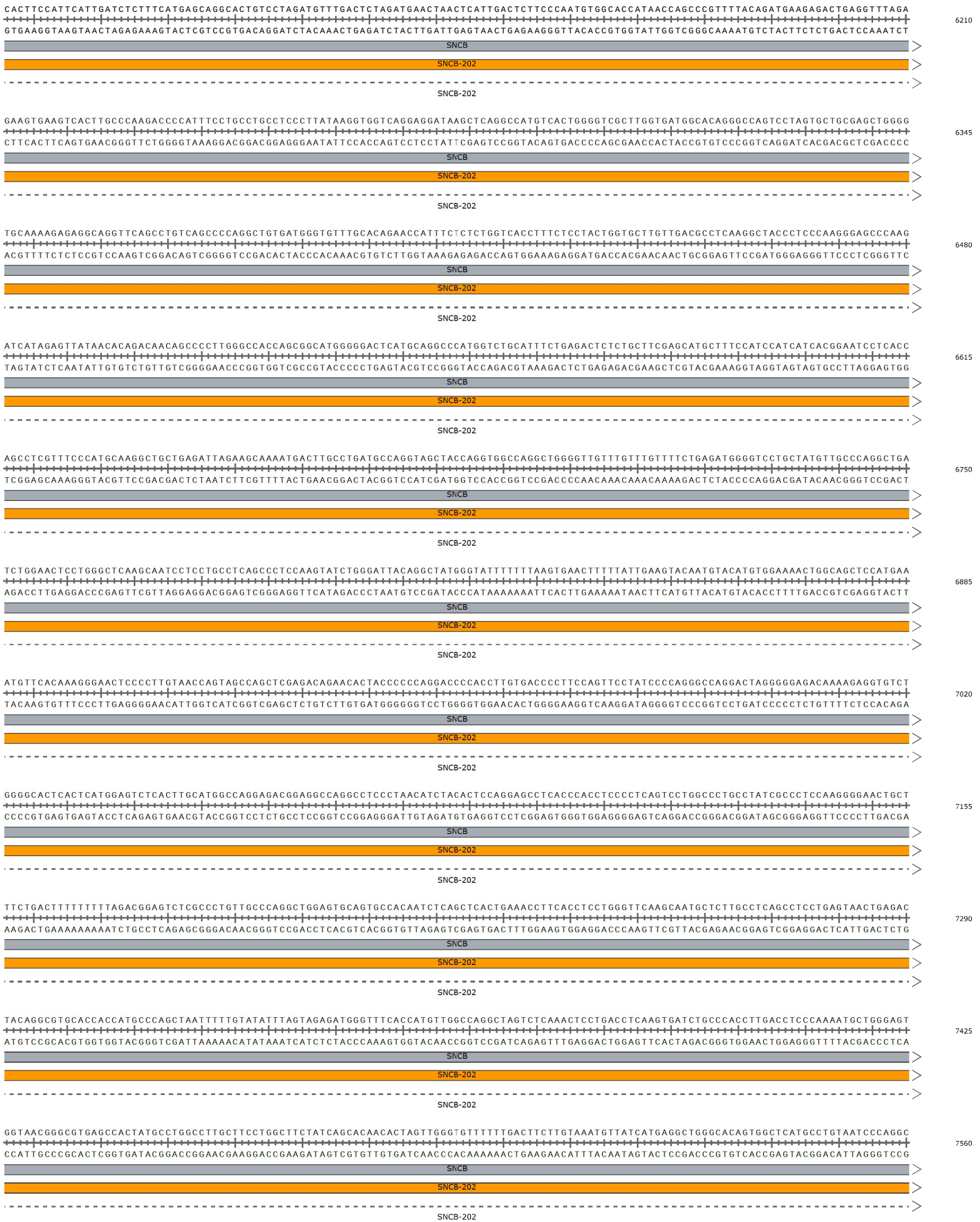
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SNCB-202

SNCB-202







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7695

SNCB

SNCB-202

SNCB-202

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7830

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SNCB-202

SNCB-202

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7965

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SNCB-202

SNCB-202

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8100

SNCB

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SNCB-202

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8235

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8370

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8505

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8640

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SNCB-202

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8775

PCR Forward  
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SNCB

SNCB-202

SNCB-202

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8910

SNCB

SNCB-202

SNCB-202

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9045

SNCB

SNCB-202

SNCB-202

PCR Forward  
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9180

SNCB

SNCB-202

SNCB-202

Sanger Sequencing Primer  
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9315

SNCB

SNCB-202

95 P E E V A Q E A A E E P L I E P L M E P E  
ENSE00003602811

Donor Template WT -> SNV

CGGTCT

Donor Template WT -> SNV

gRNA Protospacer  
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9450

SNCB

SNCB-202

120 G E S Y E D P P Q  
ENSE00003602811

SNCB-202

Donor Template WT -> SNV

Protospacer Sequence PAM

SNV

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Donor Template WT -> SNV

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9585

SNCB

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SNCB-202

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9720

SNCB

SNCB-202

SNCB-202

125 E E  
ENSE000...

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9855

SNCB

SNCB-202

130 Y Q E Y E D E A 135  
ENSE00001196517  
SNCB-202

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9990

SNCB

SNCB-202

GACACAATCACAGACAGGTAGACAG  
PCR Reverse

TGCTCCTCCATCCAGCGTCTGCGGGATGTAGCATGTTCTATGTGTTTTAAACGAAGATCCGAGCGCAGGGCTCCTCCCGATCCCGACAGTG6GCTCTCCAAGCGGGCCCGGGGAGCCCGAGACACCCG  
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10,125

SNCB

SNCB-202

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10,260

SNCB

SNCB-202



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SNCB

SNCB-202

CACGTGGCGGGGAGTCTTCCAGGTCCAGCCTACCCCTCTGTGCTCTTTGGGGTCTGGCACACACAGCCCAAGGTACAAGA  
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3'  
 10,475  
 5'

SNCB

SNCB-202

Feature	Location	Size	Color	Symbol	Type
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/note	= gene <a href="#">ENSG00000074317</a> Protein coding				
<b>SNCB-206</b>	1 .. 10,347	10,347 bp	■	→	prim_transcript
/note	= primary transcript <a href="#">ENST00000614675</a>				
<b>SNCB-202</b>	23 .. 10,475	10,453 bp	■	→	prim_transcript
/note	= primary transcript <a href="#">ENST00000393693</a>				
<b>SNCB-201</b>	193 .. 10,473	10,281 bp	■	→	prim_transcript
/note	= primary transcript <a href="#">ENST00000310112</a>				
<b>SNCB-204</b>	509 .. 9343	8835 bp	■	→	prim_transcript
/note	= primary transcript <a href="#">ENST00000508006</a> Retained intron				
<b>SNCB-203</b>	557 .. 9772	9216 bp	■	→	prim_transcript
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<b>SNCB-205</b>	563 .. 9876	9314 bp	■	→	prim_transcript
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<b>SNCB-201</b>	903 .. 9746	8844 bp	■	→	CDS
▶ 5 segments = 405 bp					
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/translation	= MDVFMKGLSMAKEGVVAAAETKQGVTEAAEKTKEGVLYV,,GSKTREGVVQGVAS,,VAEKTKEQASHLGGAVFSGAGNIAAATGLVKREEFPTDLK,,PEEVAQEAAEEPLIEPLMEPEGESYEDPPQ,,EEYQEYEP EA *				
134 amino acids = 14.3 kDa					
<b>SNCB-202</b>	903 .. 9746	8844 bp	■	→	CDS
▶ 5 segments = 405 bp					
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134 amino acids = 14.3 kDa					
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▶ 5 segments = 405 bp					
/note	= coding sequence <a href="#">ENSP00000422223</a>				
/translation	= MDVFMKGLSMAKEGVVAAAETKQGVTEAAEKTKEGVLYV,,GSKTREGVVQGVAS,,VAEKTKEQASHLGGAVFSGAGNIAAATGLVKREEFPTDLK,,PEEVAQEAAEEPLIEPLMEPEGESYEDPPQ,,EEYQEYEP EA *				
134 amino acids = 14.3 kDa					
<b>SNCB-205</b>	903 .. 9746	8844 bp	■	→	CDS
▶ 5 segments = 405 bp					
/note	= coding sequence <a href="#">ENSP00000424073</a>				
/translation	= MDVFMKGLSMAKEGVVAAAETKQGVTEAAEKTKEGVLYV,,GSKTREGVVQGVAS,,VAEKTKEQASHLGGAVFSGAGNIAAATGLVKREEFPTDLK,,PEEVAQEAAEEPLIEPLMEPEGESYEDPPQ,,EEYQEYEP EA *				
134 amino acids = 14.3 kDa					
<b>SNCB-206</b>	903 .. 9746	8844 bp	■	→	CDS
▶ 4 segments = 363 bp					
/note	= coding sequence <a href="#">ENSP00000479489</a>				
/translation	= MDVFMKGLSMAKEGVVAAAETKQGVTEAAEKTKEGVLYV,,VAEKTKEQASHLGGAVFSGAGNIAAATGLVKREEFPTDLK,,PEEVAQEAAEEPLIEPLMEPEGESYEDPPQ,,EEYQEYEP EA *				
120 amino acids = 12.9 kDa					
<b>MIR4281</b>	1057 .. 1118	62 bp	■	→	gene
/note	= gene <a href="#">ENSG00000266329</a> miRNA				
<b>MIR4281-201</b>	1057 .. 1118	62 bp	■	→	prim_transcript
/note	= primary transcript <a href="#">ENST00000580852</a> miRNA				
<b>Donor Template WT -&gt; SNV</b>	9310 .. 9409	100 bp	■	⇌	misc_feature
<b>Protospacer Sequence</b>	9328 .. 9347	20 bp	■	⇌	misc_feature
<b>SNV</b>	9339 .. 9339	1 bp	■	⇌	misc_feature
/note	= WT = C SNV = A				
<b>PAM</b>	9348 .. 9350	3 bp	■	⇌	misc_feature

Primer	Length	Binding Sites	Tm	Date Added
✓ <b>PCR Forward</b>	25-mer	8760 .. 8784 →	56°C	Jun 14, 2022
/sequence = TAAAATAGCAACAGCTGAAGAAACG 36% GC / 7725.1 Da				
✓ <b>Sanger Sequencing Primer</b>	20-mer	9228 .. 9247 →	57°C	Jun 14, 2022
/sequence = CGAGTCCTGACCTTTTCTGC 55% GC / 6035.0 Da				
✓ <b>Donor Template WT -&gt; SNV</b>	100-mer	9310 .. 9409 ←	82°C	Jun 14, 2022
/sequence = CCAGCTAGGGACGGCAGCAATCATCCTGGATTCCCAAAGTCCCGCCAGCCCTGCTGCCCCCTCACCTGGTGTGGGTCCTCATAACTCTCCCCTTCTGGC 63% GC / 30,436.6 Da				
✓ <b>gRNA Protospacer</b>	20-mer	9328 .. 9347 →	62°C	Jun 14, 2022
/sequence = TGAGGACCCACCCAGGTGA 65% GC / 6112.0 Da				
✓ <b>PCR Reverse</b>	25-mer	9883 .. 9907 ←	58°C	Jun 14, 2022
/sequence = GACAGATGGACAGACACTAACACAG 48% GC / 7702.1 Da				