



Donor Template WT -> SNV



gRNA Protospacer



SNV

**CZK2J00157 EIF2B5\_R195H\_B01\_BB**  
1105 bp

5' C T A A A T G T T A T A T G G A T T G C A G A A G G G G A A A G C T G A C G T G A A G C G T G T T C C C A G G C T A C T T T G T G T A C C T A G G C T T C A G C A G G A  
3' G A T T T A C A A T A T A C C T A A C G T C T T C C C C T T T C G A C T G C A C T T C G C A C A A G G G T C C G A T G A A A C A C A T G G G A T C C G A A G T C G T C C T

EIF2B5

PCR Forward

AGGAAATAACCATGTTTCCTAACCC

T T T T G A G T T C A A A T T G A T G T T T A G G G A C A A G G G G T C A A G G T A T G G A G A G G A A A T A A C C A T G T T T C C T A A C C C A T A A A A C A A G A C T  
A A A A C T C A A G T T T A A C T A C A A A T C C C T G T T C C C C A G T T C C A T A C C T C T C C T T T A T T G G T A C A A A G G A T T G G G T A T T T T G T T C T G A

EIF2B5

G G T G A C A C A G G A T G C T G A A A G C A T T C A T T A T A G T T C A T A A T A C T C T T T G A A G T T G A A G A C G C T G G C A A G A A C C C A A A A A A G C C A T  
C C A C T G T G T C C T A C G A C T T T C G T A A G T A A T A T C A A G T A T T A T G A G A A A C T T C A A C T T C T G C G A C C G T T C T T G G G T T T T T C G G T A

EIF2B5

C G A G A A G G A C T G T G A G T G C T G A A A G G G G G T G A A A A T G G G G A A G G C T C A A A T G A T C T T T A T G C T T G A T A C A C T C A T T C C C C T C A C  
G C T C T T C C T G A C A C T C A C G A C T T T C C C C C A C T T T T A C C C C T T C C G A G T T A C T A G A A A T A C G A A C T A T G T G A G T A A G G G G A G T G

EIF2B5

C C T C C C T T C C T T T A G G A A G T C A A A G T G G T G C C G C C T A C A T C T C T C A A T G T G G T T C G A A T A A T T A C A T C A G A G C T C T A T C G A T C A  
G G A G G G A A G G A A A T C C T T C A G T T T C A C C A C G G C G G G A T G T A G A G A G T T A C A C C A A G C T T A T T A A T G T A G T C T C G A G A T A G C T A G T

EIF2B5

Sanger Sequencing Primer

TTTGGTGCGCTCTGACTTTC

C T G G G A G A T G T C C T C C G T G A T G T T G A T G C C A A G G C T T T G G T G C G C T C T G A C T T T C T T C T G G T G T A T G G G G A T G T C A T C T C A A A C A  
G A C C C T C T A C A G G A G G C A C T A C A A C T A C G G T T C C G A A A C C A C G C G A G A C T G A A A G A A G A C C A C A T A C C C C T A C A G T A G A G T T T G T

EIF2B5

T C A A T A T C A C C A G A G C C C T T G A G G A A C A C A G G T C A G G A T G G G A A A T G A C A G G A A C A A G G G T T A A A G A C C A G C A G A G C C C T G A G A  
A G T T A T A G T G G T C T C G G G A A C T C C T T G T G T C C A G T C C T A C C C T T T A C T G T C C T T G T T C C C A A T T T C T G G T C G T C T C G G G A C T C T

EIF2B5

Donor Template WT -> SNV

CGGAAGCTAGAAAAAATGTTTCTGTGATGACGATGATCT

C T G C T T T T T T G C A G T T C T G T C C C T C C T G T C C T T T A T A G G T T G A G A C G G A A G C T A G A A A A A A T G T T T C T G T G A T G A C G A T G A T C T  
G A C G A A A A A A C G T C A A G A C A G G G A G G A C A G G A A A T A T C C A A C T C T G C C T T C G A T C T T T T T T A C A A A G A C A C T A C T G C T A C T A G A

EIF2B5

Donor Template WT -> SNV

Donor Template WT -> SNV

T C A A G G A G T C A T C C C C C A G C C A C C C A A C T C A T T G C C A C G A A G A C A A T G T G G T A G T G G C T G  
T C A A G G A G T C A T C C C C C A G C C A C C C A A C T C G T T G C C A C G A A G A C A A T G T G G T A G T G G C T G T G G A T A G T A C C A C A A A C A G G G T T C T  
A G T T C C T C A G T A G G G G T C G G T G G G T T G A G C A A C G G T G C T T C T G T T A C A C C A T C A C C G A C A C C T A T C A T G G T G T T T G T C C C A A G A

EIF2B5

Donor Template WT -> SNV



CCATTTTCAGAAGACCCAGGGTCTCCGGCGTTTTGCATTTCTCTGGTGTGTGGATATCTGGGGTCCTTTGAGATGGGGAAGTGA  
GGTAAAAGTCTTCTGGGTCCCAGAGGCCGCAAACGTAAGGAGACCACACACCTATAGACCCCAGGAAACTCTACCCCTTCACT

850

EIF2B5

CAGGCTTCAGTGGGAGAAACAAATTAAGTCCTTGTAACCTTCCCCACAGAGCCTGTTTTAGGGCAGTAGTGATGGAGTGGAGG  
GTCCGAAGTCACCCTCTTTGTTAATTTAGGAACATTGAAGAGGGGTGTCTCGGACAAAGTCCCGTCATCACTACCTCACCTCC

935

EIF2B5

TTCGATATGATTTACTGGATTGTCATATCAGCATCTGTTCTCCTCAGGTGAGCTCTTTAGGGCTGGGGCTGCACACCCAAAGAGT  
AAGCTATACTAAATGACCTAACAGTATAGTCGTAGACAAGAGGAGTCCACTCGAGAAATCCCGACCCCGACGTGTGGGTTTCTCA

1020

EIF2B5

CA

PCR Reverse

AGAACTCTGTGGGTCTGTTATTGTCCCCTTAGAAGGCCAGGGTATATTTCTTCTCCCTGTTTATTTTCATTATTTTTACTTTTAT  
TCTTGAGACACCCAGACAATAACAGGGGAATCTTCCGGTCCCATATAAAGAAGAGGGACAAATAAAAGTAATAAAAATGAAAATA

3'

1105

5'

EIF2B5

TCTTGAGACACCCAGACAATAAC

PCR Reverse

Feature	Location	Size	Start	End	Type
✓ <b>EIF2B5</b>	1 .. 1105	1105 bp	■	➔	gene
/note = gene <a href="#">ENSG00000145191</a> Protein coding					
<b>EIF2B5-DT</b>	1 .. 1105	1105 bp	■	←	gene
/note = gene <a href="#">ENSG00000272721</a> lncRNA					
<b>EIF2B5-202</b>	1 .. 1105	1105 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000432982</a> Nonsense mediated decay					
<b>EIF2B5-203</b>	1 .. 1105	1105 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000465218</a> Retained intron					
<b>EIF2B5-204</b>	1 .. 1105	1105 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000468748</a> Retained intron					
<b>EIF2B5-207</b>	1 .. 1105	1105 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000481054</a> Retained intron					
<b>EIF2B5-208</b>	1 .. 1105	1105 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000484154</a> Retained intron					
<b>EIF2B5-209</b>	1 .. 1105	1105 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000491008</a> Retained intron					
<b>EIF2B5-210</b>	1 .. 1105	1105 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000491144</a> Retained intron					
<b>EIF2B5-211</b>	1 .. 1105	1105 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000492226</a> Retained intron					
<b>EIF2B5-212</b>	1 .. 1105	1105 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000492773</a> Nonsense mediated decay					
<b>EIF2B5-215</b>	1 .. 1105	1105 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000647636</a> Nonsense mediated decay					
<b>EIF2B5-216</b>	1 .. 1105	1105 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000647909</a>					
<b>EIF2B5-217</b>	1 .. 1105	1105 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000648145</a> Nonsense mediated decay					
<b>EIF2B5-218</b>	1 .. 1105	1105 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000648189</a> Nonsense mediated decay					
<b>EIF2B5-219</b>	1 .. 1105	1105 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000648256</a> Nonsense mediated decay					
<b>EIF2B5-220</b>	1 .. 1105	1105 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000648314</a> Nonsense mediated decay					
<b>EIF2B5-221</b>	1 .. 1105	1105 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000648599</a> Nonsense mediated decay					
<b>EIF2B5-222</b>	1 .. 1105	1105 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000648630</a> Nonsense mediated decay					
<b>EIF2B5-223</b>	1 .. 1105	1105 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000648682</a> Nonsense mediated decay					

Feature	Location	Size	Start	End	Type
<b>EIF2B5-224</b>	1 .. 1105	1105 bp	■	→	prim_transcript
/note	= primary transcript <a href="#">ENST00000648882</a> Nonsense mediated decay				
<b>EIF2B5-225</b>	1 .. 1105	1105 bp	■	→	prim_transcript
/note	= primary transcript <a href="#">ENST00000648890</a> Nonsense mediated decay				
<b>EIF2B5-226</b>	1 .. 1105	1105 bp	■	→	prim_transcript
/note	= primary transcript <a href="#">ENST00000648915</a>				
<b>EIF2B5-227</b>	1 .. 1105	1105 bp	■	→	prim_transcript
/note	= primary transcript <a href="#">ENST00000649545</a> Nonsense mediated decay				
<b>EIF2B5-228</b>	1 .. 1105	1105 bp	■	→	prim_transcript
/note	= primary transcript <a href="#">ENST00000649688</a> Nonsense mediated decay				
<b>EIF2B5-229</b>	1 .. 1105	1105 bp	■	→	prim_transcript
/note	= primary transcript <a href="#">ENST00000649814</a> Retained intron				
<b>EIF2B5-231</b>	1 .. 1105	1105 bp	■	→	prim_transcript
/note	= primary transcript <a href="#">ENST00000650270</a> Nonsense mediated decay				
<b>EIF2B5-DT-201</b>	1 .. 1105	1105 bp	■	←	prim_transcript
/note	= primary transcript <a href="#">ENST00000608135</a> lncRNA				
<b>EIF2B5-DT-202</b>	1 .. 1105	1105 bp	■	←	prim_transcript
/note	= primary transcript <a href="#">ENST00000608232</a> lncRNA				
<b>EIF2B5-214</b>	1 .. 769	769 bp	■	→	prim_transcript
/note	= primary transcript <a href="#">ENST00000498831</a> protein_coding_CDS_not_defined				
<b>EIF2B5-230</b>	1 .. 678	678 bp	■	→	prim_transcript
/note	= primary transcript <a href="#">ENST00000650244</a> Nonsense mediated decay				
<b>EIF2B5-216</b>	356 .. 982	627 bp	■	→	CDS
▶ 3 segments = 469 bp					
/codon_start = 1					
/note = coding sequence <a href="#">ENSP00000498164</a>					
/translation = KSKWCRPTSLNVVRIITSELYRSLGDVLRDVKALVRSDFLLVYGDVISNITRALEEHS, , SVPPVLYRLRRKLEKNVSVMTMIFKESSPSHPTRCHEDNVVAVDSTTN RVLHFQKTQGLRRFAFPL, , SLFQGSDDGVEVRYDLLDCHISICSPQ 156 amino acids = 17.7 kDa					
<b>EIF2B5-226</b>	356 .. 982	627 bp	■	→	CDS
▶ 3 segments = 445 bp					
/codon_start = 1					
/note = coding sequence <a href="#">ENSP00000497160</a>					
/translation = KSKWCRPTSLNVVRIITSELYRSLGDVLRDVKALVRSDFLLVYGDVISNITRALEEHR, , LRRKLEKNVSVMTMIFKESSPSHPTRCHEDNVVAVDSTTNRVLHFQKT QGLRRFAFPL, , SLFQGSDDGVEVRYDLLDCHISICSPQ 148 amino acids = 16.9 kDa					
✓ <b>Donor Template WT -&gt; SNV</b>	641 .. 740	100 bp	■		misc_feature
✓ <b>PAM</b>	700 .. 702	3 bp	■		misc_feature
✓ <b>gRNA Protospacer</b>	703 .. 722	20 bp	■		misc_feature
✓ <b>SNV</b>	711 .. 711	1 bp	■		misc_feature
/note = WT = G SNV = A					

Primer	Length	Binding Sites	Tm	Date Added
✓ <b>PCR Forward</b>  /sequence = AGGAAATAACCATGTTTCCTAACCC 40% GC / 7594.0 Da	25-mer	133 .. 157 →	57°C	Sep 29, 2023
✓ <b>Sanger Sequencing Primer</b>  /sequence = TTTGGTGGCTCTGACTTTC 50% GC / 6081.0 Da	20-mer	461 .. 480 →	58°C	Sep 29, 2023
✓ <b>Donor Template WT -&gt; SNV</b>  /sequence = CGGAAGCTAGAAAAAATGTTTCTGTGATGACGATGATCTTCAAGGAGTCATCCCCAGCCACCAACTCATTGCCACGAAGACAATGTGGTAGTGGCTG 48% GC / 30,868.1 Da	100-mer	641 .. 740 →	76°C	Sep 29, 2023
✓ <b>gRNA Protospacer</b>  /sequence = CTTCGTGGCAACGAGTTGGG 60% GC / 6189.1 Da	20-mer	703 .. 722 ←	62°C	Sep 29, 2023
✓ <b>PCR Reverse</b>  /sequence = CAATAACAGACCCACAGAGTTCTAC 44% GC / 7588.0 Da	25-mer	1019 .. 1043 ←	56°C	Sep 29, 2023