

**INK2J00079\_VAPB\_D130E\_F03\_BB**  
993 bp

5' CCAGTCCCTCCACCTCAGCCTCCCAAAGTGCTGGGATTACAGGCATAAGCCACCATGCCAGCTGAAAACAAGTTTTATTTTCAT  
 3' GGTCAGGGAGGTGGAGTCCGAGGGTTTCACGACCCTAATGTCCGTATTCGGTGGTACGGGTCGACTTTTGTTCAAAATAAAAGTA

85

VAPB

VAPB-204

PCR Forward

CCTGTCCCACAGAGTAGC

AAGGAGAGCCAAGGTTACCTGGTCATCCAGACATTGTTTAGAGGATTCCTTTTTGTTTCAGTGGTGGCCTGTCCCACAGAGTAGC  
 TTCCTCTCGGTTCCAATGGACCAGTAGGTCTGTAACAAATCTCCTAAGGAAAAACAAGTCACCACCCGGACAGGGTGTCTCATCG

170

VAPB

VAPB-204

PCR Forward

AACATGG

AACATGGGGAGCATTGGTTGACTTTGAGGAGTCAGGCAGCAAGACTTCAGGGTTTATGAAGTGTAATTTTTGAGAAAGTGAGTG  
 TTGTACCCCTCGTAACCAACTGAAACTCCTCAGTCCGTCGTTCTGAAGTCCCAAATACTTCACATTATAAAACTCTTTCACCTCAC

255

VAPB

VAPB-204

TATTTAATCTTGTCATGTTACAAATATTGAAATCTCAGCAGACTTCAGTCTTTATTTGATATACATCAGGGCTTCTCATTAAAG  
 ATAAATTAGAACAGTACAATGTTTATAACTTTAGAGTCGTCTGAAGTCAAGAAATAAACTATATGTAGTCCCAGAAAGAGTAATTC

340

VAPB

VAPB-204

AGTATTTTTCTGAAATTGTCAGTTATAGGAAGAAAGTTATTCTTCCAGCAGGTTTATAATATCTTGATTATTAATTTAAATTGT  
 TCATAAAAAGACTTTAACAGTCAATATCCTTCTTTCAATAAGAAGGTCGTCCAAATATTATAGAATAATAATTTAAATTTAACA

425

VAPB

VAPB-204

Donor Template WT -> SNV

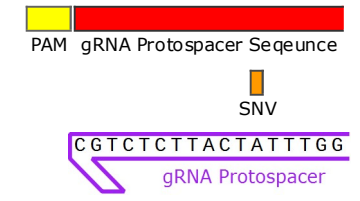
GGAAGGAGGCAAAACCGGAAGACCTTATGGATTCAAACCTTAGATGTGTGTTGAATTGCCAGCAGAGAATGA<sup>G</sup>AAACC  
 TTTAGTGAAGGAGGCAAAACCGGAAGACCTTATGGATTCAAACCTTAGATGTGTGTTGAATTGCCAGCAGAGAATGATAAACC  
 AAATCACCTTCTCCGTTTTGGCCTTCTGGAATACCTAAGTTTTGAATCTACACACAAACTTAACGGTCGTCTCTTACTATTTGG

510

VAPB

VAPB-204

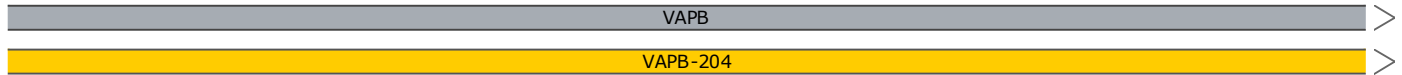
110                      115                      120                      125                      130  
 W K E A K P E D L M D S K L R C V F E L P A E N D K P  
 ENSE0003576198  
 VAPB-204  
 Donor Template Sequence WT -> SNV



Donor Template WT -> SNV  
AGTAAGTATATTTATAGTTAA

AGTAAGTATATTTATAGTTAACAATAATTGAATGTTGTAAGCTGATACTTATTTGCATACCATTTCTGCAAAACCAAGATTTAA  
 TCATTCATATAAAATATCAATTGTTATTAACCTACAACATTCGACTATGAATAAACGATGGTAAAGGACGTTTTGGTTCTAAATT

595



V S I F I V N N N \*  
 (in frame with VAPB-204)

VAPB-204

Donor Template Sequence WT -> SNV

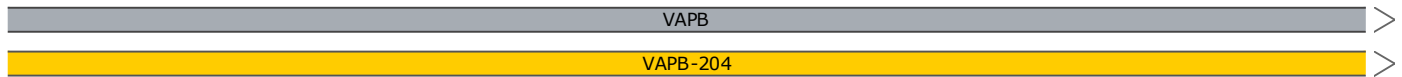
gRNA Protospacer Sequence

TCA

gRNA Protospacer

GTTGGCAAATTATTTTCTTTATCTGATGCTGAAGAAAAAATAAGCTGAAGTCAGCAAATAAGTGGGCCTTTATGAAATCAG  
 CAACCGTTAATAAAAGGAAATAGACTACAGACTTCTTTTTTTTATTCGACTTCAGTCGTTTATTCACCCGGAAATACTTTAGTC

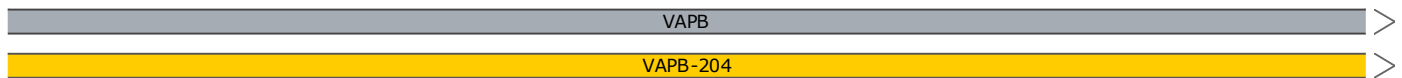
680



GGAAATAGACTACAGACTTC  
 Sanger Sequencing Primer

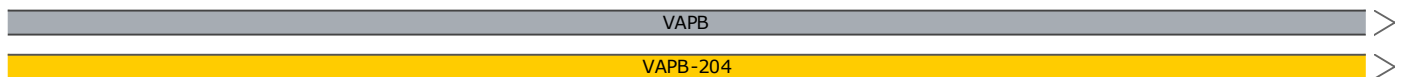
CCTTTGAAAACTCACGGAAAGACAACCTGATTGACAGTGTTTTCCCTTGAAAAGTGCAGCCCGATGGCCATTGAGATGTCATAAA  
 GGAAACTTTTTGAGTGCCCTTCTGTTGACTAACTGTCACAAAGGGGAACTTTTACGTCGGGCTACCGGTAACCTCTACAGTATTT

765



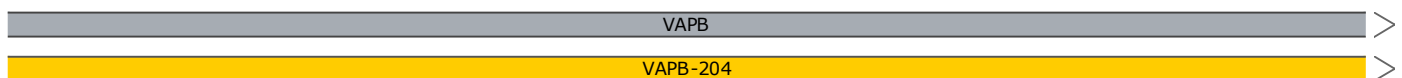
TCCTGAAGAGCTTCTGTGGCCTGGCAAAGGTATAGGTTGCTGTTAAACAGTGGGTGAGAGTGAAAGAGGGAACAATTTGCCCTTT  
 AGGACTTCTCGAAGACACCGGACCGTTTTCCATATCCAACGACAATTTGTCACCCACTCTCACTTTCTCCCTTGTTAAACGGGAAA

850



ATCATGGTGGTTGATGGACGTGTGGGAAGCTTTCAAGTTCTTTGTTTTACAAAGTGCCCTGTCAGCCTCCCTACCCCTTTTACC  
 TAGTACCACCAACTACCTGCACACCCTTCGAAAGTTCAAGAGAACAAAATGTTTCACGGGACAGTCGGAGGGATGGGGAAAATGG

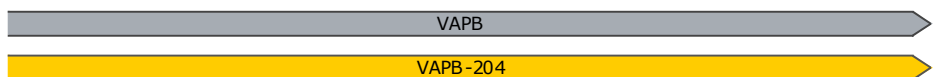
935



CCTGCACACCCTTCGAAAGTTCAAG  
 PCR Reverse

CTATCTACCTCTTCAATCAAAGGCTGCTTTTAGATGAGGATTTCTCAGCCTCAACACT  
 GATAGATGGAGAAGTTAGTTTCCGACGAAAATCTACTCCTAAAGAGTCGGAGTTGTGA

3 '  
 993  
 5 '



Feature	Location	Size	Start	End	Type
✓ <b>VAPB</b>	1 .. 993	993 bp	■	→	gene
/note = gene <a href="#">ENSG00000124164</a> Protein coding					
<b>VAPB-201</b>	1 .. 993	993 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000265619</a> protein_coding_CDS_not_defined					
<b>VAPB-202</b>	1 .. 993	993 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000395802</a>					
<b>VAPB-203</b>	1 .. 993	993 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000463370</a> protein_coding_CDS_not_defined					
✓ <b>VAPB-204</b>	1 .. 993	993 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000475243</a>					
<b>VAPB-206</b>	1 .. 993	993 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000520497</a> Nonsense mediated decay					
✓ <b>VAPB-204</b>	431 .. 511	81 bp	■	→	CDS
/note = coding sequence <a href="#">ENSP00000417175</a>					
/translation = WKEAKPEDLMDSKLRVCFELPAENDKP 27 amino acids = 3.2 kDa					
✓ <b>Donor Template Sequence WT -&gt; SNV</b>	432 .. 531	100 bp	■	⌊	misc_feature
<b>VAPB-205</b>	463 .. 993	531 bp	■	→	prim_transcript
/note = primary transcript <a href="#">ENST00000476395</a> Retained intron					
✓ <b>PAM</b>	491 .. 493	3 bp	■	⌊	misc_feature
✓ <b>gRNA Protospacer Sequence</b>	494 .. 513	20 bp	■	⌊	misc_feature
✓ <b>SNV</b>	505 .. 505	1 bp	■	⌊	misc_feature
/note = WT = T SNV = G					

Primer	Length	Binding Sites	Tm	Date Added
✓ <b>PCR Forward</b> /sequence = CCTGTCCCACAGAGTAGCAACATGG 56% GC / 7636.0 Da	25-mer	153 .. 177	62°C	Aug 15, 2023
✓ <b>Donor Template WT -&gt; SNV</b> /sequence = GGAAGGAGGCAAAACCGAAGACCTTATGGATTCAAACCTTAGATGTGTGTTTGAATTGCCAGCAGAGAATGAGAAACCAGTAAGTATATTTATAGTTAA 37% GC / 31,145.4 Da	100-mer	432 .. 531	70°C	Aug 15, 2023
✓ <b>gRNA Protospacer</b> /sequence = ACTGGTTTATCATTCTCTGC 40% GC / 6049.0 Da	20-mer	494 .. 513	52°C	Aug 15, 2023
✓ <b>Sanger Sequencing Primer</b> /sequence = CTTCAGACATCAGATAAAGG 40% GC / 6134.1 Da	20-mer	612 .. 631	51°C	Aug 15, 2023
✓ <b>PCR Reverse</b> /sequence = GAACTTGAAAGCTTCCCACACGTCC 52% GC / 7571.0 Da	25-mer	866 .. 890	61°C	Aug 15, 2023