



INK2S00025.2_APOE_R136S_A08_BB
3803 bp

ATGTTTCAAGCCCGCTGCACCTCCAAGCCTGGGGTGACAGAGCAAGACCCCTGTTTATAAATACATAAATGCTTTTCCAAGTGATTAACCCGACTCCCCCTCACCTTGCACCCATGGCTCCAAAGAAGCATTTGTGGAGAC
TACAAGTCCGGCGACGTGAGGTGCGGACCCACTGTCTCGTTCTGGGACAAATATTTATGTATTACGAAAGGTTCACTAATTTGGCTGAGGGGGGAGTGGGACGGGTGGTACCGAGGTTTCTTCGTAACACCTCGT

1890

APOE

APOE-201

APOE-201

CCTTCTGTGTGCCCTTAGGTAAGTACTAGATGCCTGGACGGGGTCAGAAGGACCCCTGACCCACCTTGAACCTTGTTCACACAGGATGCCAGGCCAAGGTGGAGCAAGCGGTGGAGACAGAGCCGGAGCCCGAGCTGCGC
GGAAGACACACGGGGATCCATGATCTACGGACCTGCCAGTCTTCTGGGACTGGGTGGAACCTTGAACAAGGTGTCTTACGGTCCGGTTCACCTCGTTTCGCCACCTCTGTCTCGGCCCTCGGGCTCGACGCG

2025

APOE

APOE-201

APOE-201

15 20 25 30
G C Q A K V E Q A V E T E P E P E L R
ENSE00000893952

CAGCAGACCAGGTGGCAGAGCGGCCAGCGCTGGGAACTGGCACTGGGTTCGCTTTTGGGATTACCTGCGCTGGGTGCGAGACTGTCTGAGCAGGTGCAGGAGGAGTGTCTAGCTCCAGGTACCCAGGAACTG
GTCGTCTGGCTACCAGTCTCGCCGGTCCGACCCCTTGACCGTGACCCAGCGAAAACCTAATGGACGGCACCACGCTGTGACAGACTGTCACGCTCCTCCTCGACGAGTCGAGGGTCCAGTGGGTCTTGAC

2160

APOE

APOE-201

APOE-201

35 40 45 50 55 60 65 70 75
Q Q T E W Q S G Q R W E L A L G R F W D Y L R W V Q T L S E Q V Q E E L L S S Q V T Q E L
ENSE00000893952

AGGTGAGTGTCCCATCTG6CCCTTACCCTCTGGTGGGCGGCTATACCTCCCCAGGTCCAAGTTTCTATTCTGCCCTGTGCTAAAGTCTTGGGGGGCTGGGTCTCTGCTGGTTCTAGCTTCTCTTCCCAT
TCCACTCACAGGGGTAGGACCGGGAACCTGGGAGGACCCCGCGGATATGGAGGGGTCCAGGTCCAAAGTAAAGACGGGACAGCGATTCAGAAACCCCGGACCCAGAGACGACCAAGATCGAAGGAGAAGGGTA

2295

APOE

APOE-201

APOE-201

TTCTGACTCCTGGCTTTAGCTCTCTG6AATTTCTCTCTCTCAGCTTTGTCTCTCTCTTCCCTTCTGACTCAGTCTCTCACACTCGTCTG6CTCTGTCTCTGTCTCTCCCTAGCTCTTTATATAGAGACAGAG
AAGACTGAGGACCGAAATCGAGAGACCTTAAGAGAGAGAGTGAACACAGAGAGAGAGAAGGGAAGACTGAGTCAAGAGAGTGTGAGCAGGACCGAGACAGAGACAGGAAAGGATCGAGAAAATATATCTGTCTC

2430

APOE

APOE-201

APOE-201

AGATGGGGTCTCACTGTGTTGCCAGGCTGGTCTTGAACCTCTGGGCTCAAGCGATCCTCCCGCTCGCCCTCCAAAGTCTGGGATTAGAGGCATGAGCCACCTTGC6GGCCTCCTAGCTCCTTCTCTGCT
TCTACC6AGAGTGACACAAACGGTCCGACAGAACTTGAAGACCCGAGTTCTGCTAGGAGGGCGGAGCCGGAGGGTTTACGACCCCTAATCTCCGTAAGTCTGGTGAACGGGCGGAGGATCGAGGAAGAAGCAGA

2565

APOE

APOE-201

APOE-201

CTGCTCTGCCCTCTGCATCTGCTCTCTG6ATCTGTCTCTGCTCTCTTCTCTG6GCTCTGCCCGTTCCTTCTCTCCCTCTGGGTTCTCTGCTGCTATCC6CATCTCGCCCGCCCATCCAGCCCTTCTCCC
GACGGAGACGGGAGACGTAGACGAGAGACGTAGACAGAGACAGAGGAAGAGAGCCGAGAGCGGGCAAGGAGAGAGGGAGAACCCAGAGAGACCAGTGGGGTAGAGCGGGCGGGGTAGGGTCTGGGAAGAGGG

2700

APOE

APOE-201

APOE-201

CGCCTCCCCTGTG6GACACCCTCCG6CCTCTCG6CCGAGGGCGCTGATGGACGAGACCATGAAGGAGTTGAAGGCTACAAATCGGAACTGGAGGAACAACCTGACCCCGGTGGCGGAGGAGACGGGGCAGC
GCGGAGGGTGAACGCTGTGGGAGGGCGGGAAGCCGGCTCCGCGACTACCTGCTCTGGTACTTCTCAACTTCCGGATGTTAGCCTTGACCTCCTTGTGACTGGGGCCACCGCTCCTCTGCGCCCGTGC

2835

APOE

APOE-201

APOE-201

80 85 90 95 100 105 110
A L M D E T M K E L K A Y K S E L E E Q L T P V A E E T R A R
ENSE00000893954

Sanger Sequencing Primer
CGGAACTGGAGGAACAAGTGA
PCR Forward
CGGAACTGGAGGAACAAGTGA

GCTGTCCAAGGAGCTG6AGGCGGCGAGGCCCGGCTGGGCGGACATG6AGGACGTGTG6GCGGCTGGTGCAGTACCGGCGAGGTTG6AGGCGATGCTCGGACAGGACACCAGGAGGCTG6GGTGGCT
CGACAGGTTCTCTGACGCTCCG6CGCTGGGCGGACCCG6CCTGTACCTCTGACACGCGGCGGAGCCAGCTCATGGGCGGCTCCAGCTCGGACGAGCGGCTCTG6GCTCCTGACGCCACAGCGGA

2970

APOE

APOE-201

APOE-201

115 120 125 130 135 140 145 150 155
L S K E L Q A A Q A R L G A D M E D V C G R L L V Q Y R G E V Q A M L G Q S T E E L R V R L
ENSE00000893954

Donor Template WT -> SNV
CAGGCCATGCTCGGCCAGAGCACCAGGAGCTCGGGTCAAGCCT

Donor Template WT -> SNV

SNV PAM

Donor Template WT -> SNV

CGCCTCCACCTGCGCAAGCTGCGTAAGCGGCTCC

CGCCTCCACCTGCGCAAGCTGCGTAAGCGGCTCCCTCCGCGATGCCGATGACCTGCAGAAAGCGCCTGGCAGTGTACCAAGCCGGGGCCCGCGAGGGCGCCGAGCGCGGCTCAGCGCCATCCGCGAGCGCCTGGG
GCGGAGGTTGGACGCGTTCGACGCATTCCGCCGAGGAGCGCTACGGCTACTGGACGCTTTCGCGGACCGTACATGGTCCGGCCCGGGCGCTCCCGGGCTCGCGCCGAGTCCGGTAGCGCTCGCGACCC

3105

APOE

APOE-201

A S H L R K L R K R L L R D A D D L Q K R L A V Y Q A G A R E G A E R G L S A I R E R L G

ENSE0000893954

APOE-201

Donor Template WT -> SNV

Protospacer Sequence

GCGGAGGTTGGACGCGTTCG

gRNA Protospacer

GCCCTGGTGAACAGGGCCGCGTGGGGCCGCGCACTGTGGGCTCCCTGGCGGCCAGCCGCTACAGGAGCGGGCCAGGCTGGGGCGAGCGGCTCGCGCGCGGATGGAGGAGATGGCGAGCCGCGGACCCGCGA
CGGGACACCTTGTCCCGGCGCACGCCGCGGTTGACACCCGAGGGACCGCGGTTCCGCGATGTCTCGCCGCGGTTCCGGACCCGCTCGCGACGCGCGCGCTACCTCCTACCCGTCGGCTGGCGGCT

3240

APOE

APOE-201

P L V E Q G R V R A A T V G S L A G Q P L Q E R A Q A W G E R L R A R M E E M G S R T R D

ENSE0000893954

APOE-201

CTACCTCCTACCCGTCG

PCR Reverse

CCGCCTGGACGAGGTGAAGGAGCAGGTGGCGGAGGTGGCGCCAAGCTGGAGGAGCAGGCCAGCAGATACGCCCTGCAGGCGGAGCCCTTCCAGGGCCGCGCTCAAGAGCTGGTTCGAGCCCTGGTGAAGACAT
GGCGGACCTGCTCCACTTCTCCTGCTCCACCGCCTCCACGCGGGTTGACACTCCTGCTCGGGTCTGCTATGCGGACGCTCGGCTCCGGAAGGTCGGGGCGGAGTTCGACCAAGCTCGGGACACCTTCTGTA

3375

APOE

APOE-201

R L D E V K E Q V A E V R A K L E E Q A Q Q I R L Q A E A F Q A R L K S W F E P L V E D M

ENSE0000893954

APOE-201

GCAGCGCAGTGGGCCGGGCTGGTGGAGAGGTGCAAGGCTGCCGTTGGGCACCAAGCGCCGCGCCCTGTGCCAGCGACAATCACTGAACGCCGAAAGCCTGCAGCCATGCGACCCACGCCACCCCGTGCCTCCTGCC
CGTCGCGTCAACCGGCCGACCACTCTCCACGTCGACGGCACCCGTTGGTCGGCGGGGACACGGGTCGCTGTTAGTGACTTGCGGCTTCGGACGTCGGTACGCTGGGTGGGGTGGGGCACGGAGGACGG

3510

APOE

APOE-201

Q R Q W A G L V E K V Q A A V G T S A A P V P S D N H

ENSE0000893954

APOE-201

TCCGCGCAGCCTGCAGCGGGAGACCTGTCCCGGCCAGCCGTCCTCCTGGGGTGGACCTAGTTTAATAAAGATTACCAAGTTTCACGCATCTGCTGGCCTCCCTGTGATTTCTCTAAGCCCCAGCCTC
AGGCGCGTCGGACGTCGCCCTCTGGGACAGGGGCGGGTCCGGCAGGAGGACCCACCTGGGATCAAATATTTCTAAGTGGTCAAAGTGCCTAGACGACCGGAGGGGGACACTAAAGGAGATTGGGGTCCGGAG

3645

APOE

APOE-201

AGTTTCTCTTTCTGCCACATACTGGCCACACAATTTCTCAGCCCTCCTCTCCTATCTGTGTCTGTGTATCTTTCTCTCTGCCCTTTTTTTTTTTTTTATAGCGGAGTCTGGCTCTGTCAACCGGAGTGAAGT
TCAAAGAGAAAGACGGGTGATGACCGGTGTGTTAAGAGTCCGGGGAGGAGGTAGACACAGACACATAGAAAGAGAGACGGGAAAAAAAAAAAAAAAAATCTGCCTCAGACCGAGACAGTGGGTCCGATCTCAC

3780

CAGTGGCAGGATCTTGGCTCACT 3'
GTCACCGTGTAGAACCGAGTGA 5'

Feature	Location	Size	Color	Strand	Type
APOE	1 .. 3603	3603 bp	grey	→	gene
/note	= gene ENSG00000130203 Protein coding				
APOE-204	1 .. 3154	3154 bp	grey	→	prim_transcript
/note	= primary transcript ENST00000446996				
APOE-201	6 .. 3603	3598 bp	yellow	→	prim_transcript
/note	= primary transcript ENST00000252486				
APOE-205	6 .. 1536	1531 bp	grey	→	prim_transcript
/note	= primary transcript ENST00000485628 Retained intron				
APOE-203	22 .. 3235	3214 bp	grey	→	prim_transcript
/note	= primary transcript ENST00000434152				
APOE-203	79 .. 3235	3157 bp	dark red	→	CDS
▶ 4 segments = 807 bp					
/note	= coding sequence ENSP00000413653				
/translation	= MSSGA SRKSWDPGNPWPP,,DWPITGRMKVLWALLVTFLA,,GCQAKVEQAVETEPEPELRQQTEWQSGQRWELALGRFWDYLRWVQTLSEQVQEELLSSQVTQELR,,ALMDETMKELKAYKSELEEQLTPVAEETRA RLSKELQAAQARLGADMEDVCGRLVQYRGEVQAMLGQSTEEELRVRLASHLRKLRKLLRDADDLQKRLAVYQAGAREGAERGLSAIRERLGPLVEQGRVRAATVGSLAGQPLQERAQAWGERLRARMEEMGSRT 269 amino acids = 30.6 kDa				
APOE-202	570 .. 3164	2595 bp	grey	→	prim_transcript
/note	= primary transcript ENST00000425718				
APOE-201	835 .. 3460	2626 bp	dark red	→	CDS
▶ 3 segments = 954 bp					
/note	= coding sequence ENSP00000252486				
/translation	= MKVLWALLVTFLA,,GCQAKVEQAVETEPEPELRQQTEWQSGQRWELALGRFWDYLRWVQTLSEQVQEELLSSQVTQELR,,ALMDETMKELKAYKSELEEQLTPVAEETRARLSKELQAAQARLGADMEDVCGRLVQ YRGEVQAMLGQSTEEELRVRLASHLRKLRKLLRDADDLQKRLAVYQAGAREGAERGLSAIRERLGPLVEQGRVRAATVGSLAGQPLQERAQAWGERLRARMEEMGSRTDRDLDEVKEQVAEVRAKLEEQAQQIRLQAE 812 amino acids = 90.2 kDa				
APOE-202	835 .. 3164	2330 bp	dark red	→	CDS
▶ 3 segments = 658 bp					
/note	= coding sequence ENSP00000410423				
/translation	= MKVLWALLVTFLA,,GCQAKVEQAVETEPEPELRQQTEWQSGQRWELALGRFWDYLRWVQTLSEQVQEELLSSQVTQELR,,ALMDETMKELKAYKSELEEQLTPVAEETRARLSKELQAAQARLGADMEDVCGRLVQ YRGEVQAMLGQSTEEELRVRLASHLRKLRKLLRDADDLQKRLAVYQAGAREGAERGLSAIRERLGPLVEQGRVRAATVGSLAGQ 219 amino acids = 24.9 kDa				
APOE-204	835 .. 3154	2320 bp	dark red	→	CDS
▶ 3 segments = 648 bp					
/note	= coding sequence ENSP00000413135				
/translation	= MKVLWALLVTFLA,,GCQAKVEQAVETEPEPELRQQTEWQSGQRWELALGRFWDYLRWVQTLSEQVQEELLSSQVTQELR,,ALMDETMKELKAYKSELEEQLTPVAEETRARLSKELQAAQARLGADMEDVCGRLVQ YRGEVQAMLGQSTEEELRVRLASHLRKLRKLLRDADDLQKRLAVYQAGAREGAERGLSAIRERLGPLVEQGRVRAATVGSLS 216 amino acids = 24.6 kDa				
Donor Template WT -> SNV	2927 .. 3005	79 bp	blue	⇌	misc_feature
SNV	2966 .. 2966	1 bp	orange	⇌	misc_feature
/note	= WT = C SNV = A				
PAM	2968 .. 2970	3 bp	yellow	⇌	misc_feature
Protospacer Sequence	2971 .. 2990	20 bp	red	⇌	misc_feature
	3585 .. 9378	5794 bp	grey	→	gene
/note	= gene ENSG00000280087 TEC				
	3585 .. 9378	5794 bp	grey	→	prim_transcript
/note	= primary transcript ENST00000623895 TEC				

Primer		Length		Binding Sites		Tm	Date Added
✓ PCR Forward		21-mer		2787 .. 2807	→	57°C	Oct 13, 2022
/sequence	=	CGGAACTGGAGGAACAACCTGA 52% GC / 6513.3 Da					
✓ Sanger Sequencing Primer		21-mer		2787 .. 2807	→	57°C	Oct 13, 2022
/sequence	=	CGGAACTGGAGGAACAACCTGA 52% GC / 6513.3 Da					
✓ Donor Template WT -> SNV		79-mer		2927 .. 3005	→	85°C	Oct 13, 2022
/sequence	=	CAGGCCATGCTCGGCCAGAGCACCGAGGAGCTGCGGGTGAGCCTCGCCTCCCACCTGCGCAAGCTGCGTAAGCGGCTCC 71% GC / 24,286.6 Da					
✓ gRNA Protospacer		20-mer		2971 .. 2990	←	67°C	Oct 13, 2022
/sequence	=	GCTTGCGCAGGTGGGAGGCG 75% GC / 6255.1 Da					
✓ PCR Reverse		19-mer		3211 .. 3229	←	59°C	Oct 13, 2022
/sequence	=	GCTGCCCATCTCCTCCATC 63% GC / 5635.7 Da					