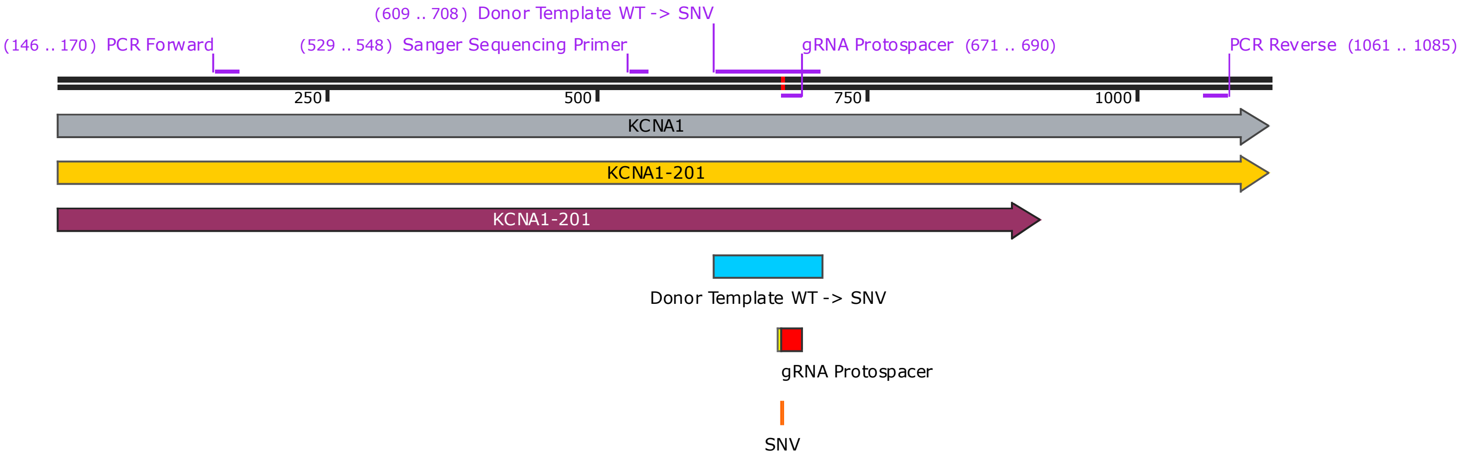

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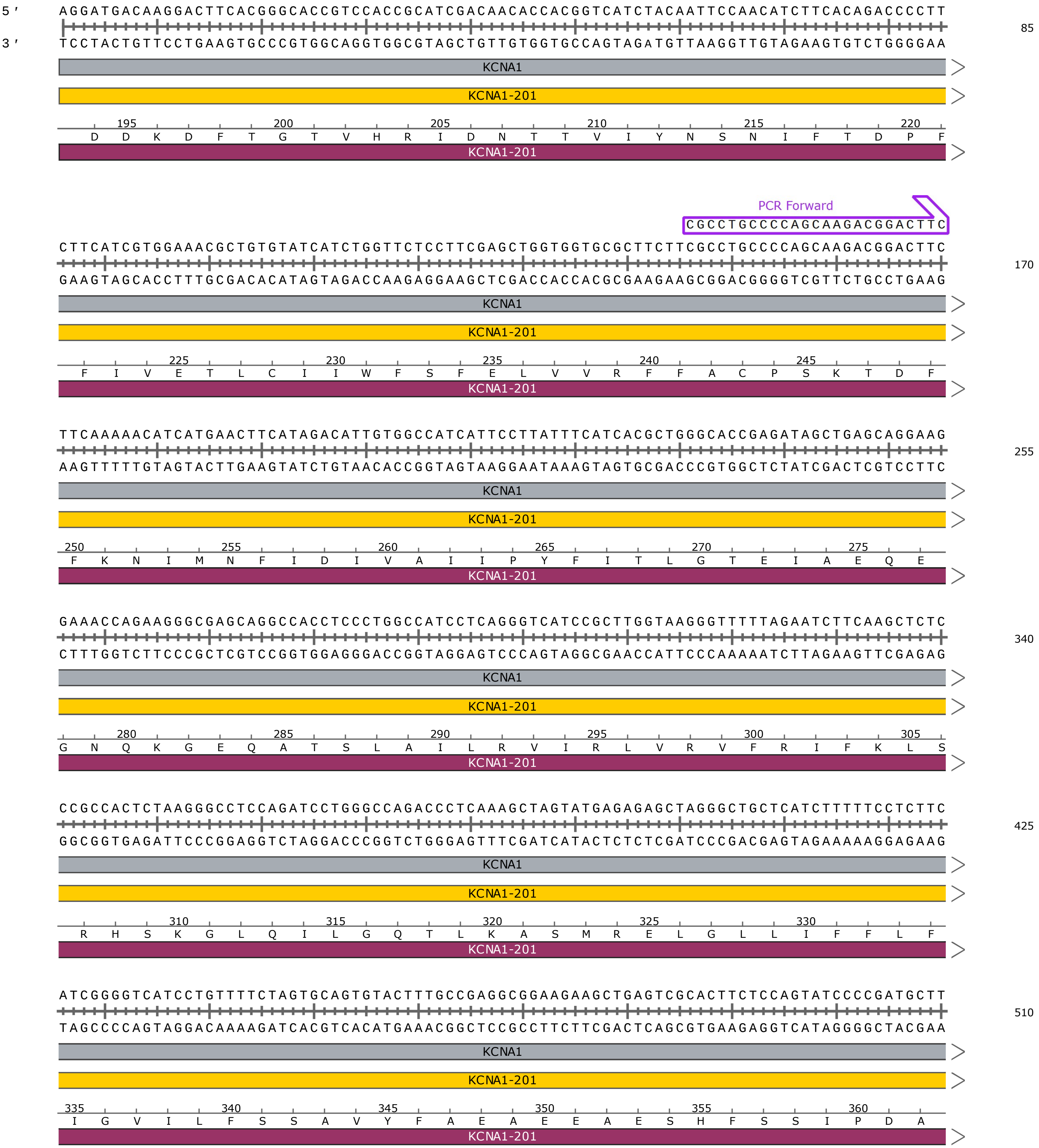

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5′ AGGATGACAAGGACTTCACGGGCACCGTCCACCGCATCGACAACACCACGGTCATCTACAATTCCAACATCTTCACAGACCCCTT  85
3′ TCCTACTGTTCCTGAAGTGCCCGTGGCAGGTGGCGTAGCTGTTGTGGTGCCAGTAGATGTTAAGGTTGTAGAAGTGTCTGGGGAA
   KCNA1
   KCNA1-201
        195        200        205        210        215        220
    D  D  K  D  F  T  G  T  V  H  R  I  D  N  T  T  V  I  Y  N  S  N  I  F  T  D  P  F
   KCNA1-201

                                                            PCR Forward
                                                            CGCCTGCCCCAGCAAGACGGACTTC
   CTTCATCGTGGAAACGCTGTGTATCATCTGGTTCTCCTTCGAGCTGGTGGTGCGCTTCTTCGCCTGCCCCAGCAAGACGGACTTC  170
   GAAGTAGCACCTTTGCGACACATAGTAGACCAAGAGGAAGCTCGACCACCACGCGAAGAAGCGGACGGGGTCGTTCTGCCTGAAG
   KCNA1
   KCNA1-201
           225        230        235        240        245
    F  I  V  E  T  L  C  I  I  W  F  S  F  E  L  V  V  R  F  F  A  C  P  S  K  T  D  F
   KCNA1-201

   TTCAAAAACATCATGAACTTCATAGACATTGTGGCCATCATTCCTTATTTCATCACGCTGGGCACCGAGATAGCTGAGCAGGAAG  255
   AAGTTTTTGTAGTACTTGAAGTATCTGTAACACCGGTAGTAAGGAATAAAGTAGTGCGACCCGTGGCTCTATCGACTCGTCCTTC
   KCNA1
   KCNA1-201
   250        255        260        265        270        275
    F  K  N  I  M  N  F  I  D  I  V  A  I  I  P  Y  F  I  T  L  G  T  E  I  A  E  Q  E
   KCNA1-201

   GAAACCAGAAGGGCGAGCAGGCCACCTCCCTGGCCATCCTCAGGGTCATCCGCTTGGTAAGGGTTTTTAGAATCTTCAAGCTCTC  340
   CTTTGGTCTTCCCGCTCGTCCGGTGGAGGGACCGGTAGGAGTCCCAGTAGGCGAACCATTCCCAAAAATCTTAGAAGTTCGAGAG
   KCNA1
   KCNA1-201
         280        285        290        295        300        305
   G  N  Q  K  G  E  Q  A  T  S  L  A  I  L  R  V  I  R  L  V  R  V  F  R  I  F  K  L  S
   KCNA1-201

   CCGCCACTCTAAGGGCCTCCAGATCCTGGGCCAGACCCTCAAAGCTAGTATGAGAGAGCTAGGGCTGCTCATCTTTTTCCTCTTC  425
   GGCGGTGAGATTCCCGGAGGTCTAGGACCCGGTCTGGGAGTTTCGATCATACTCTCTCGATCCCGACGAGTAGAAAAAGGAGAAG
   KCNA1
   KCNA1-201
           310        315        320        325        330
    R  H  S  K  G  L  Q  I  L  G  Q  T  L  K  A  S  M  R  E  L  G  L  L  I  F  F  L  F
   KCNA1-201

   ATCGGGGTCATCCTGTTTTCTAGTGCAGTGTACTTTGCCGAGGCGGAAGAAGCTGAGTCGCACTTCTCCAGTATCCCCGATGCTT  510
   TAGCCCCAGTAGGACAAAAGATCACGTCACATGAAACGGCTCCGCCTTCTTCGACTCAGCGTGAAGAGGTCATAGGGGCTACGAA
   KCNA1
   KCNA1-201
   335        340        345        350        355        360
    I  G  V  I  L  F  S  S  A  V  Y  F  A  E  A  E  E  A  E  S  H  F  S  S  I  P  D  A
   KCNA1-201
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Sanger Sequencing Primer
CCATGACCACTGTAGGATAC
TCTGGTGGGCGGTGGTGTCCATGACCACTGTAGGATACGGTGACATGTACCCTGTGACAATTGGAGGCAAGATCGTGGGCTCCTT   595
AGACCACCCGCCACCACAGGTACTGGTGACATCCTATGCCACTGTACATGGGACACTGTTAACCTCCGTTCTAGCACCCGAGGAA
  365                370                375                380                385                390
F W W A V V S M T T V G Y G D M Y P V T I G G K I V G S L

Donor Template WT -> SNV
                          GGTGTGCTAACAATTGCCCTGCCCGTACCTGTCATTGTGTCCAATTTCAACTATTTCTACCACTGAGAAACT
GTGTGCCATCGCTGGTGTGCTAACAATTGCCCTGCCCGTACCTGTCATTGTGTCCAATTTCAACTATTTCTACCACCGAGAAACT   680
CACACGGTAGCGACCACACGATTGTTAACGGGACGGGCATGGACAGTAACACAGGTTAAAGTTGATAAAGATGGTGGCTCTTTGA
        395                400                405                410                415
C A I A G V L T I A L P V P V I V S N F N Y F Y H R E T
GGCTCTTTGA
gRNA Protospacer

Donor Template WT -> SNV
GAGGGGGAAGAGCAGGCTCAGTTGCTCC
GAGGGGGAAGAGCAGGCTCAGTTGCTCCACGTCAGTTCCCCTAACTTAGCCTCTGACAGTGACCTCAGTCGCCGCAGTTCCTCTA   765
CTCCCCCTTCTCGTCCGAGTCAACGAGGTGCAGTCAAGGGGATTGAATCGGAGACTGTCACTGGAGTCAGCGGCGTCAAGGAGAT
420                425                430                435                440                445
E G E E Q A Q L L H V S S P N L A S D S D L S R R S S S
CTCCCCCTTC
gRNA Protospacer

CTATGAGCAAGTCTGAGTACATGGAGATCGAAGAGGATATGAATAATAGCATAGCCCATTATAGACAGGTCAATATCAGAACTGC   850
GATACTCGTTCAGACTCATGTACCTCTAGCTTCTCCTATACTTATTATCGTATCGGGTAATATCTGTCCAGTTATAGTCTTGACG
  450                455                460                465                470                475
T M S K S E Y M E I E E D M N N S I A H Y R Q V N I R T A
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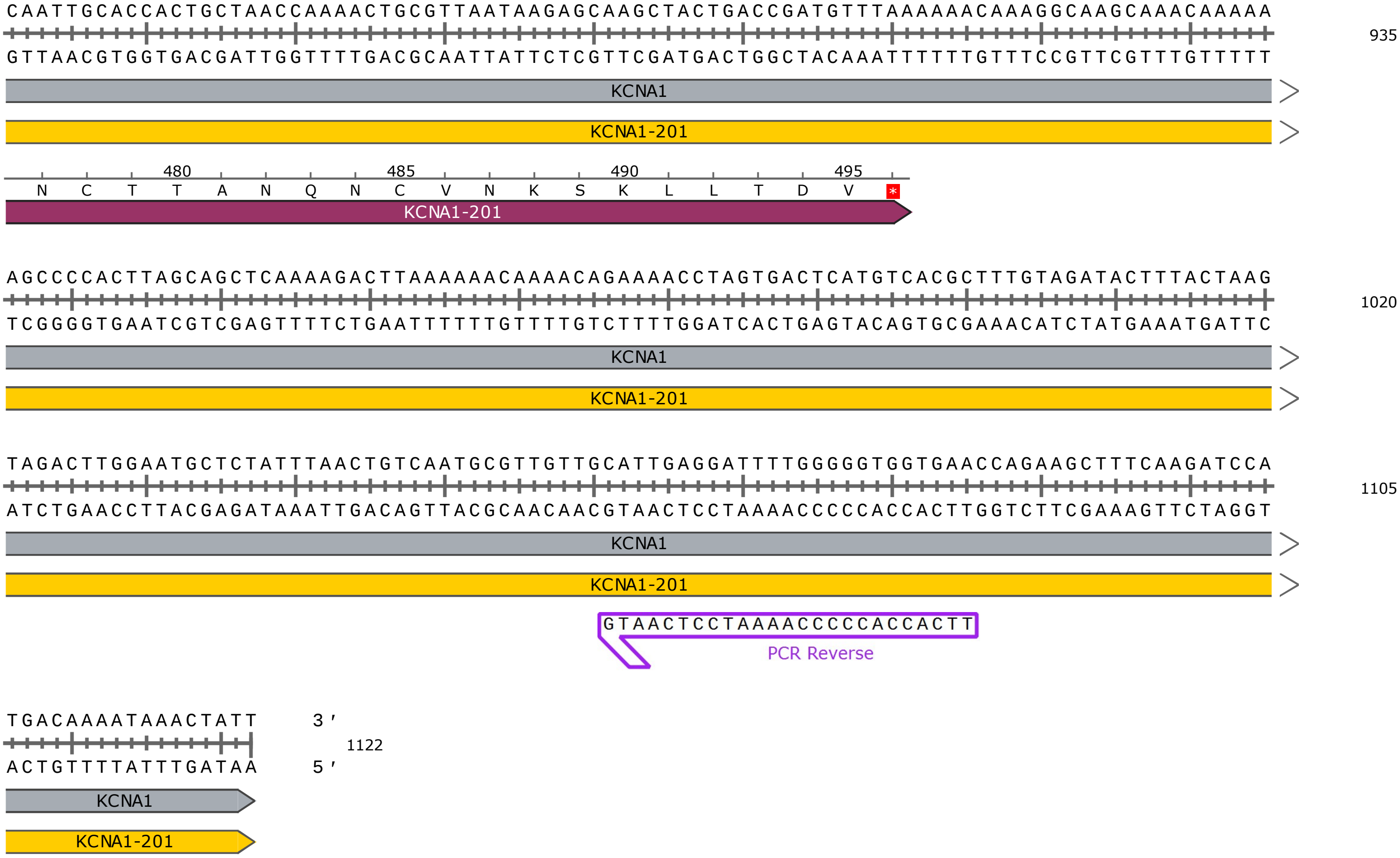

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AGCCCCACTTAGCAGCTCAAAAGACTTAAAAAACAAAACAGAAAACCTAGTGACTCATGTCACGCTTTGTAGATACTTTACTAAG   1020
TCGGGGTGAATCGTCGAGTTTTCTGAATTTTTTGTTTTGTCTTTTGGATCACTGAGTACAGTGCGAAACATCTATGAAATGATTC

TAGACTTGGAATGCTCTATTTAACTGTCAATGCGTTGTTGCATTGAGGATTTTGGGGGTGGTGAACCAGAAGCTTTCAAGATCCA   1105
ATCTGAACCTTACGAGATAAATTGACAGTTACGCAACAACGTAACTCCTAAAACCCCCACCACTTGGTCTTCGAAAGTTCTAGGT

TGACAAAATAAACTATT   3'
                        1122
ACTGTTTTATTTGATAA   5'
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	Feature		Location	Size		⇄	Type
			1 .. 108,878	108,878 bp	■	→	gene
	/note	= gene ENSG00000256654 lncRNA					
			1 .. 108,878	108,878 bp	■	→	prim_transcript
	/note	= primary transcript ENST00000640877 lncRNA					
			1 .. 108,856	108,856 bp	■	→	prim_transcript
	/note	= primary transcript ENST00000640962 lncRNA					
			1 .. 94,611	94,611 bp	■	→	prim_transcript
	/note	= primary transcript ENST00000638821 lncRNA					
			1 .. 14,227	14,227 bp	■	→	prim_transcript
	/note	= primary transcript ENST00000541095 lncRNA					
✓	**KCNA1**		1 .. 1122	1122 bp	■	→	gene
	/note	= gene ENSG00000111262 Protein coding					
✓	**KCNA1-201**		1 .. 1122	1122 bp	🟨	→	prim_transcript
	/note	= primary transcript ENST00000382545					
	KCNA1-202		1 .. 1122	1122 bp	■	→	prim_transcript
	/note	= primary transcript ENST00000543874 protein_coding_CDS_not_defined					
	KCNA1-203		1 .. 1122	1122 bp	■	→	prim_transcript
	/note	= primary transcript ENST00000639306 Nonsense mediated decay					
✓	**KCNA1-201**		1 .. 911	911 bp	🟪	→	CDS
	/codon_start	= 1					
	/note	= coding sequence ENSP00000371985					
	/translation	= DDKDFTGTVHRIDNTTVIYNSNIFTDPFFIVETLCIIWFSFELVVRFFACPSKTDFFKNIMNFIDIVAIIPYFITLGTEIAEQEGNQKGEQATSLAILRVIRLVRVFRIFKLS RHSKGLQILGQTLKASMRELGLLIFFLFIGVILFSSAVYFAEAEEAESHFSSIPDAFWWAVVSMTTVGYGDMYPVTIGGKIVGSLCAIAGVLTIALPVPVIVSNFNYFYHRET EGEEQAQLLHVSSPNLASDSDLSRRSSSTMSKSEYMEIEEDMNNSIAHYRQVNIRTANCTTANQNCVNKSKLLTDV* 302 amino acids = 34.0 kDa					
	KCNA1-204		236 .. 1122	887 bp	■	→	prim_transcript
	/note	= primary transcript ENST00000639680					
	KCNA1-204		236 .. 311	76 bp	🟪	→	CDS
	/note	= coding sequence ENSP00000492218					
	/translation	= RDS*AGRKPEGRAGHLPGHPQGHPL 25 codons (1 internal stop codon)					
✓	**Donor Template WT -> SNV**		609 .. 708	100 bp	🟦	⊢⊣	misc_feature
✓	**PAM**		668 .. 670	3 bp	🟨	⊢⊣	misc_feature
✓	**gRNA Protospacer**		671 .. 690	20 bp	🟥	⊢⊣	misc_feature
✓	**SNV**		672 .. 672	1 bp	🟧	⊢⊣	misc_feature
	/note	= WT = C SNV = T					

	Primer	Length		Binding Sites	⇄	Tm	Date Added
✓	**PCR Forward** /sequence = CGCCTGCCCCAGCAAGACGGACTTC 68% GC / 7572.9 Da	25-mer		146 .. 170	→	70°C	Jun 13, 2023
✓	**Sanger Sequencing Primer** /sequence = CCATGACCACTGTAGGATAC 50% GC / 6086.0 Da	20-mer		529 .. 548	→	54°C	Jun 13, 2023
✓	**Donor Template WT -> SNV** /sequence = GGTGTGCTAACAATTGCCCTGCCCGTACCTGTCATTGTGTCCAATTTCAACTATTTCTACCACTGAGAAACTGAGGGGGAAGAGCAGGCTCAGTTGCTCC 50% GC / 30,775.0 Da	100-mer		609 .. 708	→	77°C	Jun 13, 2023
✓	**gRNA Protospacer** /sequence = CTTCCCCCTCAGTTTCTCGG 60% GC / 5970.9 Da	20-mer		671 .. 690	←	60°C	Jun 13, 2023
✓	**PCR Reverse** /sequence = TTCACCACCCCCAAAATCCTCAATG 48% GC / 7474.9 Da	25-mer		1061 .. 1085	←	62°C	Jun 13, 2023