

Pradas-Juni et al. Supplementary Data 14

For each off-target candidate, the result figures are organized as follows:

(A) Sanger sequences of each off-target PCR amplicon are showed, including both forward (Fw) and reverse (Rv) primers used for the PCR reaction as well as the alternative PAM region.

(B) The alternative PAM region (highlighted in light blue) is visualized within the Sanger PCR amplicon at the Genome Browser site and DNA sequence trace chromatogram confirms no mismatches or rearrangements.

(C) The comparison between the control sequence (“Genomic”) and the Sanger-seq indicates 100% identity between the two sequences.

Sanger Seq

>#1_3prime_intron_Ivd_Sanger-Seq

```
GGTGTGACCCAGAGAGACTCCGCCTCTAACCAAAGTGACAGAGGTAAGTAACATCAGTGT
TCTGTGCTCTGCCCCAGGAGCATGCGGATAAGCAGAGACCAAAGCGGATAAGCAGGGACCAAAG
AAGGTAGCCAGAGAGGCTGGGTCAAGTTTGAAGACAGTAAACTGTCCACAGAGTTGAGGAAGAG
GAAAATGGAAAAGAAGCCAGGAGCAGGA
```

>Fw

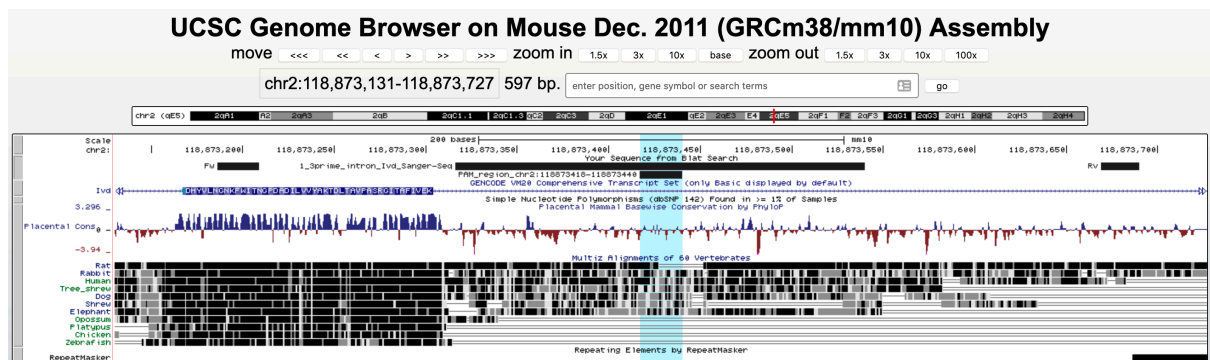
```
TGGCAACAAGTTCTGGATCACCA
```

>Rv

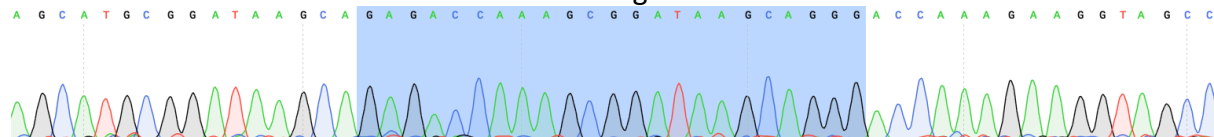
```
CACCTGTGTGGCTGGATTTGT
```

>PAM_region_chr2:118,873,418-118,873,440

```
GAGACCAAAGCGGATAAGCAGGG
```



PAM region



Genomic chr2 :

```
tgatgctgat atcctagtcg tgtatgcaa gacagattg accgctgtgc 118873266
cagcttctcg gggcatcaca gccttcattg tggagaaggt gagggtggtg 118873316
GGTGTGACCC AGAGAGACTC CGCCTCTAAC CAAAGTGACA GAGGTAACTA 118873366
AGATTAACAT CAGTGTCTG TGCTCTGCC CCAGGAGCAT GCGGATAAGC 118873416
AGAGACCAA GCGGATAAGC AGGGACCAA GAAGGTAGCC AGAGAGGCTG 118873466
GGTCAAGTTT GAAGACAGTA AAAGTGCCA CAGAGTTGAG GAAGAGGAAA 118873516
ATGGAAAAGA AGCCAGGAGC AGGAactgca ggggaggctg aggcattgggt 118873566
gggaatgtga atgagacctg caaacctctg atagtaagga caagacaaga 118873616
cacatcagga gttaaaactc gagg
```

cDNA 1_3prime_intron_Ivd_Sanger-Seq

```
GGTGTGACCC AGAGAGACTC CGCCTCTAAC CAAAGTGACA GAGGTAACTA 50
AGATTAACAT CAGTGTCTG TGCTCTGCC CCAGGAGCAT GCGGATAAGC 100
AGAGACCAA GCGGATAAGC AGGGACCAA GAAGGTAGCC AGAGAGGCTG 150
GGTCAAGTTT GAAGACAGTA AAAGTGCCA CAGAGTTGAG GAAGAGGAAA 200
ATGGAAAAGA AGCCAGGAGC AGGA
```

Sanger Seq

>#2_3prime_intron_Ikzf4_Sanger-Seq

```
CCTTTTACCCTGGAAAGGTATATGACTTGAAACCAGAACTTTGGTAGAAGACCTTCCAAAAGACCAT
TGCCCAAATAGCTCAGGGAGTCCTGTTTGACCCAAGACTGAGGAGGATAAGCAGGGGCGCTGTAC
TCCCAATACCCTCCATTTACATTTTGAGATAAGGCCCCAGAATTCTGTCCCTTTTTGACTAAAAATCT
CTCCCCACTTCTGCAAACCTTT
```

>Fw

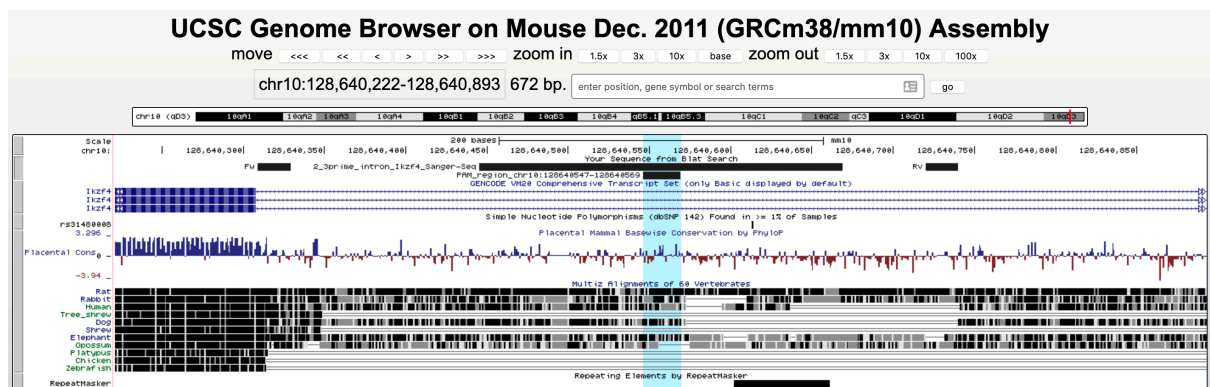
TGCAGGTGAAAGATCAGG

>Rv

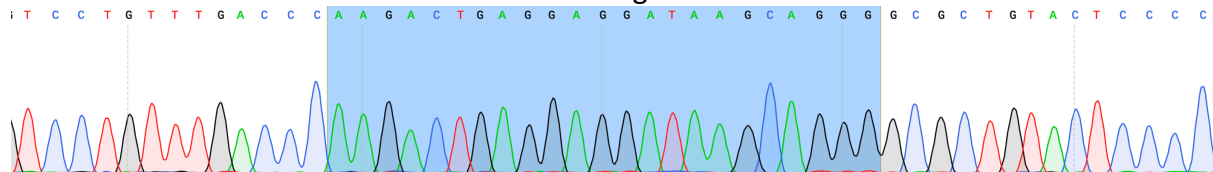
TGGCGCTTCAGTGTAGAT

>PAM_region_chr10:128,640,547-128,640,569

AAGACTGAGGAGGATAAGCAGGG



PAM region



Genomic chr10 :

```
aggggtgtgg aagagcggag ggcaagcttt ctgagcgcac tctggagggg 128640395
cagtggtgtc tacacatacc aggctatgt tatagagtc acctgggggc 128640445
CCTTTTACCC TGGAAAGGTA TATGACTTGA AACCAGAACT TTGGTAGAAG 128640495
ACCTTCCAAA AGACCATTGC CCAAATAGC TCAGGGAGTC CTGTTGACC 128640545
CAAGACTGAG GAGGATAAGC AGGGGCGCTG TACTCCCAA TACCCTCCAT 128640595
TTACATTTTG AGATAAGGCC CCAGAATTCT GTCCCTTTTT GACTAAAAAT 128640645
CTCTCCCAC TTCTGCAAAC CTTTtttggg gttgagatcc accacaaggc 128640695
cttacacgtg gacacacccc accgccgatc tacactgaag cgccaagaat 128640745
ccttcagag ggcccagtac ccgt
```

cDNA 2_3prime_intron_Ikzf4_Sanger-Seq

```
CCTTTTACCC TGGAAAGGTA TATGACTTGA AACCAGAACT TTGGTAGAAG 50
ACCTTCCAAA AGACCATTGC CCAAATAGC TCAGGGAGTC CTGTTGACC 100
CAAGACTGAG GAGGATAAGC AGGGGCGCTG TACTCCCAA TACCCTCCAT 150
TTACATTTTG AGATAAGGCC CCAGAATTCT GTCCCTTTTT GACTAAAAAT 200
CTCTCCCAC TTCTGCAAAC CTTT
```

Sanger Seq

>#3_prime_exon_Cyp4a14_Sanger-Seq

AGACCACATAGGGGCATAAGTGATCAGAAAATAAAAATATCAATATGTTCCAACCCTCCATCCTTTCT
 CTTCAACACTTGCCCACCATTACCTTGATCCTCCTGAGAATGGCAGATAAGCATGGCTATGGTGAGA
 AGAATCTGGTGCAAATCTAGAGGGGTCAAACACCTGCAGAGAGAGCACAGGGCCAGGATGGATGG
 TGCAAGCCCATTTCCAATGCAAA

>Fw

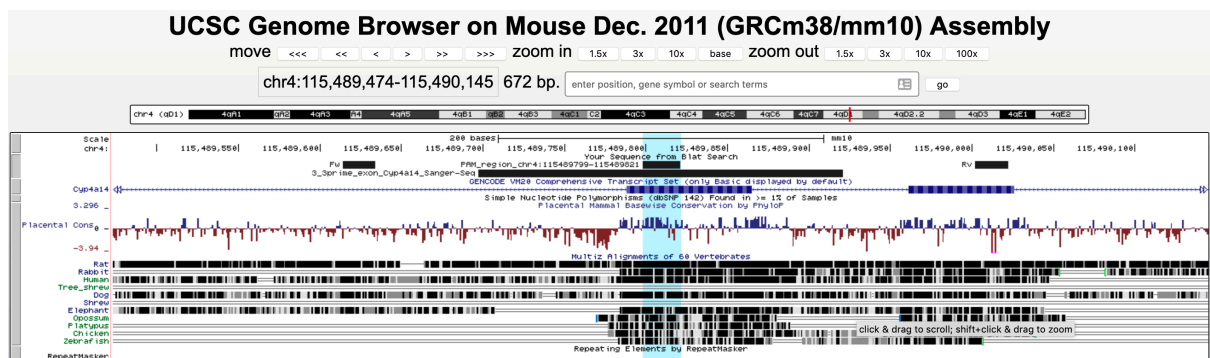
CCTTTTGGCACAAAACAC

>Rv

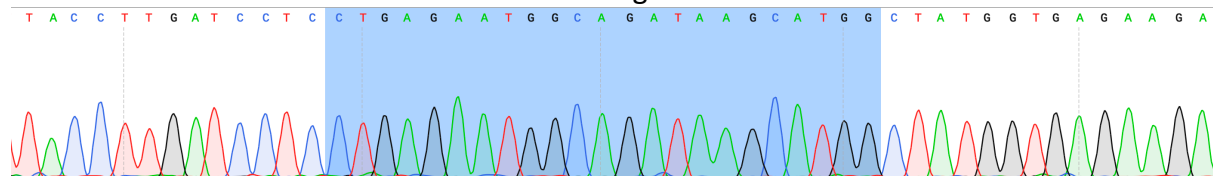
CACAGCCACAATTTCCAT

>PAM_region_chr4:115,489,799-115,489,821

CTGAGAATGGCAGATAAGCATGG



PAM region



Genomic chr4 :

```

gaattgctgc tgtctttcct tttggcacia aacacatata catcatata 115489647
tcccccccc acacacacac acacatagac caacagaaat acatgcaat 115489697
AGACCACATA GGGGCATAAG TGATCAGAAA TATAAAATAT CAATATGTTC 115489747
CAACCCCTCA TCCTTCTCT TCAACACTTG CCCACCATTA CCTTGATCCT 115489797
CCTGAGAATG GCAGATAAGC ATGGCTATGG TGAGAAGAAT CTGGTGCAAA 115489847
TCTAGAGGGG TCAAACACCT GCAGAGAGAG CACAGGGCCA GGATGGATGG 115489897
TGCAAGCCC ATTTCCAATG CAAAggggta cagtatggtt acagtcttct 115489947
ccagatttcc atacctttgg gtttggccag aaocgtgggt tatgatgtag 115489997
accataaata qaaattatqg ctat
  
```

cDNA 3_prime_exon_Cyp4a14_Sanger-Seq

```

AGACCACATA GGGGCATAAG TGATCAGAAA TATAAAATAT CAATATGTTC 50
CAACCCCTCA TCCTTCTCT TCAACACTTG CCCACCATTA CCTTGATCCT 100
CCTGAGAATG GCAGATAAGC ATGGCTATGG TGAGAAGAAT CTGGTGCAAA 150
TCTAGAGGGG TCAAACACCT GCAGAGAGAG CACAGGGCCA GGATGGATGG 200
TGCAAGCCC ATTTCCAATG CAAA
  
```

Sanger Seq

>#4_3prime_intergenic_Gm25335-Magi2_Sanger-Seq

TTTTGGTGATAATTAATAGGAGAAAGGAAATTTAAAAGGTTAAACTATCTTAGCTTGCCACAATGCAA
 AACTTAAAGATAAAATATCTGTAGCTAAAAGATACTCTGGTAAACAACTATGAAGGATAATCAAG
 GATGAAGAAAACCTGGAAGAAGATAAAATACTTCCAAAATATAAGATCATTTTTAGTATTGTAATTT
 GTAGCAAGTTATTTAATTA

>Fw

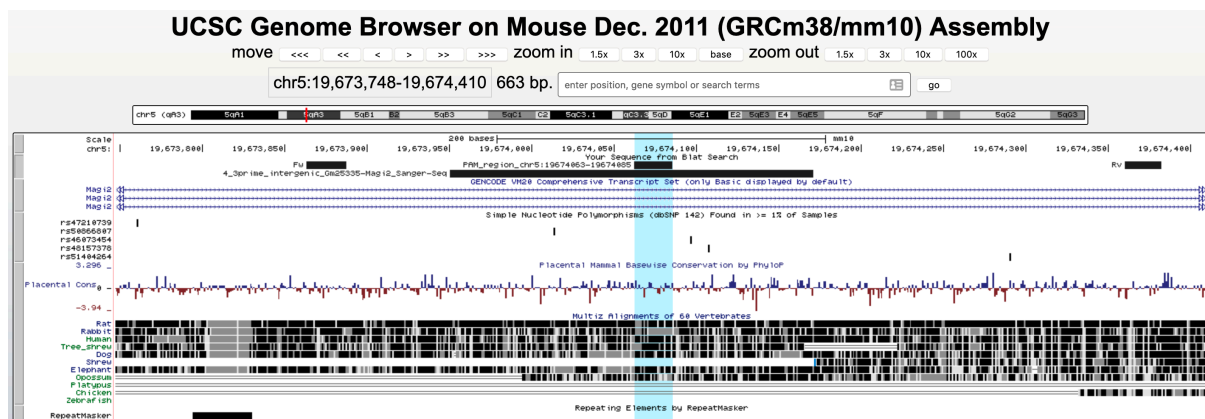
GTGAGGCTCTGGACTGAGTACTGT

>Rv

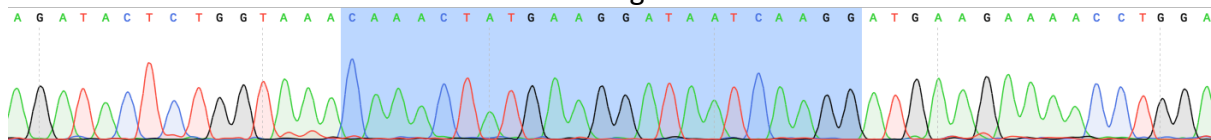
AGTCCAGCATCATTTTTGCCCCC

>PAM_region_chr5:19,674,063-19,674,085

CAAACATGAAGGATAATCAAGG



PAM region



Genomic chr5 :

```

gacagctaga aaagtgaggc tctggactga gtactgtttt agatctggag 19673900
tgaatgtccc atgactgagc atatttatgt acaaaactaa ataaaaatta 19673950
TTTTGGTGAT AATTAATAGG AGAAAGGAAA TAAAAGGTT AAACATCTTT 19674000
AGCTTGCCAC AATGCAAACA CTTAAAGATA AAATATCTGT AGCTAAAAGA 19674050
TACTCTGGTA AACAACTAT GAAGGATAAT CAAGGATGAA GAAAACCTGG 19674100
AAGAAGATAA AATACTTCCA AAATATAAGA TCATTTTTAG TATTGTAATT 19674150
TGTAGCAAGT TATTTAATTA Atgaaggaaa tttcacactt attcttaaaa 19674200
gcttcttcaa gttcttctt ggacggaaaa tacaactagc cactgaaaaa 19674250
ggttgctaaa catttggtaa t
  
```

cDNA 4_3prime_intergenic_Gm25335-Magi2_Sanger-Seq

```

TTTTGGTGAT AATTAATAGG AGAAAGGAAA TAAAAGGTT AAACATCTTT 50
AGCTTGCCAC AATGCAAACA CTTAAAGATA AAATATCTGT AGCTAAAAGA 100
TACTCTGGTA AACAACTAT GAAGGATAAT CAAGGATGAA GAAAACCTGG 150
AAGAAGATAA AATACTTCCA AAATATAAGA TCATTTTTAG TATTGTAATT 200
TGTAGCAAGT TATTTAATTA A
  
```

Sanger Seq

>#5_3prime_exon_Plxna3_Sanger-Seq

```
CCCATTGATAAGCCTCCTAGCTTCCGAGGTGAGGGGTGACATGGGGCCAAGGAGCATGGCTCCTGG
GGCAGGATTGGCACTGCTGGGGGCAAGTCTGTAGGTCCTAGAGCCCCCTGTTTTCTGCCATTGCCT
GCTCCATCAGTAGTCTCCTGTGCCTGCAGCCCTCTTTACAAGTGCTGGGCTCAGCGGCCTAGCTGTG
GCCTCTGCCTCAAGGCTGATCCCC
```

>Fw

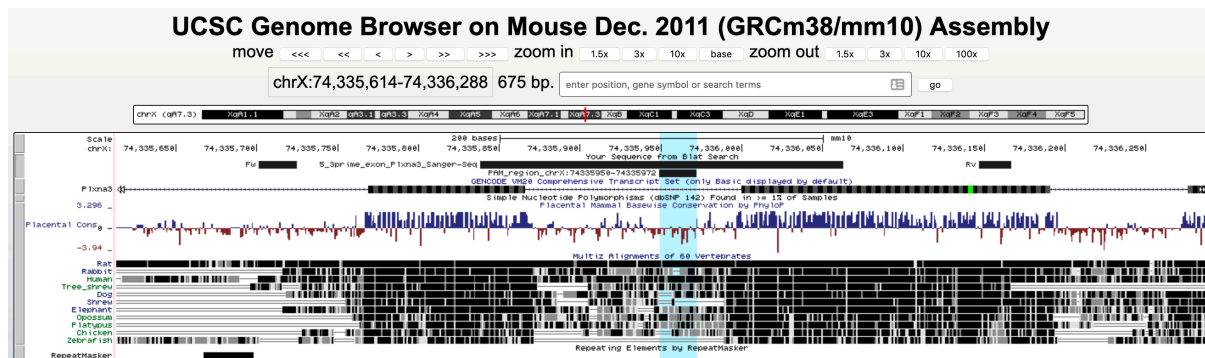
```
AGGGATTCTAAAGTGGGCAAGGA
```

>Rv

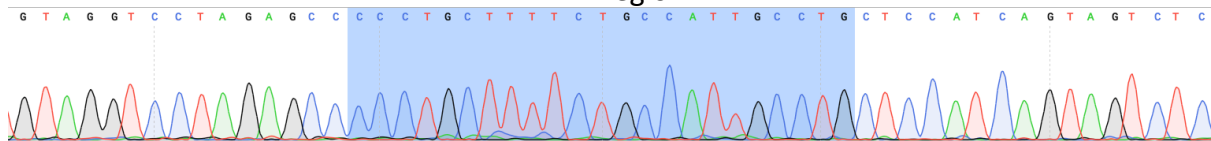
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TCGGGCACCCTTCTGGCTTG
```

>PAM_region_chrX:74,335,950-74,335,972

```
CCCTGCTTTTCTGCCATTGCCTG
```



PAM region



Genomic chrX :

```
atgtgtgctc atgtgggtcc atttttctta gtacttctat gaagtgatg 74335788
agtttgggta caccgaactg gacttctctg tggtttggga tggagatttc 74335838
CCCATTGATA AGCCTCCTAG CTTCCGAGGT GAGGGGTGAC ATGGGGCCAA 74335888
GGAGCATGGC TCCTGGGGCA GGATTGGCAC TGCTGGGGGC AAGTCTGTAG 74335938
GTCTATAGAC CCCCTGCTTT TCTGCCATTG CCTGCTCCAT CAGTAGTCTC 74335988
CTGTGCCTGC AGCCCTTCTT TACAAGTGCT GGGCTCAGCG GCCTAGCTGT 74336038
GGCCTCTGCC TCAAGGCTGA TCCCgattc aactgtggct ggtgcatctc 74336088
agagcacagg tgccagctga gggctcactg cccagctccc aaaagtaact 74336138
ggatgcacc aagccagaag ggtgc
```

cDNA 5_3prime_exon_Plxna3_Sanger-Seq

```
CCCATTGATA AGCCTCCTAG CTTCCGAGGT GAGGGGTGAC ATGGGGCCAA 50
GGAGCATGGC TCCTGGGGCA GGATTGGCAC TGCTGGGGGC AAGTCTGTAG 100
GTCTATAGAC CCCCTGCTTT TCTGCCATTG CCTGCTCCAT CAGTAGTCTC 150
CTGTGCCTGC AGCCCTTCTT TACAAGTGCT GGGCTCAGCG GCCTAGCTGT 200
GGCCTCTGCC TCAAGGCTGA TCCC
```

Sanger Seq

>#1_intergenic_Zfp428-Irgq

ACGCAGTAAGCTTAGCGTTGTACGGAGCACACAACCAACGTCATCATCACGCACCCCTGTACAGAAA
 AGGACCTCTCCACTTGGCCTTGCAGAACTTGCCTGCCGTGACTATATTAGTGCCGGACTGCAACCTT
 GAACCTGACTCGGGACAATGGTGGTGAACAGATCACTGGAGTCTGGAGACTAGGAAAACTATC
 CCGCTCTCGTCCGGTGGCAGAG

>Fw

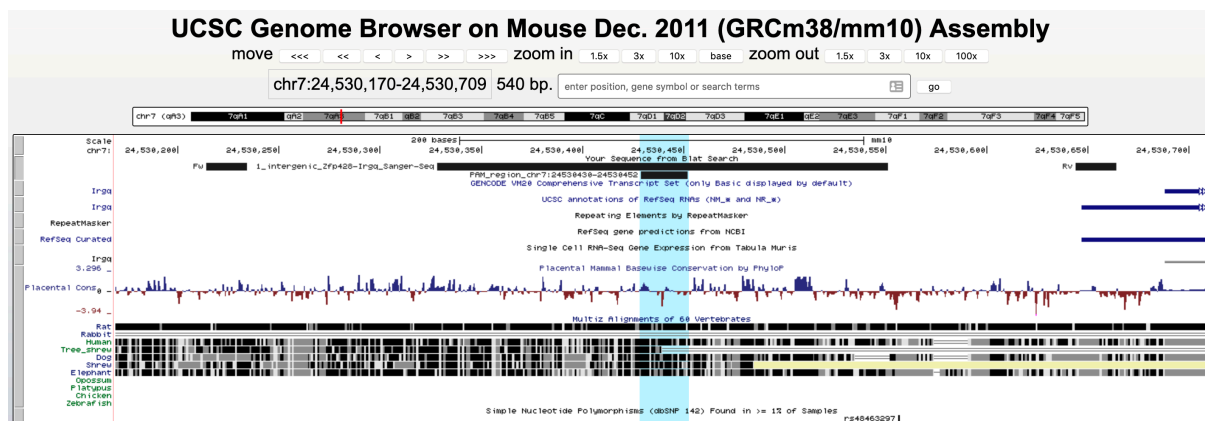
TCACGCTGCTCCCATGCACT

>Rv

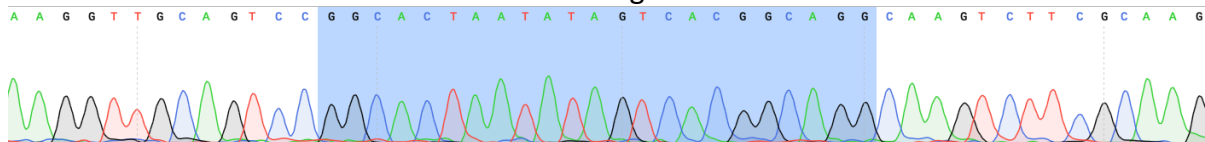
ATGCGCGTGCGCAGAAAGTT

>PAM_region_chr7:24,530,430-24,530,452

GGCCTAATATAGTCACGGCAGG



PAM region



Genomic chr7 (reverse strand):

gaaagttttg	gggcggtgcc	ccaccaagac	cagcaaccaa	tagcctgcg	24530602
cagtacgtgc	agggtttgta	cggc aaagg	ggacaggaag	cgatgagccc	24530552
ACGCAGTAAG	CTTAGCGTTG	TACGGAGCAC	ACAACCAACG	TCATCATCAC	24530502
GCACCCCTGT	ACAGAAAAGG	ACCTCTCCAC	TTGCCCTTGC	GAAGACTTGC	24530452
CTGCCGTGAC	TATATTAGTG	CCGGACTGCA	ACCTTGAACC	TGACTCGGGG	24530402
ACAATGTTGG	TGCAACAGAT	CACTGGAGTC	TGGAGACTAG	GAAAACTAT	24530352
CCCCTCTCG	TCCGGTGGCA	GAGctgtggc	gaccaactgt	caggcgcagc	24530302
taagggacag	tcttcgggga	ggaagcaag	aggcgtccgt	gaccatcaag	24530252
aactaagacg	agaaggcagt	gca			

cDNA 1_intergenic_Zfp428-Irgq_Sanger-Seq

ACGCAGTAAG	CTTAGCGTTG	TACGGAGCAC	ACAACCAACG	TCATCATCAC	50
GCACCCCTGT	ACAGAAAAGG	ACCTCTCCAC	TTGCCCTTGC	GAAGACTTGC	100
CTGCCGTGAC	TATATTAGTG	CCGGACTGCA	ACCTTGAACC	TGACTCGGGG	150
ACAATGTTGG	TGCAACAGAT	CACTGGAGTC	TGGAGACTAG	GAAAACTAT	200
CCCCTCTCG	TCCGGTGGCA	GAG			

Sanger Seq

>#2_5prime_intergenic_Gm12705-Dock7_Sanger-Seq

GCCTCTTCTCTGTGAATGGAGATATCAGATGGAAGTCTCAAGACAGAAAACAGACTCTGTTGTG
GAAAGCAGCCTCAAACGTGAGTCAGGCTAGCCTTGGAAAGTTACAAAATCAAGGCAGGGAAATGAA
GCCTGTGCTGTTGCAGGAGACGTTTAGGTAGACATGGCAGTGATGAGTGATTGCGAGTGGAAAGTTG
AGGAAGAGTGGGGCCATGCGCCTTTC

>Fw

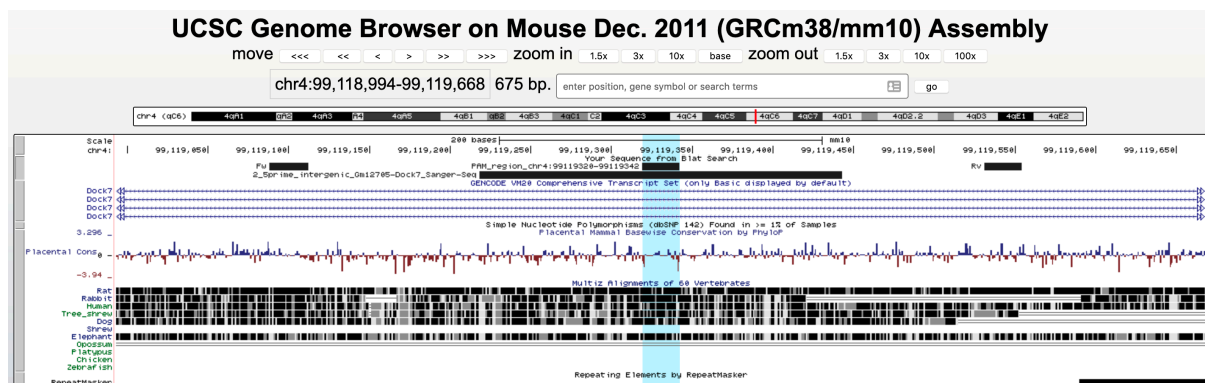
CACCTGAATCCTCACTTCCATCCA

>Rv

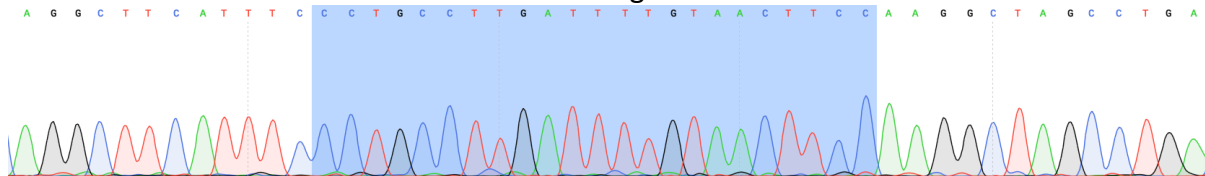
AAGGCATGCCATTAAGGACACA

>PAM_region_chr4:99,119,320-99,119,342

CCTGCCTTGATTTTGTAACTTCC



PAM region



Genomic chr4 (reverse strand):

ttaaaggaca	cattttgtac	aattctttta	aatagatcct	agagtttata	99119494
tctcataaag	taagtacacc	cttgagggta	aggtatgaaa	tatagatagt	99119444
GCCTCTTCTC	TGTGAATGGA	GATATCAGAT	GGAAGTCTC	TCAAGACAGA	99119394
AAACAGACTC	TGTTGTGGAA	AGCAGCCTCA	AACGTGAGTC	AGGCTAGCCT	99119344
TGGAAGTTAC	AAAATCAAGG	CAGGGAATG	AAGCCTGTGC	TGTTGCAGGA	99119294
GACGTTTAGG	TAGACATGGC	AGTGATGAGT	GATTGCGAGT	GGAAGTTGAG	99119244
GAAGAGTGGG	GCCATGCGCC	TTTCtgccca	cccagctttc	cagctgtgca	99119194
gccagctgaa	tgatagaagg	acatctactc	atatctagag	ctgtgcatag	99119144
gaggaagtgg	tttccgaatt	gggga			

cDNA 2_5prime_intergenic_Gm12705-Dock7_Sanger-Seq

GCCTCTTCTC	TGTGAATGGA	GATATCAGAT	GGAAGTCTC	TCAAGACAGA	50
AAACAGACTC	TGTTGTGGAA	AGCAGCCTCA	AACGTGAGTC	AGGCTAGCCT	100
TGGAAGTTAC	AAAATCAAGG	CAGGGAATG	AAGCCTGTGC	TGTTGCAGGA	150
GACGTTTAGG	TAGACATGGC	AGTGATGAGT	GATTGCGAGT	GGAAGTTGAG	200
GAAGAGTGGG	GCCATGCGCC	TTTC			

Sanger Seq

>#3_5prime_intron_Pcsk1_Sanger-Seq

ATCTGCCCTAGAATCCATGTTTTAAGCAGGCATCCAAAGCCTTTCAATGCTTGGAGTACTTTAATCAA
TACTCTTAAAATTTTTTCTACAACCTTCATCCAGCTGTGATTATGAAACTGTCCAATAGGCCACTA
AATAGACAAATTCTGGAGAAAGAAATTCATTACATAAATGCCAGTGGCACCATAATTAGTGTGCATT
AAATACATCTAAGGAAGTTTG

>Fw

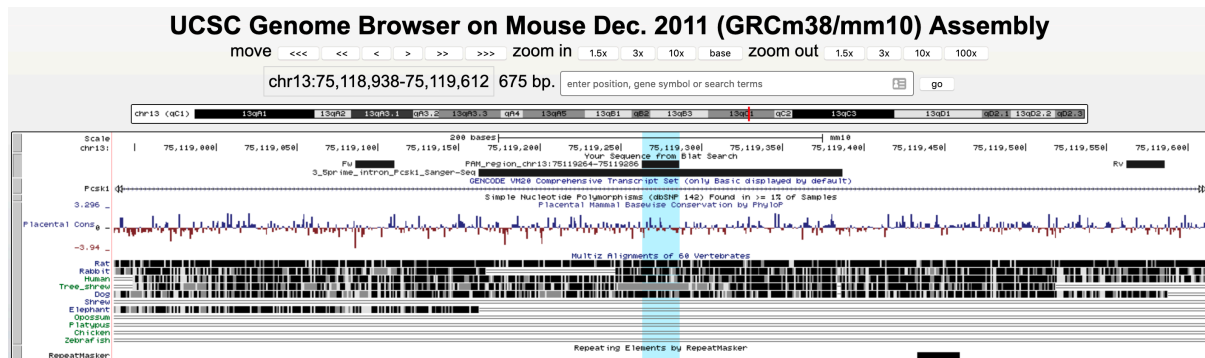
TACAGTCAAGCACCAGAGTAGGTG

>Rv

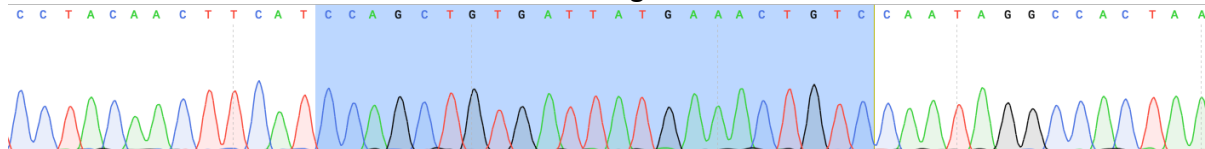
TCCCCTAAAGACTTTGTGCAACAC

>PAM_region_chr13:75,119,264-75,119,286

CCAGCTGTGATTATGAAACTGTC



PAM region



Genomic chr13 :

```
cttccttagt cagttactct tgaatacagt caagcaccag agtagtgat 75119112
tttccatgta cgttcccaaa tcccattgct aaggactcag attctgaagt 75119162
ATCTGCCCTA GAATCCATGT TTTAAGCAGG CATCCAAAGC CTTTCAATGC 75119212
TTGGAGTACT TTAATCAATT ACTCTTAAA TTTTTCCTCC TACAACCTCA 75119262
TCCAGCTGTG ATTATGAAAC TGTCCAATAG GCCACTAAAT AGACAAATTC 75119312
TGGAGAAAGA AATTCATTAC ATAAATGCCA GTGGCACCAT AATTAGTGTG 75119362
CATTAAATAC ATCTAAGGAA GTTTGcacac gatcacctct ctcttcagag 75119412
gcaagatcag aagaatcagg gtaaatTTTA ataataataa aaaaaatctc 75119462
aagtgaacca ttggatacaa tcaca
```

cDNA 3_5prime_intron_Pcsk1_Sanger-Seq

```
ATCTGCCCTA GAATCCATGT TTTAAGCAGG CATCCAAAGC CTTTCAATGC 50
TTGGAGTACT TTAATCAATT ACTCTTAAA TTTTTCCTCC TACAACCTCA 100
TCCAGCTGTG ATTATGAAAC TGTCCAATAG GCCACTAAAT AGACAAATTC 150
TGGAGAAAGA AATTCATTAC ATAAATGCCA GTGGCACCAT AATTAGTGTG 200
CATTAAATAC ATCTAAGGAA GTTTG
```

Sanger Seq

>#4_5prime_exon_Snx1_Sanger-Seq

```
ATTCATTCATCTTGATGGTCATTTTGCTGACTGCATCTGTAGCTTTGTTGAACATCTTAAGGAGACCA
GCGCCACTCAAAGCCTGCGTGCCACAGCTCGAGGCAGCTACAAGATCAAGGCAGGATACATGTTA
TCTGCTGCCCTTTAGGTTCAACAAGAATTAGTATCCACCATGGCATGCCCAAACCTTGTCAACAAGTCA
CTTGACTCTAGACTGCTGCATTC
```

>Fw

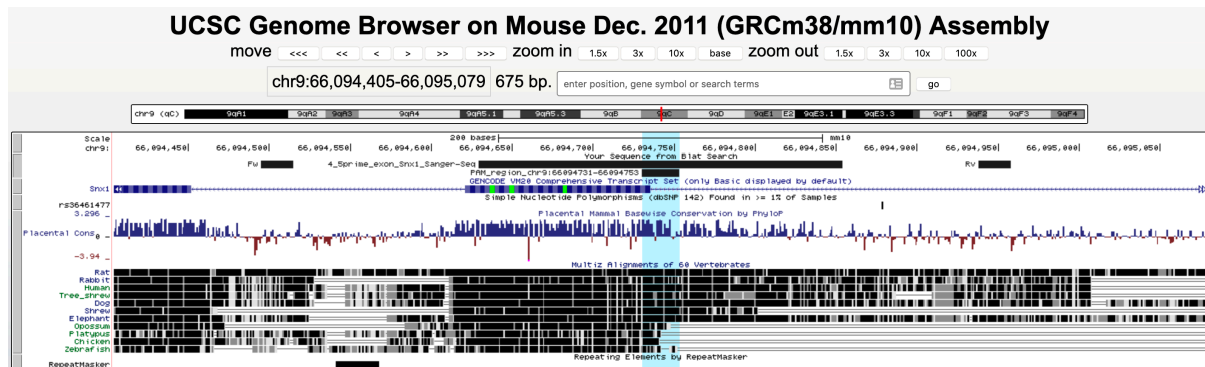
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GTGCAGGGAGCTGCCTTTCT
```

>Rv

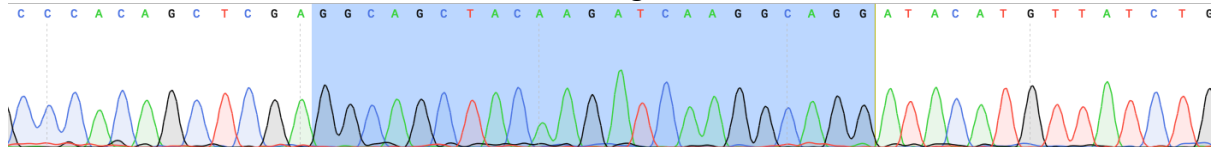
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TGGGCCTGACCACAGAGCAC
```

>PAM_region_chr9:66,094,731-66,094,753

```
GGCAGCTACAAGATCAAGGCAGG
```



PAM region



Genomic chr9 :

```
caaggcgtat tttttgttg tttgtttgtt tgtttgttt attcgaaag 66094579
aaagtaactc cagatatttc aggacttctc agggctactc acaatgtctg 66094629
ATTCATTCAT CTGATGGTC ATTTTGCTGA CTGCATCTGT AGCTTTGTTG 66094679
AACATCTTAA GGAGACCAGC GCCACTCAA GCCTGCGTGC CCACAGCTCG 66094729
AGGCAGCTAC AAGATCAAG CAGGATACAT GTTATCTGCT GCCCTTTAGG 66094779
TTCACAAGAA TTAGTATCCA CCATGGCATG CCCAAAACCT GTCAACAAGT 66094829
CACTTGACTC TAGACTGCTG CATTGgaagg cacttacagg aatctaagat 66094879
taggaaatga gaagcaaac caaacaccca catactattc acaaaaggaa 66094929
gcacagagag tgctctgtg tcagg
```

cDNA 4_5prime_exon_Snx1_Sanger-Seq

```
ATTCATTCAT CTGATGGTC ATTTTGCTGA CTGCATCTGT AGCTTTGTTG 50
AACATCTTAA GGAGACCAGC GCCACTCAA GCCTGCGTGC CCACAGCTCG 100
AGGCAGCTAC AAGATCAAG CAGGATACAT GTTATCTGCT GCCCTTTAGG 150
TTCACAAGAA TTAGTATCCA CCATGGCATG CCCAAAACCT GTCAACAAGT 200
CACTTGACTC TAGACTGCTG CATTG
```

Sanger Seq

>#5_5prime_intergenic_C430042M11Rik-Gm23905_Sanger-Seq

```
GGCTGTCATTATTTTGAGGAAGATCTCTTGAAGCTCAGGCTGGCTTCAGATTAGTTACATAATCAAG
GCTGGCCTTAAGGGCTGCTTCTTACCTTCATCATCCGAGTACTGGAATTATGGGCAAAGCATACA
ACTGTGGCCTGGCTTATATGATAGTTAAAAATTATTAGAATATAGTAGTTGTATCTAATAGTGGGCTC
CATTGCAATATTTTCATACA
```

>Fw

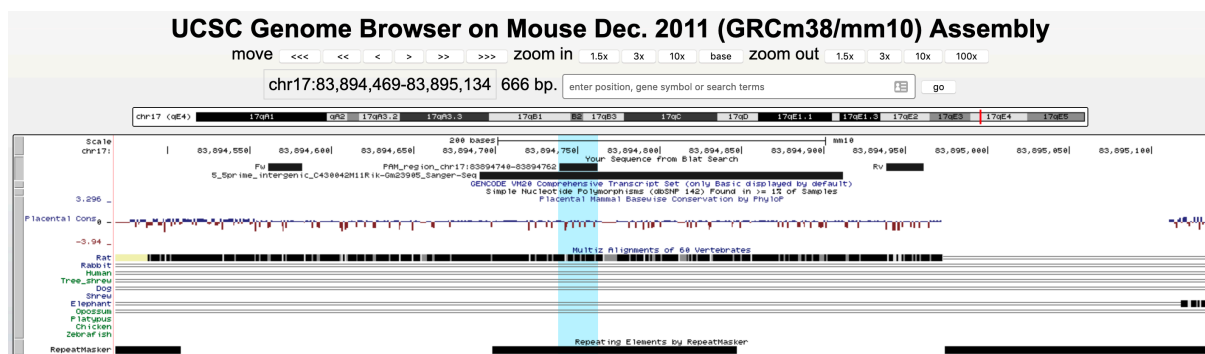
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TGCCCTTATGTGCAGCTCCC
```

>Rv

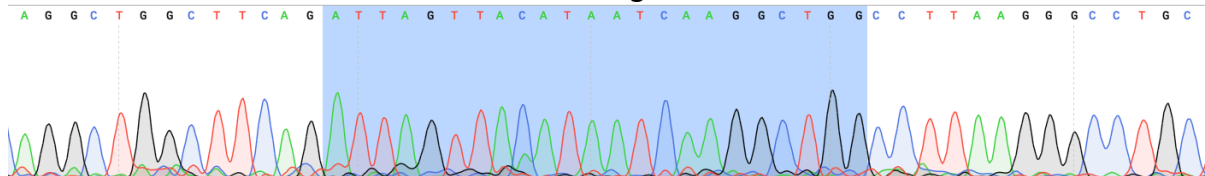
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AGGGGGATGAGATGGTAGTGGGC
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>PAM_region_chr17:83,894,740-83,894,762

```
ATTAGTTACATAATCAAGGCTGG
```



PAM region



Genomic chr17 :

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cacctctct gccccttcgt ctgccgaagt gacctctagc ctacacttgg 83894640
gctttctcaa tgtccgtggt tgttcttttc taaagctaca tgcaagtttt 83894690
GGCTGTCATT ATTTTGAGGA AGATCTCTTG AAGCTCAGGC TGGCTTCAGA 83894740
TTAGTTACAT AATCAAGGCT GGCCCTAAGG GCCTGCTTCT CTTACCTTCA 83894790
TCATCCGAGT ACTGGAATTA TGGGCAAAGC ATACAACGT GGCCTGGCTT 83894840
ATATGATAGT TAAAAATTAT TAGAATATAG TAGTTGTATC TAATAGTGGG 83894890
CTCCATTGCA ATATTCATA CAtacataat acattttgat catactcagc 83894940
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CTCCATTGCA ATATTCATA CA
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