

***Tpm1 p.E180G* Mouse Model Strategy**

-CRISPR/Cas9 technology

Designer	Qin Xia
Reviewer	Yanhua Shen
Date	2022-3-21

Project Overview

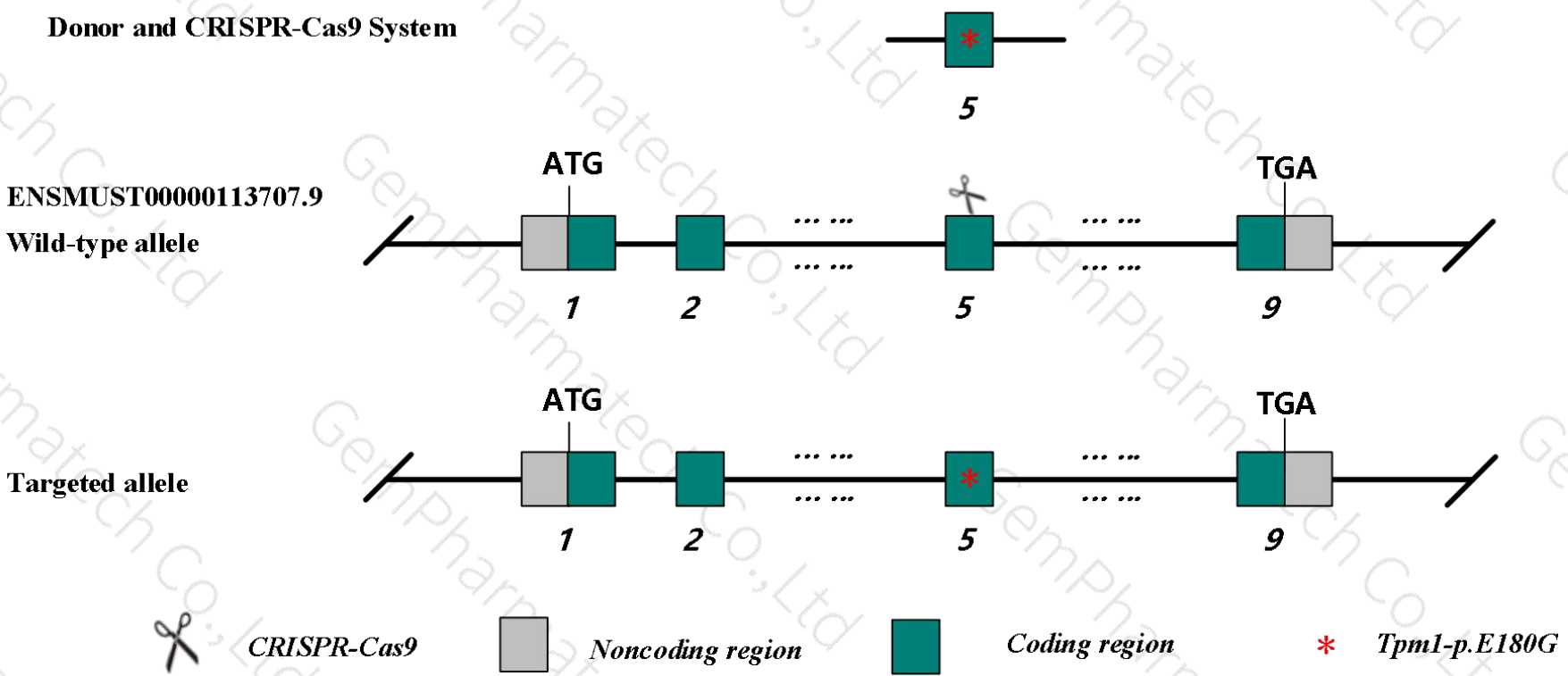
Project Name	Tpm1 p.E180G
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Project Type	Cas9-KI(PM)
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Background	N000013 C57BL/6JGpt
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Project cycle	5-8months
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This model uses CRISPR-Cas9 technology to edit the *Tpm1* gene and the schematic diagram is as follow:



Technical Description

- According to the data of Ensembl, mouse *Tpm1* gene has 21 transcripts.
- The mouse model will introduce p.E180G point mutation in exon 5 of *Tpm1-216* (ENSMUST00000113707.9). The 180th amino acid of *Tpm1* is mutated from E to G, with the codon mutated from GAG to GGG.
- *Tpm1-216* has 9 exons, the translation start codon ATG is located in exon 1, and the translation stop codon TGA is located in exon 9, which encodes 284 amino acids.
- In this project, *Tpm1* gene will be modified by CRISPR-Cas9 technology. The brief process is as follows: CRISPR-Cas9 system and donor vector were microinjected into the fertilized eggs of C57BL/6JGpt mice, and obtained positive F0 generation mice. The F0 positive mice were mated with C57BL/6JGpt mice, the pups will be genotyped by PCR, followed by sequence analysis.

- According to the data from MGI, mice homozygous for disruptions in this gene display embryonic lethality.
- One or two synonymous mutations of amino acids maybe introduced on exon5 of mouse *Tpm1* gene.
- The effect on *Tpm1-218* and *Tpm1-220* transcripts is unknown.
- Mouse *Tpm1* gene is located on Chr9. Please take the loci in consideration when breeding this knockin mice with other gene modified strains, if the other gene is also on the same chromosome, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Sequence homology Analysis

		Section 1				
	(1)	1	10	20	30	46
hTPM1	(1)	MDA IKKKMOMLKL DKENALDRAE OAEADKKAEDRSKOLEDELVSL				
mTpm1	(1)	MDA IKKKMOMLKL DKENALDRAE OAEADKKAEDRSKOLEDELVSL				
Consensus	(1)	MDA IKKKMOMLKL DKENALDRAE OAEADKKAEDRSKOLEDELVSL				
		Section 2				
	(47)	47	60	70	80	92
hTPM1	(47)	OKKLKGTEDELDKYSEALKDAOEKL ELAEKKATDAEADVASLNRR I				
mTpm1	(47)	OKKLKGTEDELDKYSEALKDAOEKL ELAEKKATDAEADVASLNRR I				
Consensus	(47)	OKKLKGTEDELDKYSEALKDAOEKL ELAEKKATDAEADVASLNRR I				
		Section 3				
	(93)	93	100	110	120	138
hTPM1	(93)	OLVEEELDRAOERLATALOKLEEAEKAADES ERGMKVIESRAOKDE				
mTpm1	(93)	OLVEEELDRAOERLATALOKLEEAEKAADES ERGMKVIESRAOKDE				
Consensus	(93)	OLVEEELDRAOERLATALOKLEEAEKAADES ERGMKVIESRAOKDE				
		Section 4				
	(139)	139	150	160	170	184
hTPM1	(139)	EKMEIOE IOLKEAKHIAEDADRKYEEVARKLVI IESDLERAEERAE				
mTpm1	(139)	EKMEIOE IOLKEAKHIAEDADRKYEEVARKLVI IESDLERAEERAE				
Consensus	(139)	EKMEIOE IOLKEAKHIAEDADRKYEEVARKLVI IESDLERAEERAE				
		Section 5				
	(185)	185	190	200	210	230
hTPM1	(185)	LSEGQVRQLEEQLRINDOTL KALMAAEDKYSOKEDRYEEEI KVLSD				
mTpm1	(185)	LSE GKCAEEELKTITNNLKSLEAOAEKYSOKEDRYEEEI KVLSD				
Consensus	(185)	LSEG LEE LK M N LKAL A DKYSOKEDRYEEEI KVLSD				
		Section 6				
	(231)	231	240	250	260	276
hTPM1	(231)	KLKEAETRAEFAERSVT KLEKSIDDLEEKVAHAKEENLSMHOMLDO				
mTpm1	(231)	KLKEAETRAEFAERSVT KLEKSIDDLEEKVAHAKEENLSMHOMLDO				
Consensus	(231)	KLKEAETRAEFAERSVT KLEKSIDDLEEKVAHAKEENLSMHOMLDO				
		Section 7				
	(277)	277	284			
hTPM1	(277)	TLLELNNM				
mTpm1	(277)	TLLELNNM				
Consensus	(277)	TLLELNNM				

consensus positions: 96.1%

identity positions: 94.0%

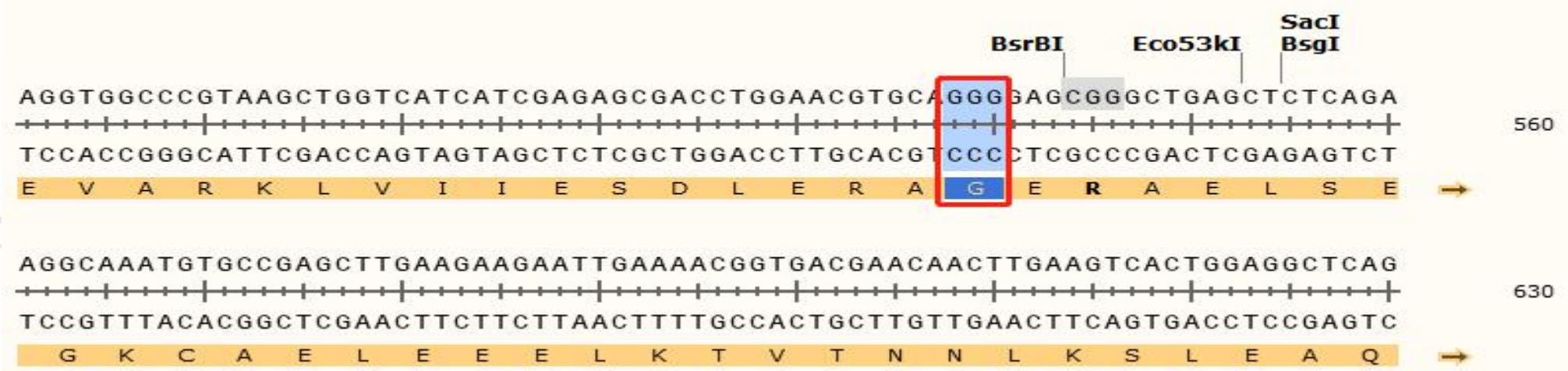
The red box is the mutation site, the 180th histidine (E) of the human gene TPM1 corresponds to the 180rd histidine (E) of the mouse gene Tpm1.

Mutation Site

Before mutation



After mutation



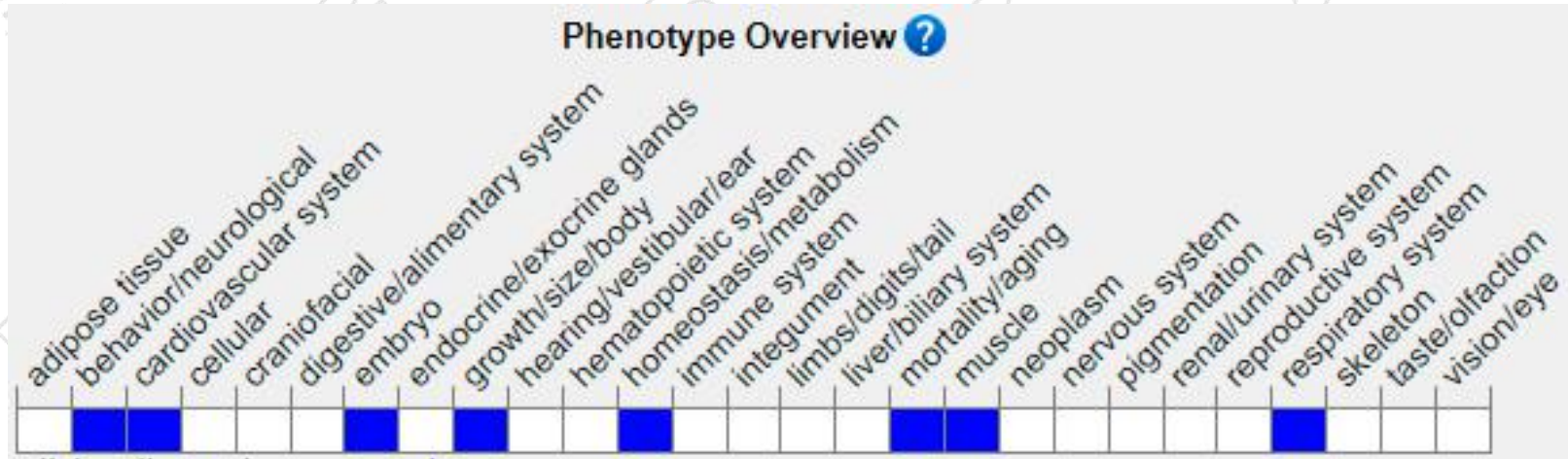
Red marks the mutation site, the codon GAG corresponding to the 180th amino acid in the mouse is mutated to GGG, and the corresponding amino acid E (Glu) will be mutated to G (Gly).

Existing model information

Export: Text File Excel File						
Allele Symbol Gene; Allele Name	Chr	Synonyms	Category	Abnormal Phenotypes Reported in these Systems	Human Disease Models	
In(9)26Rk inversion, Chr 9, Roderick 26; inversion, Chr 9, Roderick 26	9	In26Rk	Chemically induced (other) Involves 742 genes (Gcom1 , Gm23791 , Gm23759 ...) View all	reproductive		
Tg(Myh6-Tpm1*D175N)#Dfw transgene insertion, David F Wieczorek; transgene insertion, David F Wieczorek	UN	alpha-TM 175	Transgenic (Inserted expressed sequence)	cardiovascular, homeostasis, muscle	hypertrophic cardiomyopathy 3 (IDs)	
Tg(Myh6-Tpm1*D230N)HJcf transgene insertion H, Jil C Tardiff; transgene insertion H, Jil C Tardiff	UN	D230N-high	Transgenic (Inserted expressed sequence)	cardiovascular, muscle	dilated cardiomyopathy 1Y (IDs)	
Tg(Myh6-Tpm1*E54K)30Dfw transgene insertion 30, David F Wieczorek; transgene insertion 30, David F Wieczorek	UN	alpha-TM54	Transgenic (Inserted expressed sequence)	cardiovascular, homeostasis, mortality/aging, muscle	dilated cardiomyopathy 1Y (IDs)	
Tg(Myh6-Tpm1*E54K)67Dfw transgene insertion 67, David F Wieczorek; transgene insertion 67, David F Wieczorek	UN	alpha-TM54	Transgenic (Inserted expressed sequence)	cardiovascular, growth/size/body, homeostasis, mortality/aging, muscle, respiratory	dilated cardiomyopathy 1Y (IDs)	
Tg(Myh6-Tpm1*E180G)57Dfw transgene insertion 57, David F Wieczorek; transgene insertion 57, David F Wieczorek	UN	FHC alpha-TM 180, TM180, Tm-E180G	Transgenic (Inserted expressed sequence)	behavior, cardiovascular, homeostasis, mortality/aging, muscle, respiratory	hypertrophic cardiomyopathy 3 (IDs)	
Tpm1^{em1Clo} tropomyosin 1, alpha; endonuclease- mediated mutation 1, Cecilia Lo	9	Tpm1 ^{K5del}	Endonuclease-mediated (Dominant negative, Humanized sequence, Null/knockout)	cardiovascular, homeostasis, mortality/aging		

<http://www.informatics.jax.org/allele/summary?markerId=MGI:98809>

Phenotype Overview(MGI)



<http://www.informatics.jax.org/marker/MGI:98809>

Mice homozygous for disruptions in this gene display embryonic lethality.

Basic Information of Mouse *Tpm1* Gene



Target Gene	Mouse <i>Tpm1</i> Gene
Gene ID	22003
Link(NCBI)	https://www.ncbi.nlm.nih.gov/gene/22003
Link(Ensembl)	http://asia.ensembl.org/Mus_musculus/Gene/Summary?db=core;g=ENSMUSG00000032366;r=9:66929872-66956688
Location	Chr 9

Mouse *Tpm1* Gene (NCBI)

Tpm1 tropomyosin 1, alpha [*Mus musculus* (house mouse)]

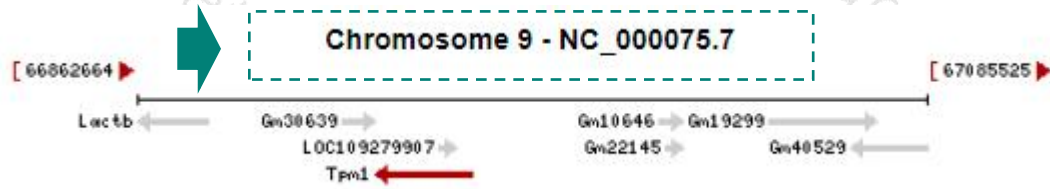
Gene ID: 22003, updated on 15-Mar-2022



Download Datasets

Summary

- Official Symbol** Tpm1 provided by MGI
- Official Full Name** tropomyosin 1, alpha provided by MGI
- Primary source** MGI:MGI:98809
- See related** Ensembl:ENSMUSG00000032366 AllianceGenome:MGI:98809
- Gene type** protein coding
- RefSeq status** VALIDATED
- Organism** *Mus musculus*
- Lineage** Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
- Also known as** TM2; Tm3; Tmpa; Tpm-1; AA986836; AI854628; alpha-TM; TPM1kappa
- Summary** Enables actin filament binding activity. Involved in cardiac muscle contraction; positive regulation of heart rate by epinephrine; and ventricular cardiac muscle tissue morphogenesis. Acts upstream of or within in utero embryonic development. Located in myofibril. Is expressed in several structures, including central nervous system; gut; heart; limb segment; and musculature. Used to study dilated cardiomyopathy 1Y and hypertrophic cardiomyopathy 3. Human ortholog(s) of this gene implicated in dilated cardiomyopathy 1Y; familial hypertrophic cardiomyopathy; and hypertrophic cardiomyopathy 3. Orthologous to human TPM1 (tropomyosin 1). [provided by Alliance of Genome Resources, Nov 2021]
- Expression** Biased expression in bladder adult (RPKM 766.9), heart adult (RPKM 589.0) and 6 other tissues [See more](#)
- Orthologs** [human](#) [all](#)



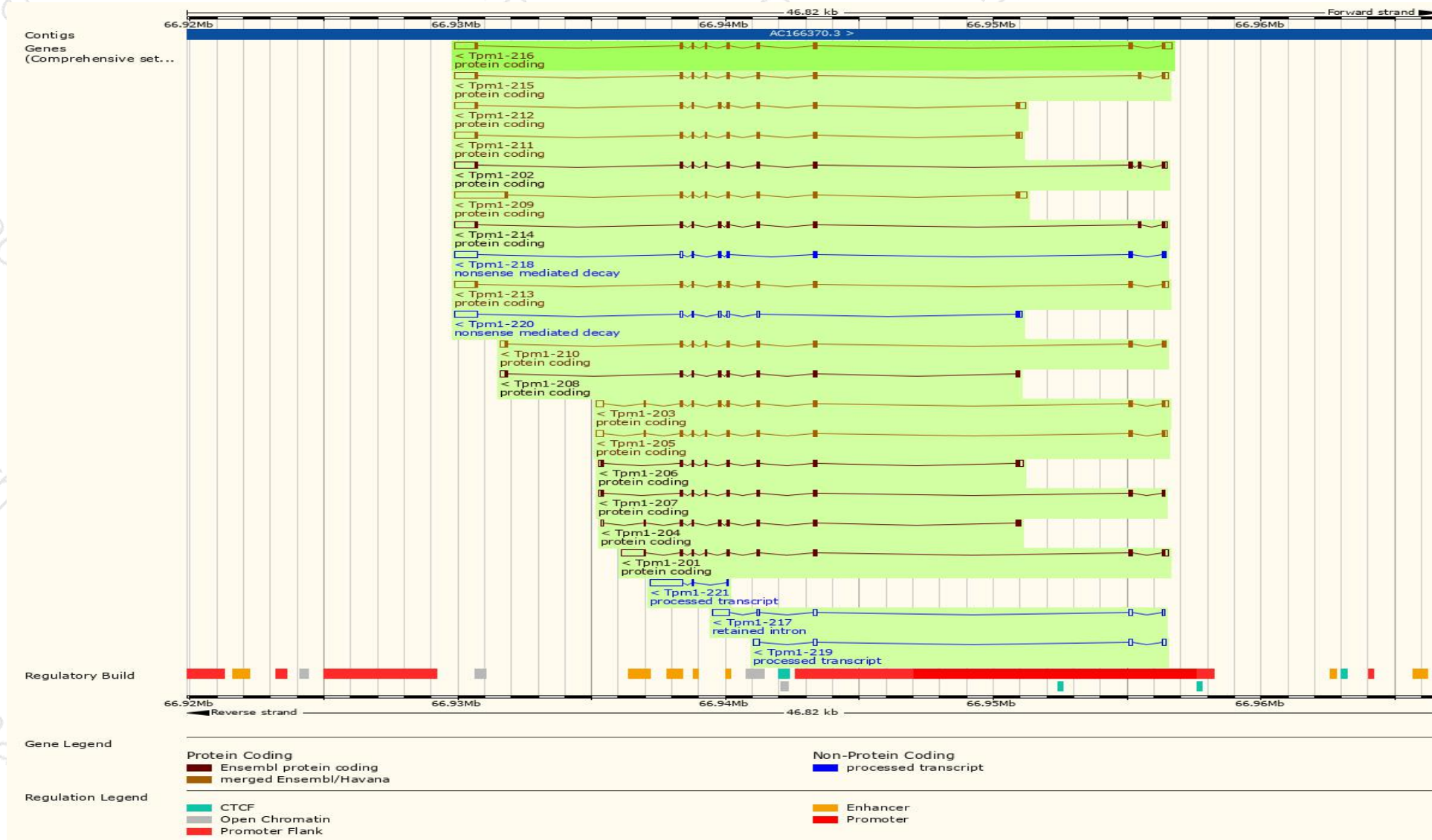
The gene has 21 transcripts, and all transcripts are shown below:

Show/hide columns (1 hidden)		Filter					
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000139046.8	Tpm1-220	1419	49aa	Nonsense mediated decay	-	S4R261	TSL:5
ENSMUST00000129733.8	Tpm1-218	1544	157aa	Nonsense mediated decay	-	S4R2U0	TSL:5
ENSMUST00000113690.8	Tpm1-209	2890	245aa	Protein coding	CCDS52842	G5E8R0	GENCODE basic TSL:3
ENSMUST00000113689.8	Tpm1-208	904	245aa	Protein coding	-	E9Q453	GENCODE basic TSL:5
ENSMUST00000113696.8	Tpm1-212	1714	248aa	Protein coding	CCDS52843	G5E8R2	GENCODE basic TSL:2
ENSMUST00000113695.8	Tpm1-211	1607	248aa	Protein coding	CCDS52841	G5E8R1	GENCODE basic TSL:3
ENSMUST00000113684.8	Tpm1-204	887	248aa	Protein coding	-	E9Q456	GENCODE basic TSL:5
ENSMUST00000113686.8	Tpm1-206	998	251aa	Protein coding	-	E9Q455	GENCODE basic TSL:5
ENSMUST00000113693.8	Tpm1-210	1054	281aa	Protein coding	CCDS52844	E9Q452	GENCODE basic TSL:5
ENSMUST00000113707.9	Tpm1-216	1898	284aa	Protein coding	CCDS23311	P58771-2	GENCODE basic APPRIS P3 TSL:1
ENSMUST00000113705.8	Tpm1-215	1759	284aa	Protein coding	CCDS52848	B7ZNL3	GENCODE basic TSL:1
ENSMUST00000113697.8	Tpm1-213	1742	284aa	Protein coding	CCDS52846	E9Q450	GENCODE basic TSL:2
ENSMUST00000050905.16	Tpm1-203	1259	284aa	Protein coding	CCDS52847	Q8BP43	GENCODE basic APPRIS ALT1 TSL:1
ENSMUST00000113685.10	Tpm1-205	1208	284aa	Protein coding	CCDS52845	P58771-1	GENCODE basic APPRIS ALT1 TSL:1
ENSMUST00000030185.5	Tpm1-201	1797	284aa	Protein coding	-	Q8BSH3	GENCODE basic APPRIS ALT1 TSL:2
ENSMUST00000113701.8	Tpm1-214	1677	284aa	Protein coding	-	E9Q448	GENCODE basic TSL:5
ENSMUST00000113687.8	Tpm1-207	962	287aa	Protein coding	-	E9Q454	GENCODE basic TSL:5
ENSMUST00000034928.12	Tpm1-202	1824	326aa	Protein coding	-	F8WID5	GENCODE basic TSL:5
ENSMUST00000156899.2	Tpm1-221	1274	No protein	Processed transcript	-	-	TSL:1
ENSMUST00000131279.2	Tpm1-219	649	No protein	Processed transcript	-	-	TSL:2
ENSMUST00000129466.8	Tpm1-217	1123	No protein	Retained intron	-	-	TSL:2

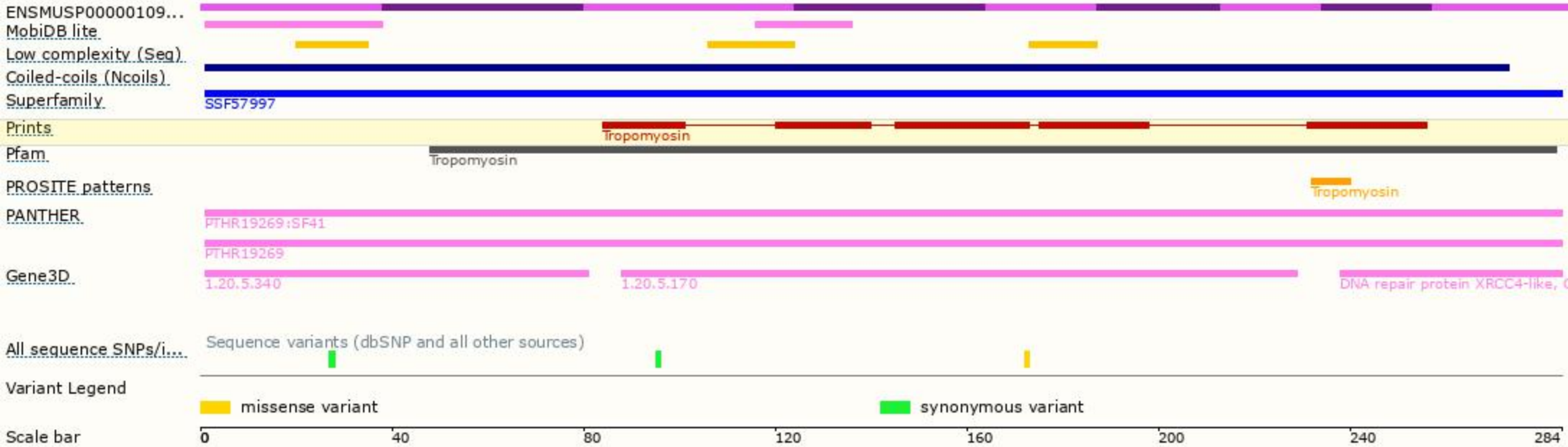
The strategy is based on the design of *Tpm1-216* transcript, the transcription is shown below:



Mouse Genomic location distribution



Mouse Protein domain



If you have any questions, please feel free to contact us.

Tel: 025-5864 1534



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