

Ptk7-P690R cas9-ki(PM) Mouse Model Strategy

-CRISPR/Cas9 technology

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Reviewer: Jia Yu

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Project Overview

Project Name	Ptk7-P690R
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Project type	cas9-ki(PM)
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Strain background	C57BL/6JGpt
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Project cycles	5-8 months
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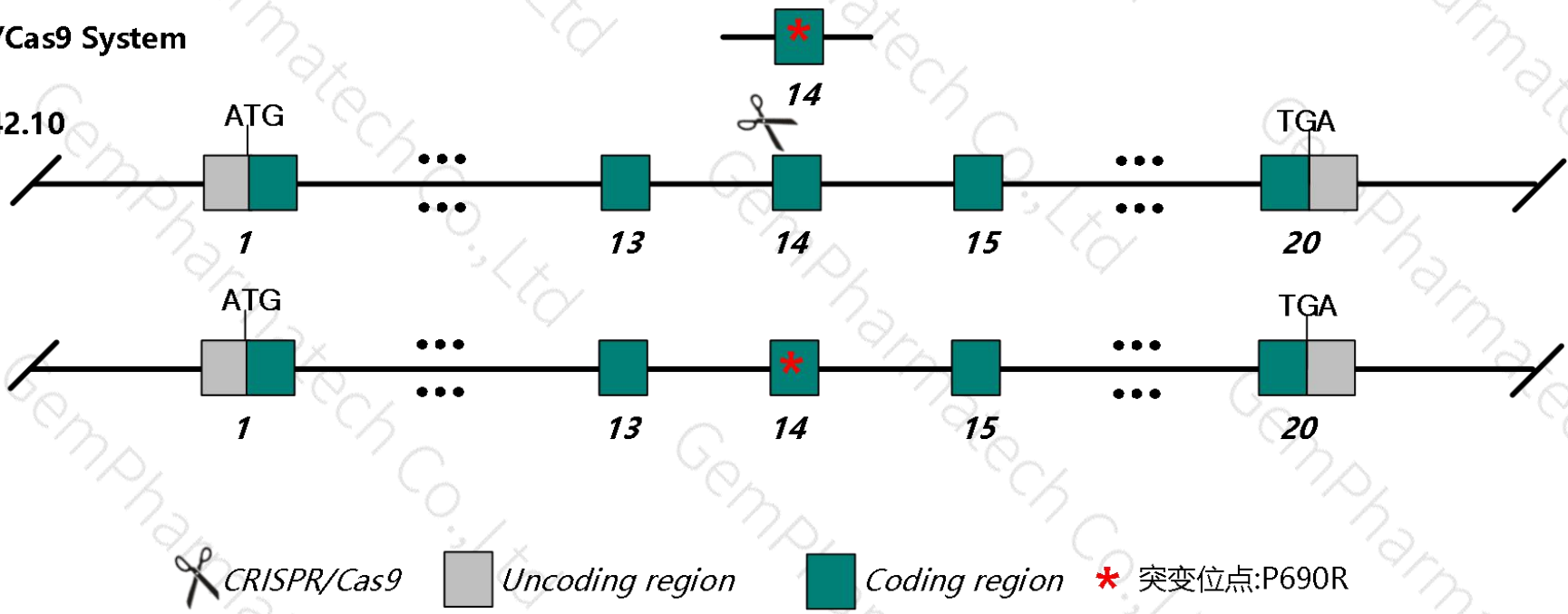
This model uses CRISPR/Cas9 technology to edit the *Ptk7* gene and the schematic diagram is as follow:

Donor and CRISPR/Cas9 System

ENSMUST00000044442.10

Wild-type allele

Targeted allele



Technical Description

- The mouse *Ptk7* gene has 2 transcripts. The human *PTK7* gene has 20 transcripts.
- According to the structure of *Ptk7* gene and requirements of customer, the 698th amino acid(P) of human *PTK7*-202(NM_002821.5) gene corresponds to the 690th amino acid(P) of mouse *Ptk7* gene after comparing homology of mouse *Ptk7* gene and human *PTK7* gene. This project produced *Ptk7*-P690R point mutation on exon14 of the transcript of *Ptk7*-201(ENSMUST00000044442.10, NM_175168.4). The 690th amino acids will be mutated from P to R, and the corresponding codon will be mutated to CGC by the CCA.
- The mouse *Ptk7*-201 transcript contains 20 exons. The translation initiation site ATG is located at exon1, and the translation termination site TGA is located at exon20, encoding 1062aa.
- In this project, *Ptk7* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: In vitro, CRISPR/Cas9 system and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice. Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

A comparison of the aa homology of human and mouse *Ptk7* gene

	698	700	710	720	730	740	750	760	770
Human PTK7-202 protein	698	PYKMIQTIGLSVGAAVAYII IAVLGLMFYCKKRCKAKRLQKQPEGEEPEMECLNGGPLQNGQPSAEIQEEVALTSLGS							
Mouse Ptk7-201 protein	690	PYKMIQTIGLSVGAAVAYII IAVLGLMFYCKKRCKAKRLQKQPEGEEPEMECLNGGPLQNGQPSAEIQEEVALTSLGS							

The 698th amino acid(P) of human *PTK7* gene corresponds to the 690th amino acid(P) of mouse *Ptk7* gene after comparing homology of mouse *Ptk7* gene and human *PTK7* gene.

Mutation Site

Before mutation

+2	K P V M E D S E G P G S P P P Y K M I Q T I G																			
57001	CTGTCCTGAC	CTCTCCGTGT	GTCTCTTAGA	CAAGCCAGTG	ATGGAAGACT	CGGAGGGGCC	AGGCAGCCCT	CCCCCATACA	AGATGATCCA	GACCATCGGG										
	GACAGGACTG	GAGAGGCACA	CAGAGAATCT	GTTCGGTCAC	TACCTTCTGA	GCCTCCCGGG	TCCGTCGGGA	GGGGGTATGT	TCTACTAGGT	CTGGTAGCCC										
+2	L S V G A A V A Y I I A V L G L M F Y C K K R C K A K R L Q K Q P E ?																			
57101	CTGTCGGTGG	GCGCAGCGGT	AGCCTACATC	ATCGCGGTCC	TGGGCCTCAT	GTTCTACTGC	AAGAAGCGGT	GTAAAGCCAA	GCGGCTGCAG	AAACAGCCTG										
	GACAGCCACC	CGCGTCGCCA	TCGGATGTAG	TAGCGCCAGG	ACCCGGAGTA	CAAGATGACG	TTCTTCGCCA	CATTTCGGTT	CGCCGACGTC	TTTGTCGGAC										
+2	? E G E E P E M E C L N																			
57201	AGGGGGAGGA	GCCAGAGATG	GAGTGCCTCA	ATGTTGGGTC	CCCCTGTAGC	GGGAGGTGGA	CCGGTCTGGG	TACAGAGCAC	CCTCCCGTGC	CAGCTGTGGA										
	TCCCCCTCCT	CGGTCTCTAC	CTCACGGAGT	TACCACCCAG	GGGGACATCG	CCCTCCACCT	GGCCAGACCC	ATGTCTCGTG	GGAGGGCACG	GTCGACACCT										

After mutation

+2	K P V M E D S E G P G S P P R Y K M I Q T I G																			
57001	CTGTCCTGAC	CTCTCCGTGT	GTCTCTTAGA	CAAGCCAGTG	ATGGAAGACT	CGGAGGGGCC	AGGCAGCCCT	CCCCCGTACA	AGATGATCCA	GACCATCGGG										
	GACAGGACTG	GAGAGGCACA	CAGAGAATCT	GTTCGGTCAC	TACCTTCTGA	GCCTCCCGGG	TCCGTCGGGA	GGGGCGATGT	TCTACTAGGT	CTGGTAGCCC										
+2	L S V G A A V A Y I I A V L G L M F Y C K K R C K A K R L Q K Q P E ?																			
57101	CTGTCGGTGG	GCGCAGCGGT	AGCCTACATC	ATCGCGGTCC	TGGGCCTCAT	GTTCTACTGC	AAGAAGCGGT	GTAAAGCCAA	GCGGCTGCAG	AAACAGCCTG										
	GACAGCCACC	CGCGTCGCCA	TCGGATGTAG	TAGCGCCAGG	ACCCGGAGTA	CAAGATGACG	TTCTTCGCCA	CATTTCGGTT	CGCCGACGTC	TTTGTCGGAC										
+2	? E G E E P E M E C L N																			
57201	AGGGGGAGGA	GCCAGAGATG	GAGTGCCTCA	ATGTTGGGTC	CCCCTGTAGC	GGGAGGTGGA	CCGGTCTGGG	TACAGAGCAC	CCTCCCGTGC	CAGCTGTGGA										
	TCCCCCTCCT	CGGTCTCTAC	CTCACGGAGT	TACCACCCAG	GGGGACATCG	CCCTCCACCT	GGCCAGACCC	ATGTCTCGTG	GGAGGGCACG	GTCGACACCT										

The yellow region is exon14 of *Ptk7-201*, the red region represents the mutation site.

- According to the data of MGI, Mice homozygous for a gene trapped allele die perinatally with defects in neural tube closure and planar cell polarity in the ear. ENU-induced mutant mice show omphalocele, impaired neural tube, heart and lung development, rib defects, polydactyly, failed eyelid closure and altered cell polarity.
- One or Two synonymous mutations of amino acids will be introduced on exon14 of *Ptk7*.
- Mouse *Ptk7* gene is located on Chr17. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr17, 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.

Gene name and location (NCBI)

Ptk7 PTK7 protein tyrosine kinase 7 [*Mus musculus* (house mouse)]

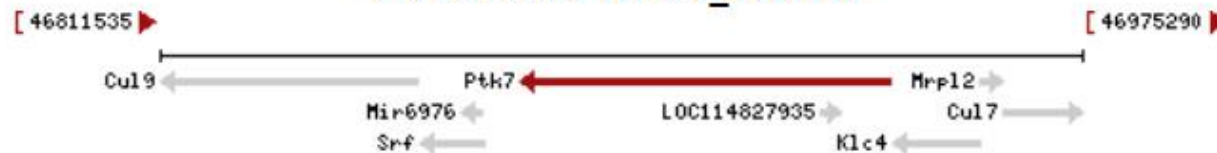
[Download Datasets](#)

Gene ID: 71461, updated on 23-Jun-2021

Summary

Official Symbol	Ptk7 provided by MGI
Official Full Name	PTK7 protein tyrosine kinase 7 provided by MGI
Primary source	MGI:MGI:1918711
See related	Ensembl:ENSMUSG00000023972
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	chz; mPTK7/CCK; mPTK7/CCK4; 8430404F20Rik
Expression	Broad expression in limb E14.5 (RPKM 30.4), ovary adult (RPKM 29.7) and 22 other tissues See more
Orthologs	human all

Chromosome 17 - NC_000083.7

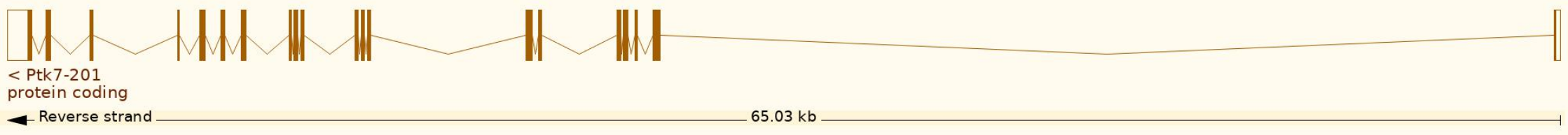


Transcript information (Ensembl)

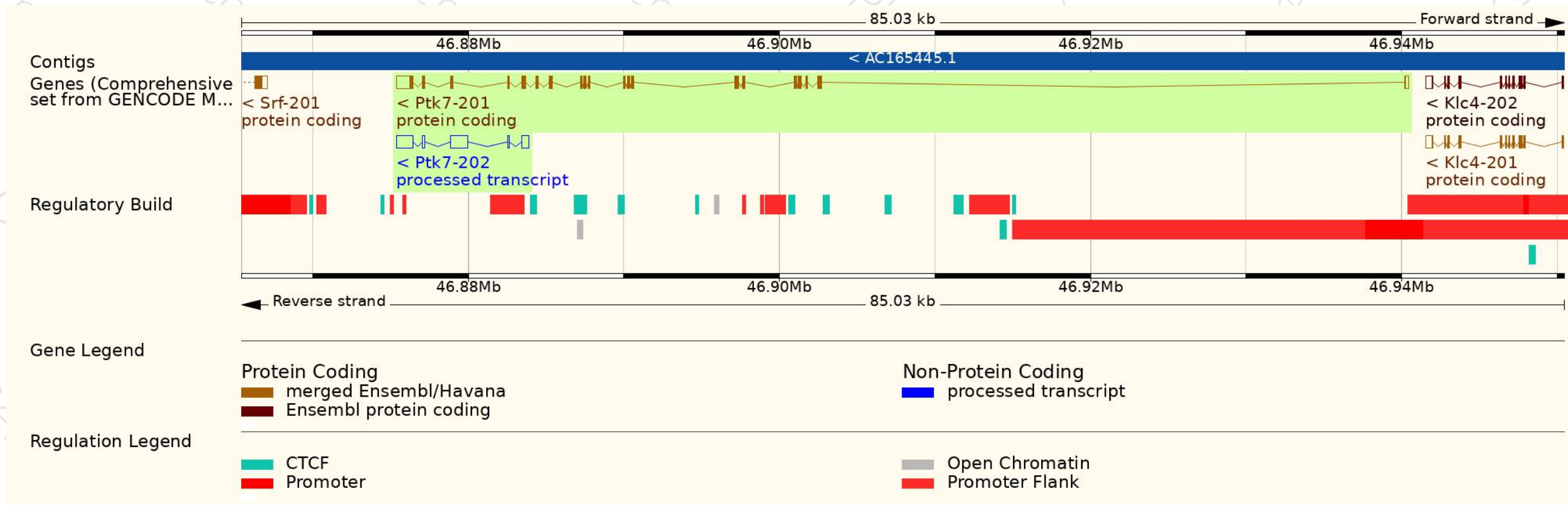
The gene has 2 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Ptk7-201	ENSMUST00000044442.10	4235	1062aa	Protein coding	CCDS37637	Q8BKG3	GENCODE basic APPRIS P1 TSL:1
Ptk7-202	ENSMUST00000232855.2	2819	No protein	Processed transcript	-	-	-

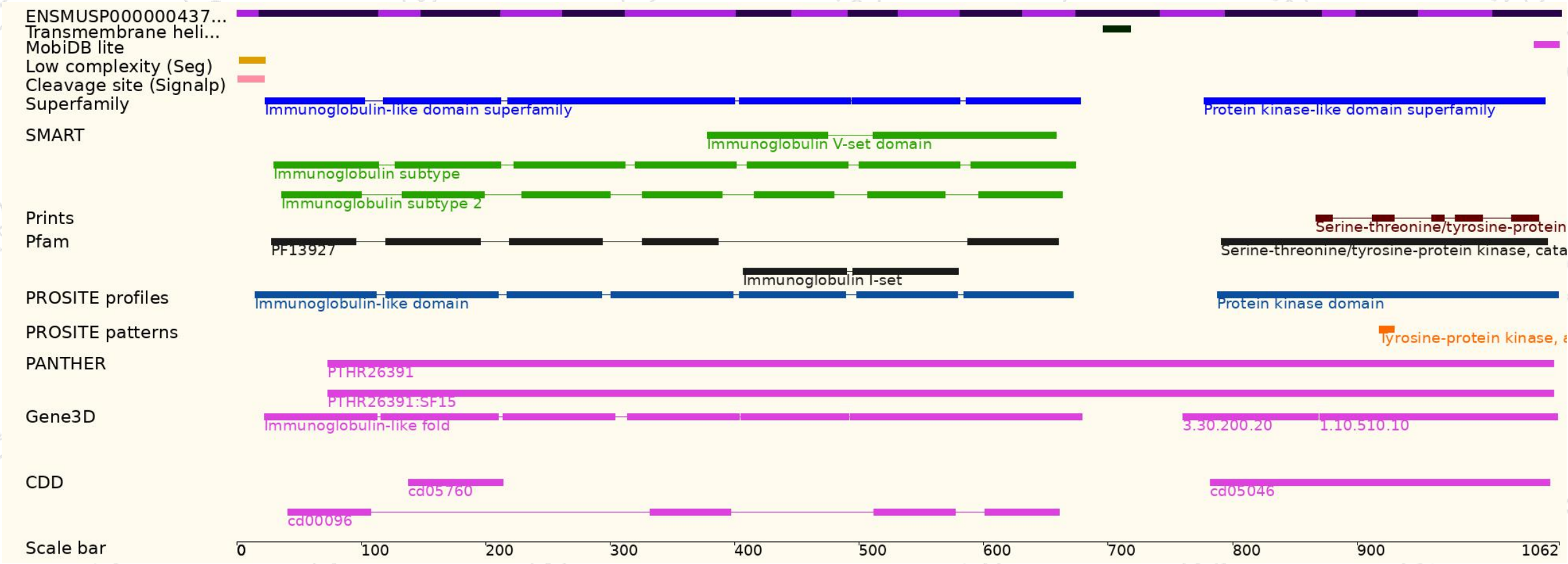
The strategy is based on the design of *Ptk7-201* transcript, the transcription is shown below:



Genomic location distribution



Protein domain

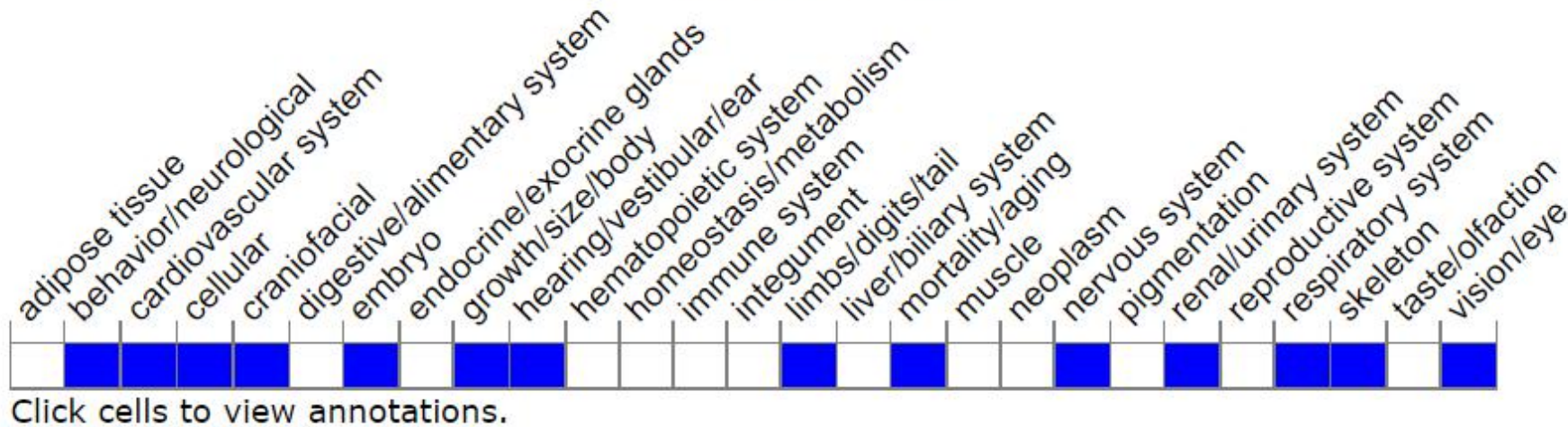


Mouse phenotype description(MGI)

URL link is as follows:

<http://www.informatics.jax.org/marker/MGI:1918711>

Phenotype Overview ?



Mice homozygous for a gene trapped allele die perinatally with defects in neural tube closure and planar cell polarity in the ear. ENU-induced mutant mice show omphalocele, impaired neural tube, heart and lung development, rib defects, polydactyly, failed eyelid closure and altered cell polarity.

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

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