Pus7-D293A cas9-ki(PM) Mouse Model Strategy-CRISPR/Cas9 technology

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Reviewer: Jia Yu
Design Date: 2021-3-22

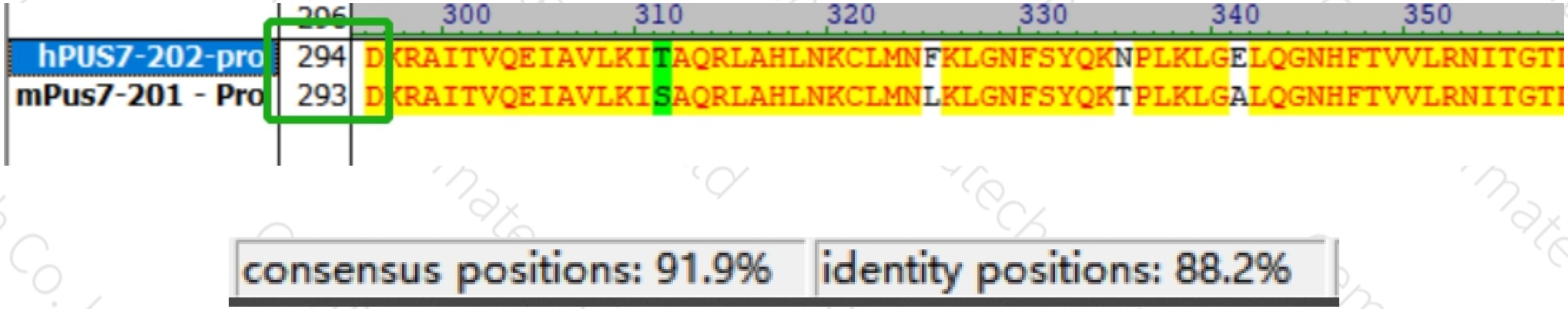
Project Overview

Project Name	**Pus7-D293A**
Project type	**cas9-ki(PM)**
Strain background	**C57BL/6JGpt**

Technical Description

- The mouse *Pus7* gene has 9 transcripts.The human *PUS7* gene has 6 transcripts.
- According to the structure of *Pus7* gene and requirements of customer, the 294th amino acid(D) of human *PUS7* gene corresponds to the 293th amino acid(D) of mouse *Pus7* gene after comparing homology of mouse *Pus7* gene and human *PUS7* gene.This project produced *Pus7*-D293A point mutation on exon7 of the transcript of *Pus7*-201(ENSMUST00000119946.8,NM_178403.5 → NP_848490.2, 660aa). The 293th amino acids will be mutated from D to A, and the corresponding codon will be mutated to GCC by the GAC.
- The mouse *Pus7*-201 transcript contains 16 exons. The translation initiation site ATG is located at exon2, and the translation termination site TGA is located at exon16, encoding 660aa.
- In this project, *Pus7* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: In vitro, sgRNA and donor vectors were constructed. Cas9, sgRNA and donor were injected into the fertilized eggs of C57BL/6JGpt mice for homologous recombination, and obtained positive F0 mice identified by PCR and sequencing analysis. The stable inheritable positive F1 mice model was obtained by mating F0 mice with C57BL/6JGpt mice.

A comparison of the aa homology of human and mouse *Pus7* gene



The 294th amino acid(D) of human *PUS7* gene corresponds to the 293th amino acid(D) of mouse *Pus7* gene after comparing homology of mouse *Pus7* gene and human *PUS7* gene.

Targeted Mutation Site

集萃药康 GemPharmatech

Before mutation

```
      +1                  V  K  P  N  I  F  S  Y  M  G  T  K  D  K  R  A  I  T  V  Q  E  I  A  V  L
43401 GTGTCTCATC AGGGTAAAGC CAAACATATT CTCCTATATG GGAACCAAAG ACAAAAGGGC AATCACAGTC CAGGAGATTG CTGTTCTCAA GTAAGTGGAG
      CACAGAGTAG TCCCATTTCG GTTTGTATAA GAGGATATAC CCTTGGTTTC TGTTTTCCCG TTAGTGTCAG GTCCTCTAAC GACAAGAGTT CATTCACCTC

43501 TGCCCGCCCA GACTGACAGT GCACAGCCTC AGCTCTGCTT TAACGAACTT ACCGCTTTCA GCTTTGCATG GTCTCTCTGC CTTTTAAAAT CTTGTAGACG
      ACGGGCGGGT CTGACTGTCA CGTGTCGGAG TCGAGACGAA ATTGCTTGAA TGGCGAAAGT CGAAACGTAC CAGAGAGACG GAAAATTTTA GAACATCTGC
```

After mutation

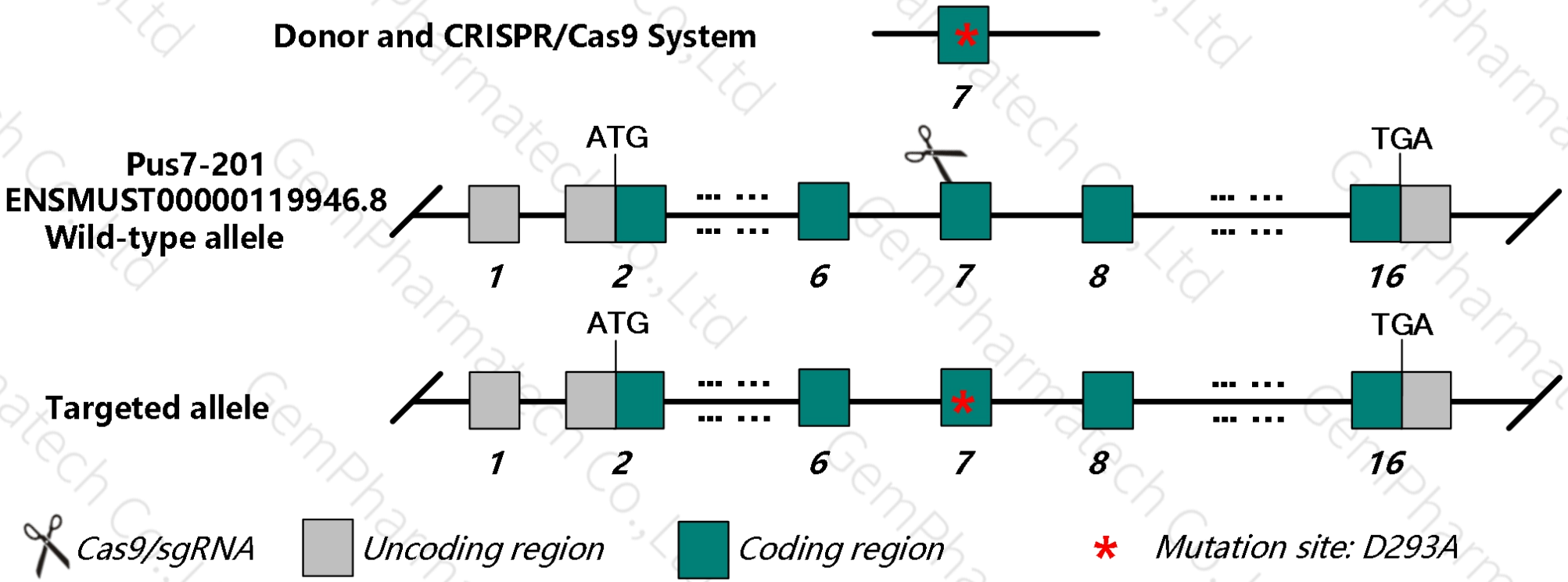
```
      +1                  V  K  P  N  I  F  S  Y  M  G  T  K  A  K  R  A  I  T  V  Q  E  I  A  V  L
43401 GTGTCTCATC AGGGTAAAGC CAAACATATT CTCCTATATG GGAACCAAAG CCAAAAGGGC AATCACAGTC CAGGAGATTG CTGTTCTCAA GTAAGTGGAG
      CACAGAGTAG TCCCATTTCG GTTTGTATAA GAGGATATAC CCTTGGTTTC GGTTTTCCCG TTAGTGTCAG GTCCTCTAAC GACAAGAGTT CATTCACCTC

43501 TGCCCGCCCA GACTGACAGT GCACAGCCTC AGCTCTGCTT TAACGAACTT ACCGCTTTCA GCTTTGCATG GTCTCTCTGC CTTTTAAAAT CTTGTAGACG
      ACGGGCGGGT CTGACTGTCA CGTGTCGGAG TCGAGACGAA ATTGCTTGAA TGGCGAAAGT CGAAACGTAC CAGAGAGACG GAAAATTTTA GAACATCTGC
```

The green region is exon7 of *Pus7-201,* the yellow region represents the mutation site.

Strategy

This model uses CRISPR/Cas9 technology to edit the *Pus7* gene and the schematic diagram is as follow:



Notice

- One or Two synonymous mutations of amino acids will be intronduced on exon7 of *Pus7*.
- Mouse *Pus7* gene is located on Chr5. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr5, 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)

Pus7 pseudouridylate synthase 7 [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 78697, updated on 17-Dec-2020

Summary

Official Symbol	Pus7 provided by MGI
Official Full Name	pseudouridylate synthase 7 provided by MGI
Primary source	MGI:MGI:1925947
See related	Ensembl:ENSMUSG00000057541
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	C330017I15Rik
Expression	Ubiquitous expression in bladder adult (RPKM 4.8), CNS E11.5 (RPKM 4.6) and 26 other tissues See more
Orthologs	human all

NEW **Try the new Gene table**

Try the new Transcript table

Genomic context

Location: 5; 5 A3

See Pus7 in Genome Data Viewer

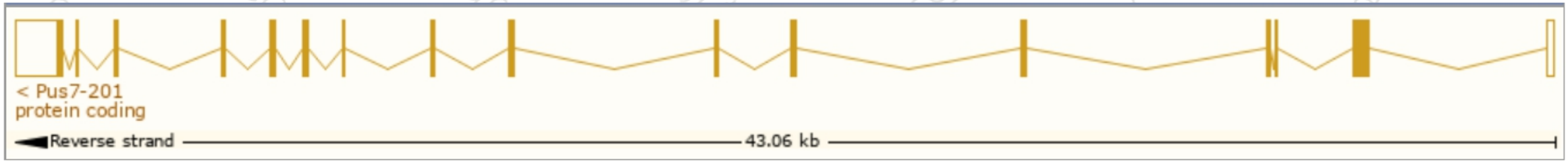
Exon count: 19

Transcript information （Ensembl）

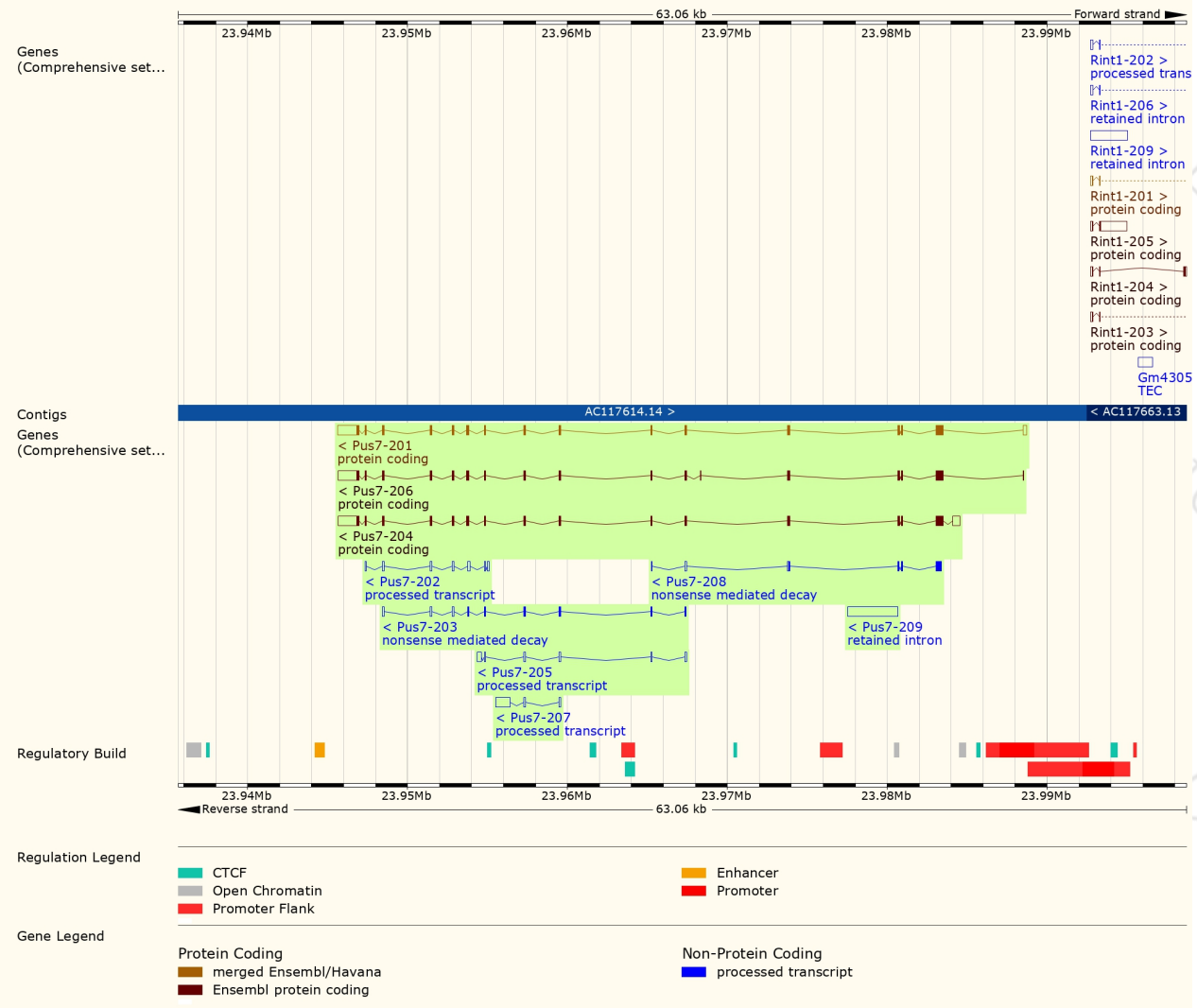
The gene has 9 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Pus7-204	ENSMUST00000131992.8	3645	660aa	Protein coding	CCDS51431	Q91VU7-1	TSL:1 GENCODE basic APPRIS P3
Pus7-201	ENSMUST00000119946.8	3407	660aa	Protein coding	CCDS51431	Q91VU7-1	TSL:1 GENCODE basic APPRIS P3
Pus7-206	ENSMUST00000148618.8	3266	666aa	Protein coding	CCDS80219	Q91VU7-2	TSL:1 GENCODE basic APPRIS ALT2
Pus7-203	ENSMUST00000131404.2	888	185aa	Nonsense mediated decay	-	F7CG42	CDS 5' incomplete TSL:5
Pus7-208	ENSMUST00000151449.2	750	176aa	Nonsense mediated decay	-	F6XMS4	CDS 5' incomplete TSL:3
Pus7-207	ENSMUST00000148921.2	1114	No protein	Processed transcript	-	-	TSL:3
Pus7-202	ENSMUST00000129848.8	773	No protein	Processed transcript	-	-	TSL:3
Pus7-205	ENSMUST00000147922.8	721	No protein	Processed transcript	-	-	TSL:3
Pus7-209	ENSMUST00000195909.2	3118	No protein	Retained intron	-	-	TSL:NA

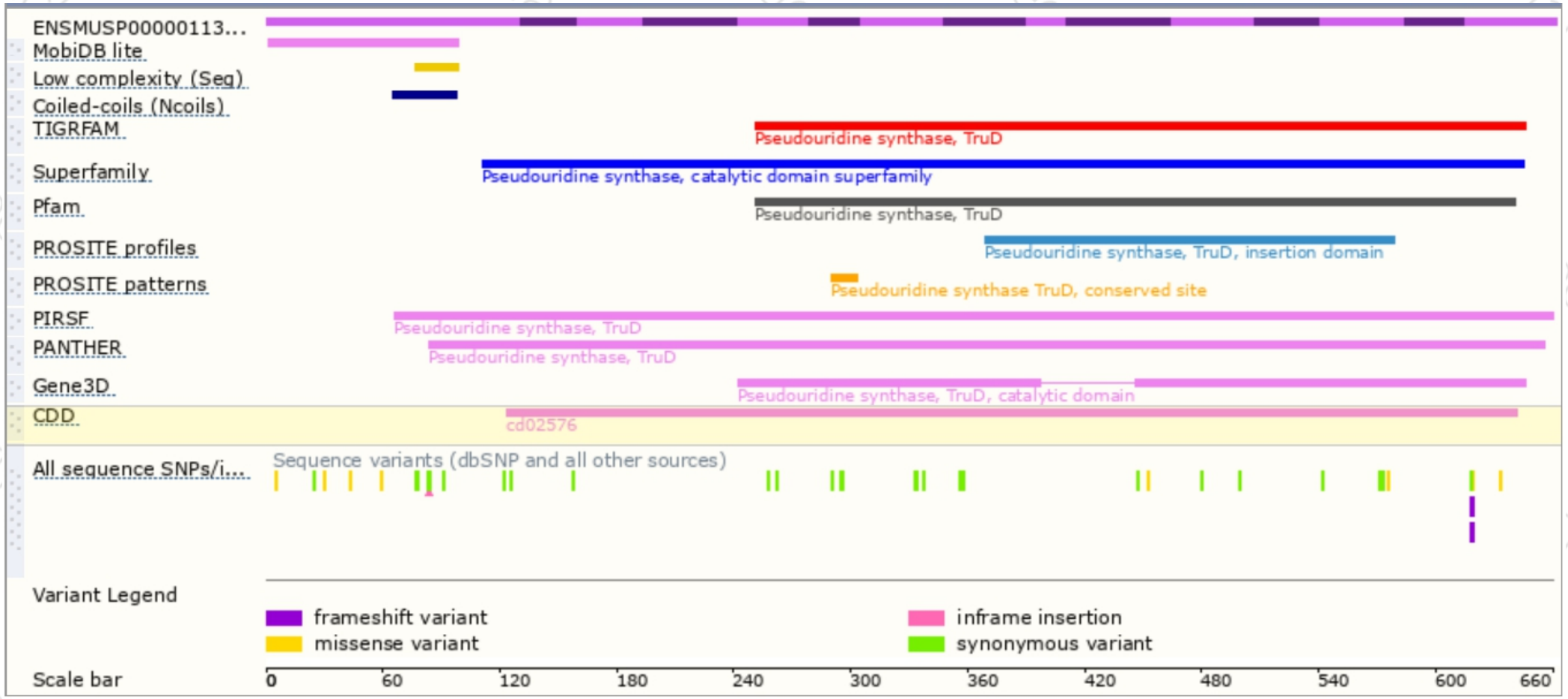
The strategy is based on the design of *Pus7*-201 transcript, the transcription is shown below:



Genomic location distribution

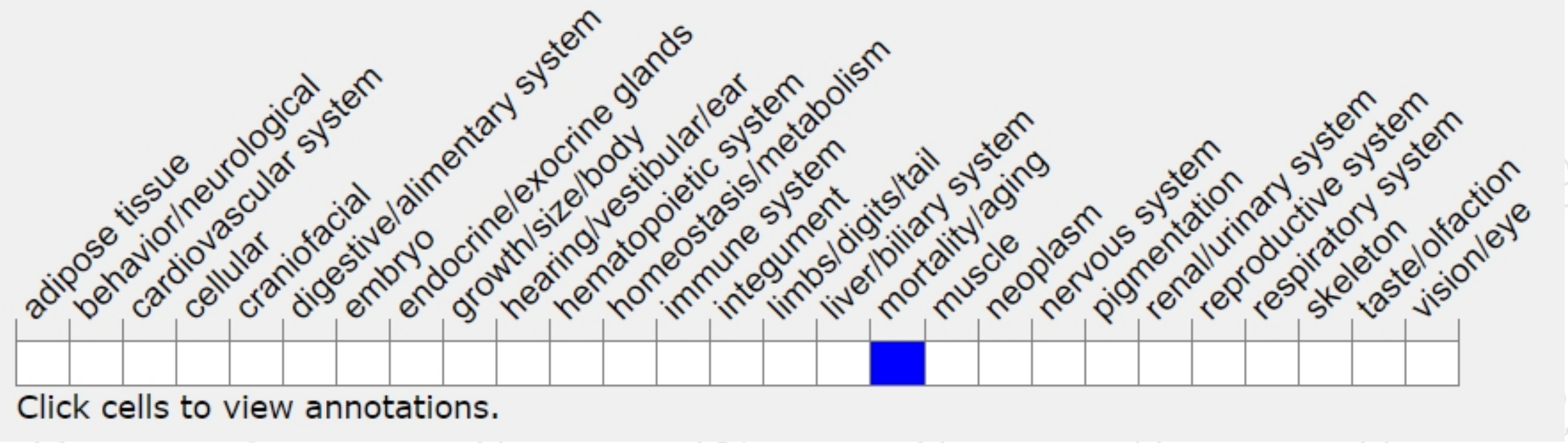


Protein domain



Mouse phenotype description(MGI)

URL link is as follows:
http://www.informatics.jax.org/marker/MGI:1925947



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