Tsc1-c.2500-2503 delAACA Mouse Model Strategy

-CRISPR/Cas9 technology

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Reviewer: Yanhua Shen

Design Date: 2020-11-12

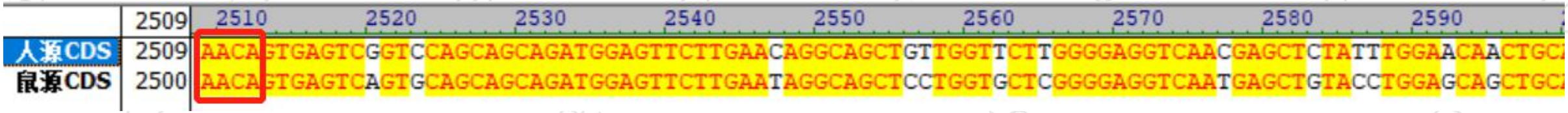
Project Overview

Project Name	**Tsc1-c.2500-2503 delAACA**
Project type	**Cas9-ki(PM)**
Strain background	**C57BL/6JGpt**

Technical Description

- The mouse *Tsc1* gene has 11 transcripts.The human *TSC1* gene has 47 transcripts.
- According to the structure of *Tsc1* gene and requirements of customer, the 2509-2512th base(AACA) of human *TSC1* gene corresponds to the 2500-2503th base(AACA) of mouse *Tsc1* gene after comparing homology of mouse *Tsc1* gene and human *TSC1* gene.This project produced *Tsc1*-c.2500-2503 delAACA point mutation on exon20 of the transcript of *Tsc1*-203(ENSMUST00000113869.7).
- The mouse *Tsc1*-203 transcript contains 23 exons. The translation initiation site ATG is located at exon3, and the translation termination site TGA is located at exon23, encoding 1161aa.
- In this project, *Tsc1* 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 *Tsc1* gene



The 2509-2512th base(AACA) of human *TSC1* gene corresponds to the 2500-2503th base(AACA) of mouse *Tsc1* gene after comparing homology of mouse *Tsc1* gene and human *TSC1* gene.

Mutation Site

集萃药康
GemPharmatech

Before mutation

```
+1                                                                                                  L  S  N  S  E  S?
60601  ACTGTGCTCC CCAGTAGTTA CTGTTGCTGT CTGTTGCCTA GTTTTGCCAT GCTGAACTTG CACTTTCTCA CTTCCATCTG GCAGCTCTCC AACAGTGAGT
       TGACACGAGG GGTCATCAAT GACAACGACA GACAACGGAT CAAAACGGTA CGACTTGAAC GTGAAAGAGT GAAGGTAGAC CGTCGAGAGG TTGTCACTCA
+1     ?S V  Q  Q  Q  M  E  F  L  N  R  Q  L  L  V  L  G  E  V  N  E  L  Y  L  E  Q  L  Q  S  K  H  P  D  T?
60701  CAGTGCAGCA GCAGATGGAG TTCTTGAATA GGCAGCTCCT GGTGCTCGGG GAGGTCAATG AGCTGTACCT GGAGCAGCTG CAGAGCAAGC ATCCTGACAC
       GTCACGTCGT CGTCTACCTC AAGAACTTAT CCGTCGAGGA CCACGAGCCC CTCCAGTTAC TCGACATGGA CCTCGTCGAC GTCTCGTTCG TAGGACTGTG
+1     ?T T  K
60801  CACCAAGGTA AGTGGCCCGG GCACTGAGAC ACAAAACTGC ACTGCCCGGC CCCGTGTTCT GCAGAGCAGC CAGGCCCAGC CACATTGCAG TGTCCAGGCA
       GTGGTTCCAT TCACCGGGCC CGTGACTCTG TGTTTTGACG TGACGGGCCG GGGCACAAGA CGTCTCGTCG GTCCGGGTCG GTGTAACGTC ACAGGTCCGT
```

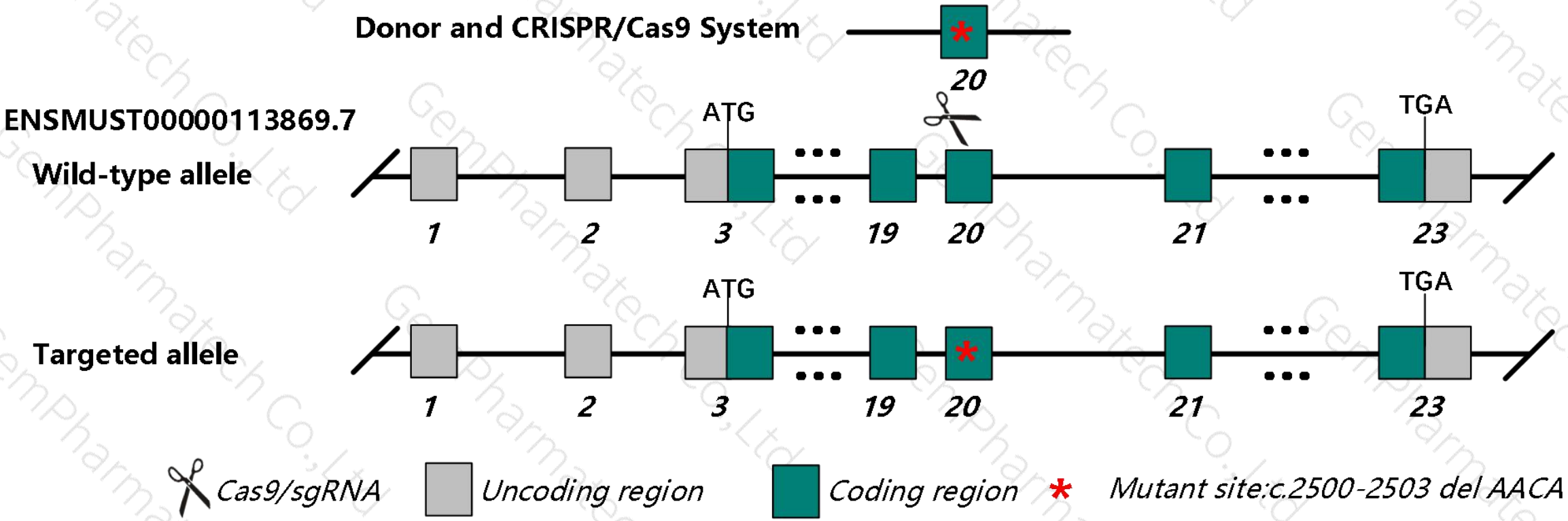
After mutation

```
+1                                                                                                  L  S  V  S  Q  C?
60601  ACTGTGCTCC CCAGTAGTTA CTGTTGCTGT CTGTTGCCTA GTTTTGCCAT GCTGAACTTG CACTTTCTCA CTTCCATCTG GCAGCTCTCC GTGAGTCAGT
       TGACACGAGG GGTCATCAAT GACAACGACA GACAACGGAT CAAAACGGTA CGACTTGAAC GTGAAAGAGT GAAGGTAGAC CGTCGAGAGG CACTCAGTCA
+1     ?C S  S  R  W  S  S  '  I  G  S  S  W  C  S  G  R  S  M  S  C  T  W  S  S  C  R  A  S  I  L  T  P  P?
60701  GCAGCAGCAG ATGGAGTTCT TGAATAGGCA GCTCCTGGTG CTCGGGGAGG TCAATGAGCT GTACCTGGAG CAGCTGCAGA GCAAGCATCC TGACACCACC
       CGTCGTCGTC TACCTCAAGA ACTTATCCGT CGAGGACCAC GAGCCCCTCC AGTTACTCGA CATGGACCTC GTCGACGTCT CGTTCGTAGG ACTGTGGTGG
+1     ?P
60801  AAGGTAAGTG GCCCGGGCAC TGAGACACAA AACTGCACTG CCCGGCCCCG TGTTCTGCAG AGCAGCCAGG CCCAGCCACA TTGCAGTGTC CAGGCACAGG
       TTCCATTCAC CGGGCCCGTG ACTCTGTGTT TTGACGTGAC GGGCCGGGGC ACAAGACGTC TCGTCGGTCC GGGTCGGTGT AACGTCACAG GTCCGTGTCC
```

The blue region is exon20 of *Tsc1-203*, the yellow region represents the mutation site.

Strategy

This model uses CRISPR/Cas9 technology to edit the *Tsc1* gene and the schematic diagram is as follow:



Notice

- According to the data of MGI,homozygous null mutants show liver hypoplasia, open neural tube and die by embryonic day 10.5-11.5. Heterozygotes develop kidney cystadenomas and liver hemangiomas. Conditional astrocyte-specific nulls show increased astrocyte numbers and seizures.This model may have the same phenotype.

- One or Two synonymous mutations of amino acids will be intronduced on exon20 of *Tsc1*.

- Mouse *Tsc1* gene is located on Chr2. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr2, 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)

集萃药康 GemPharmatech

Tsc1 TSC complex subunit 1 [*Mus musculus* (house mouse)]

Gene ID: 64930, updated on 8-Dec-2020

Summary

Official Symbol	Tsc1 provided by MGI
Official Full Name	TSC complex subunit 1 provided by MGI
Primary source	MGI:MGI:1929183
See related	Ensembl:ENSMUSG00000026812
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	ham
Expression	Ubiquitous expression in CNS E18 (RPKM 10.9), whole brain E14.5 (RPKM 9.8) and 28 other tissues See more
Orthologs	human all

Genomic context

Location: 2; 2 A3 — See Tsc1 in Genome Data Viewer

Exon count: 25

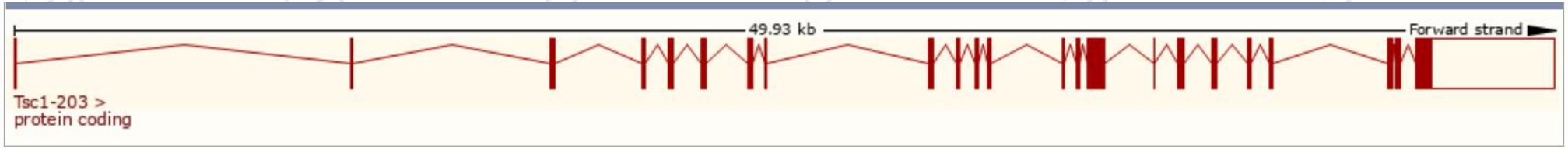
Annotation release	Status	Assembly	Chr	Location
109	current	GRCm39 (GCF_000001635.27)	2	NC_000068.8 (28531005..28581184)
108.20200622	previous assembly	GRCm38.p6 (GCF_000001635.26)	2	NC_000068.7 (28640993..28691172)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	2	NC_000068.6 (28496763..28546687)

Transcript information （Ensembl）

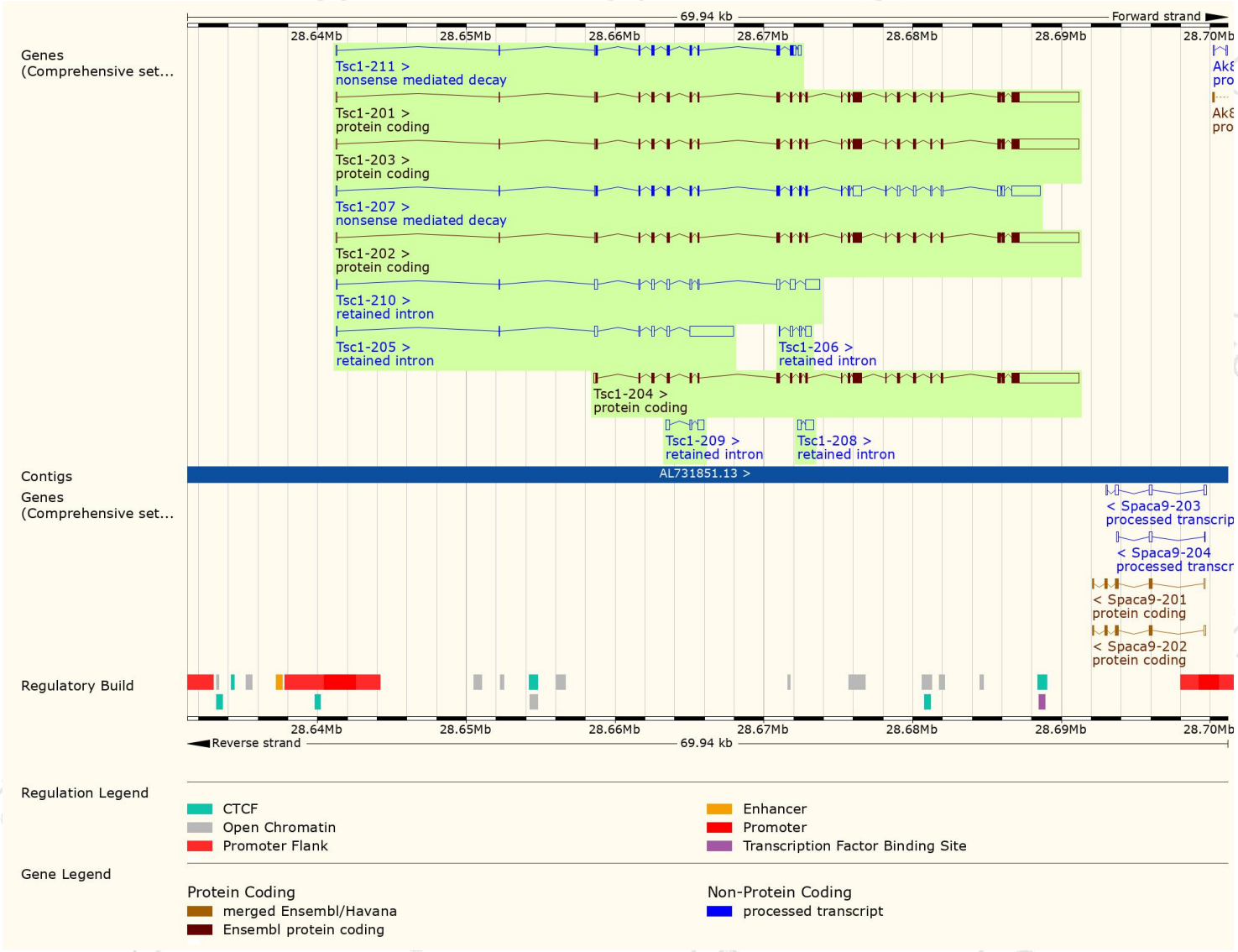
The gene has 11 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt ▲	Flags
Tsc1-211	ENSMUST00000156857.7	1711	368aa	Nonsense mediated decay	-	F2Z3W0	TSL:5
Tsc1-207	ENSMUST00000133565.7	5103	459aa	Nonsense mediated decay	-	F2Z3X2	TSL:5
Tsc1-203	ENSMUST00000113869.7	7702	1161aa	Protein coding	CCDS79762	Q9EP53	TSL:1 GENCODE basic APPRIS ALT2
Tsc1-202	ENSMUST00000113867.8	7682	1155aa	Protein coding	CCDS71013	Q9EP53	TSL:1 GENCODE basic APPRIS ALT2
Tsc1-201	ENSMUST00000028155.11	7674	1160aa	Protein coding	CCDS15844	Q9EP53	TSL:1 GENCODE basic APPRIS P3
Tsc1-204	ENSMUST00000113870.2	7595	1160aa	Protein coding	CCDS15844	Q9EP53	TSL:1 GENCODE basic APPRIS P3
Tsc1-205	ENSMUST00000124507.1	3640	No protein	Retained intron	-	-	TSL:1
Tsc1-210	ENSMUST00000153625.7	2388	No protein	Retained intron	-	-	TSL:1
Tsc1-209	ENSMUST00000150274.1	818	No protein	Retained intron	-	-	TSL:3
Tsc1-208	ENSMUST00000139642.1	772	No protein	Retained intron	-	-	TSL:2
Tsc1-206	ENSMUST00000125715.1	703	No protein	Retained intron	-	-	TSL:3

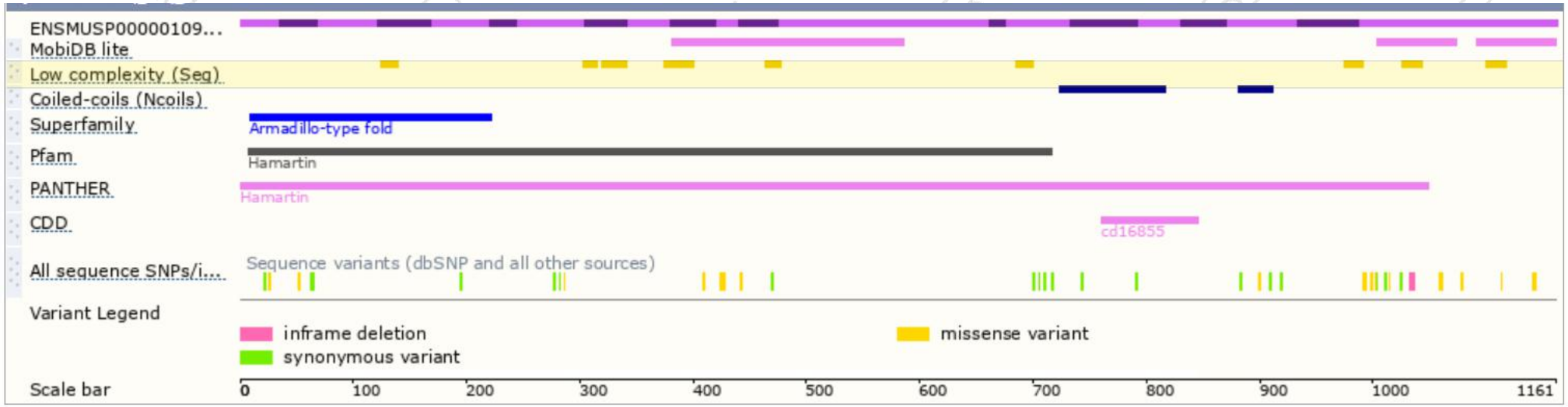
The strategy is based on the design of *Tsc1*-203 transcript, the transcription is shown below:



Genomic location distribution

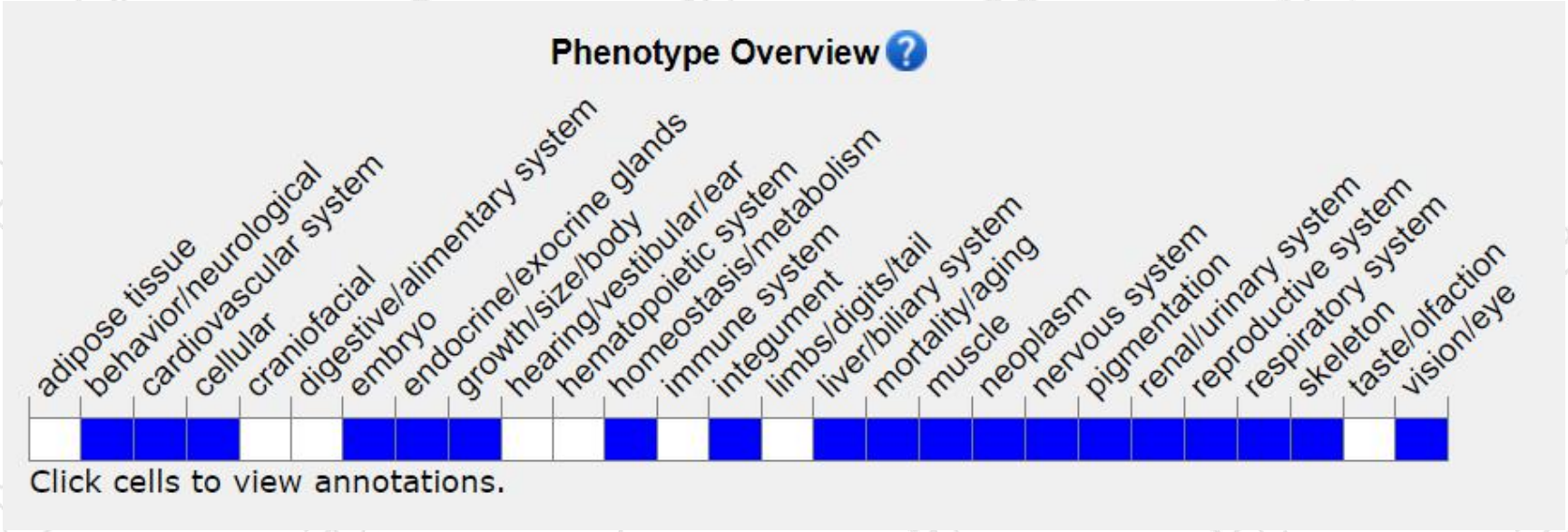


Protein domain



Mouse phenotype description(MGI)

URL link is as follows:
http://www.informatics.jax.org/marker/MGI:1929183



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