Tsc2-c.1113 delA Mouse Model Strategy

-CRISPR/Cas9 technology

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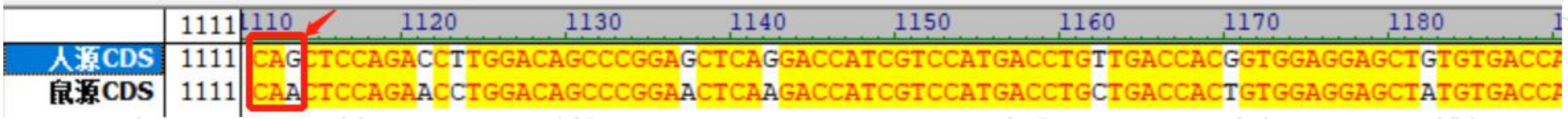
Project Overview

Project Name	**Tsc2-c.1113 delA**
Project type	**Cas9-ki(PM)**
Strain background	**C57BL/6JGpt**

Technical Description

- The mouse *Tsc2* gene has 22 transcripts.The human *TSC2* gene has 69 transcripts.
- According to the structure of *Tsc2* gene and requirements of customer, the c.1113 G of human *TSC2* gene corresponds to the c.1113 A of mouse *Tsc2* gene after comparing homology of mouse *Tsc2* gene and human *TSC2* gene.This project produced *Tsc2*-c.1113 delA point mutation on exon11 of the transcript of *Tsc2*-201(ENSMUST00000097373.1).
- The mouse *Tsc2*-201 transcript contains 40 exons. The translation initiation site ATG is located at exon2, and the translation termination site TGA is located at exon40, encoding 1742aa.
- In this project, *Tsc2* 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 *Tsc2* gene



The c.1113 G of human *TSC2* gene corresponds to the c.1113 A of mouse *Tsc2* gene after comparing homology of mouse *Tsc2* gene and human *TSC2* gene.

Mutation Site

集萃药康 GemPharmatech

Before mutation

```
 +2                                                                                                A  M  T  C  P  N  E  V  V  S  Y  E  I?
29901  AGGAGACAGG ATGCTCTCAT CCTCTATGAG GCATAACACT GCCCCATGGT ACTGTGTTTC AGGCTATGAC CTGTCCCAAT GAGGTGGTGT CATATGAGAT
       TCCTCTGTCC TACGAGAGTA GGAGATACTC CGTATTGTGA CGGGGTACCA TGACACAAAG TCCGATACTG GACAGGGTTA CTCCACCACA GTATACTCTA

 +2  ?I  V  L  S  I  T  R  L  I  K  K  Y  R  K  E  L  Q  A  V  T  W  D  I  L  L  D  I  I  E  R  L  L  Q  Q
30001  TGTTCTGTCC ATAACAAGAC TCATCAAGAA GTATAGGAAG GAGCTCCAGG CTGTGACATG GGATATTCTG CTGGACATCA TTGAACGACT ACTTCAGCAA
       ACAAGACAGG TATTGTTCTG AGTAGTTCTT CATATCCTTC CTCGAGGTCC GACACTGTAC CCTATAAGAC GACCTGTAGT AACTTGCTGA TGAAGTCGTT

 +2  L  Q
30101  CTCCAGGTAA GTCAGGAATG CCAGAAAGGA CTAAGATCCC AGAGAGGCGG GCTTGTCCCT GCTGGGAGGT GTACATGCGT TCTGTGCGTC TGGGCCCTTT
       GAGGTCCATT CAGTCCTTAC GGTCTTTCCT GATTCTAGGG TCTCTCCGCC CGAACAGGGA CGACCCTCCA CATGTACGCA AGACACGCAG ACCCGGGAAA
```

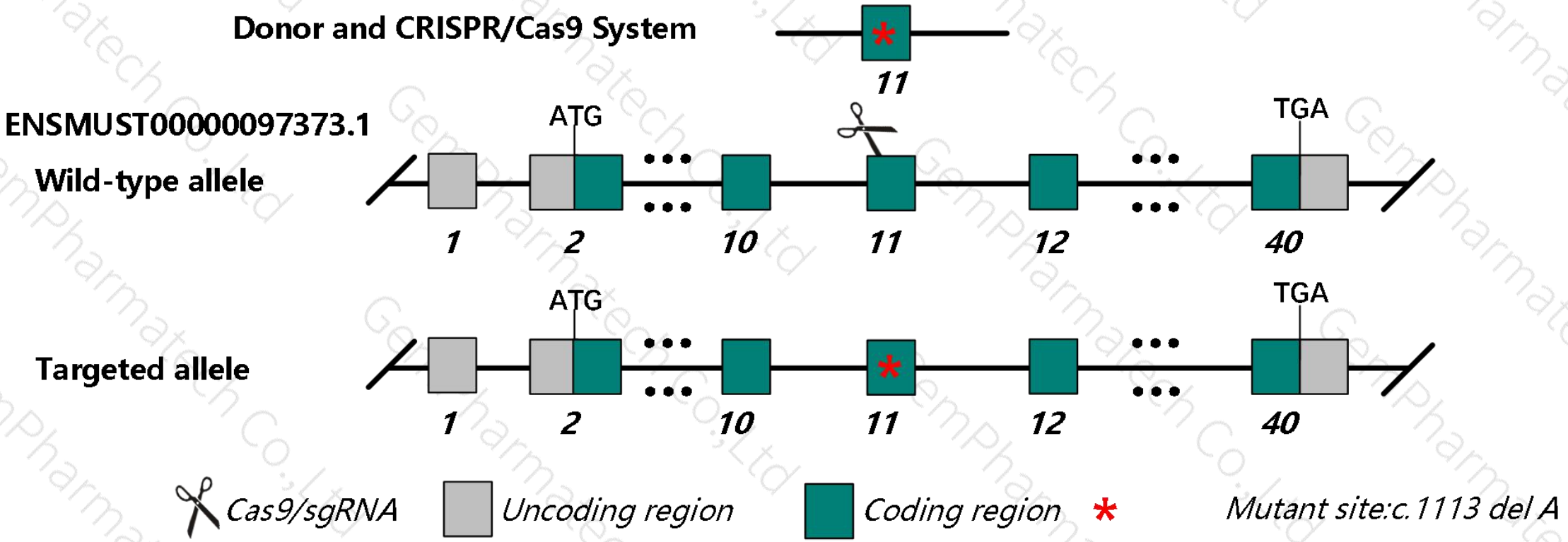
After mutation

```
 +2                                                                                                A  M  T  C  P  N  E  V  V  S  Y  E  I?
29901  AGGAGACAGG ATGCTCTCAT CCTCTATGAG GCATAACACT GCCCCATGGT ACTGTGTTTC AGGCTATGAC CTGTCCCAAT GAGGTGGTGT CATATGAGAT
       TCCTCTGTCC TACGAGAGTA GGAGATACTC CGTATTGTGA CGGGGTACCA TGACACAAAG TCCGATACTG GACAGGGTTA CTCCACCACA GTATACTCTA

 +2  ?I  V  L  S  I  T  R  L  I  K  K  Y  R  K  E  L  Q  A  V  T  W  D  I  L  L  D  I  I  E  R  L  L  Q  H
30001  TGTTCTGTCC ATAACAAGAC TCATCAAGAA GTATAGGAAG GAGCTCCAGG CTGTGACATG GGATATTCTG CTGGACATCA TTGAACGACT ACTTCAGCAC
       ACAAGACAGG TATTGTTCTG AGTAGTTCTT CATATCCTTC CTCGAGGTCC GACACTGTAC CCTATAAGAC GACCTGTAGT AACTTGCTGA TGAAGTCGTG

 +2  S
30101  TCCAGGTAAG TCAGGAATGC CAGAAAGGAC TAAGATCCCA GAGAGGCGGG CTTGTCCCTG CTGGGAGGTG TACATGCGTT CTGTGCGTCT GGGCCCTTTC
       AGGTCCATTC AGTCCTTACG GTCTTTCCTG ATTCTAGGGT CTCTCCGCCC GAACAGGGAC GACCCTCCAC ATGTACGCAA GACACGCAGA CCCGGGAAAG
```

The blue region is exon11 of *Tsc2-201,* the yellow region represents the mutation site.

Strategy

This model uses CRISPR/Cas9 technology to edit the *Tsc2* gene and the schematic diagram is as follow:



Notice

- According to the data of MGI,homozygous null mutants exhibit liver hypoplasia, open neural tube, thickened myocardium and die by embryonic day 9.5-12.5. Heterozygotes develop renal cystadenomas, liver hemangiomas (sometimes resulting in fatal bleeding) and lung adenomas.This model may have the same phenotype.
- One or Two synonymous mutations of amino acids will be intronduced on exon11 of *Tsc2*.
- The effect on the transcript *Tsc2*-213&219 is unknown.
- Mouse *Tsc2* 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.

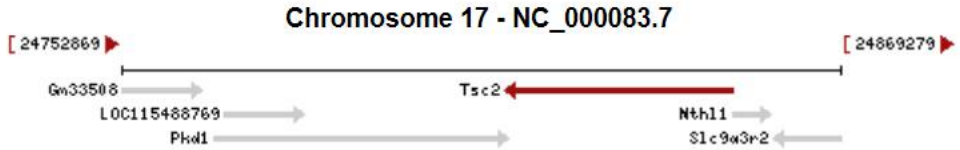
Gene name and location （NCBI)

Tsc2 TSC complex subunit 2 [*Mus musculus* (house mouse)]

Gene ID: 22084, updated on 13-Oct-2020

Summary

Official Symbol	Tsc2 provided by MGI
Official Full Name	TSC complex subunit 2 provided by MGI
Primary source	MGI:MGI:102548
See related	Ensembl:ENSMUSG00000002496
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	Na; Tcs2; tube; Nafld
Expression	Ubiquitous expression in lung adult (RPKM 31.9), ovary adult (RPKM 27.3) and 28 other tissues See more
Orthologs	human all

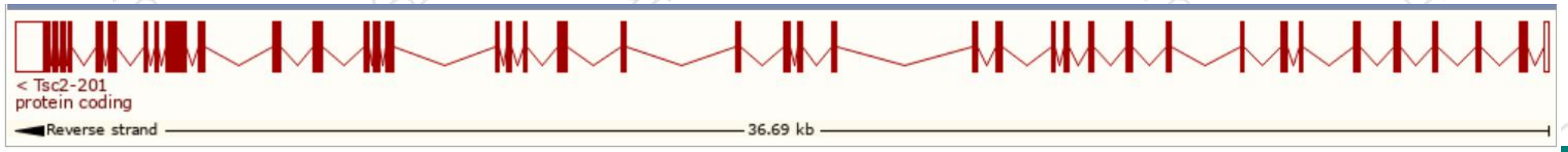


Transcript information （Ensembl）

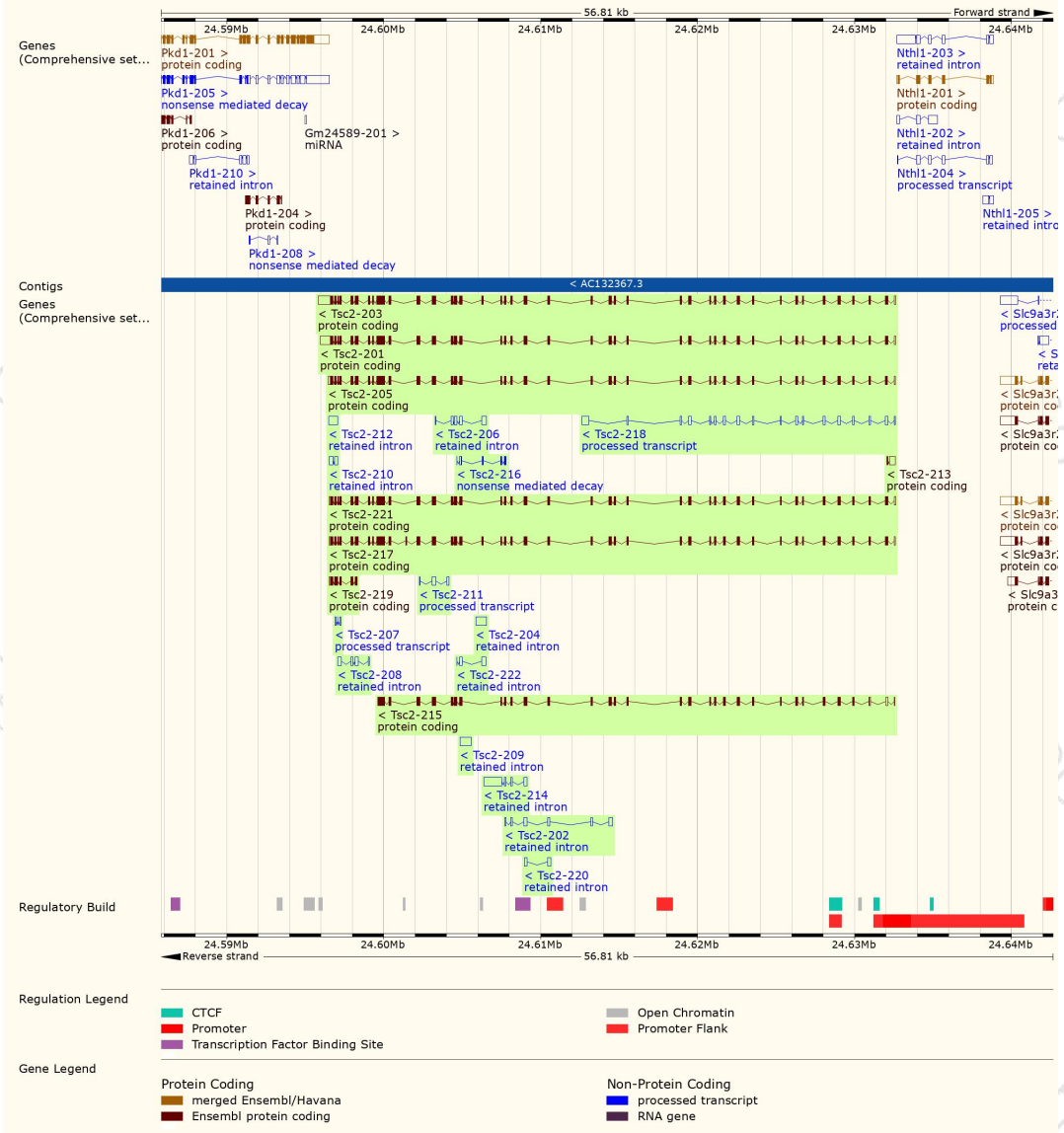
The gene has 22 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tsc2-215	ENSMUST00000227607.1	4269	1359aa	Protein coding	-	A0A2I3BPE9	CDS 3' incomplete
Tsc2-203	ENSMUST00000226284.1	6266	1785aa	Protein coding	-	A0A2I3BPP1	GENCODE basic APPRIS ALT2
Tsc2-216	ENSMUST00000227658.1	393	97aa	Nonsense mediated decay	-	A0A2I3BPR7	CDS 5' incomplete
Tsc2-213	ENSMUST00000227509.1	432	7aa	Protein coding	-	A0A2I3BRA1	CDS 3' incomplete
Tsc2-221	ENSMUST00000228412.1	5432	1741aa	Protein coding	-	A0A2I3BRT5	GENCODE basic APPRIS ALT2
Tsc2-219	ENSMUST00000227804.1	798	237aa	Protein coding	-	Q3TQ10	CDS 5' incomplete
Tsc2-217	ENSMUST00000227745.1	5598	1808aa	Protein coding	-	Q3UHB2	GENCODE basic APPRIS ALT2
Tsc2-201	ENSMUST00000097373.1	6018	1742aa	Protein coding	CCDS28486	Q7TT21	TSL:1 GENCODE basic APPRIS P2
Tsc2-205	ENSMUST00000226398.1	5459	1742aa	Protein coding	CCDS28486	Q7TT21	GENCODE basic APPRIS P2
Tsc2-218	ENSMUST00000227754.1	2357	No protein	Processed transcript	-	-	-
Tsc2-211	ENSMUST00000227330.1	354	No protein	Processed transcript	-	-	-
Tsc2-207	ENSMUST00000226473.1	137	No protein	Processed transcript	-	-	-
Tsc2-214	ENSMUST00000227543.1	1545	No protein	Retained intron	-	-	-
Tsc2-202	ENSMUST00000226242.1	859	No protein	Retained intron	-	-	-
Tsc2-206	ENSMUST00000226428.1	782	No protein	Retained intron	-	-	-
Tsc2-209	ENSMUST00000226985.1	699	No protein	Retained intron	-	-	-
Tsc2-204	ENSMUST00000226309.1	677	No protein	Retained intron	-	-	-
Tsc2-208	ENSMUST00000226691.1	623	No protein	Retained intron	-	-	-
Tsc2-212	ENSMUST00000227432.1	570	No protein	Retained intron	-	-	-
Tsc2-222	ENSMUST00000228729.1	461	No protein	Retained intron	-	-	-
Tsc2-210	ENSMUST00000227094.1	454	No protein	Retained intron	-	-	-
Tsc2-220	ENSMUST00000228220.1	376	No protein	Retained intron	-	-	-

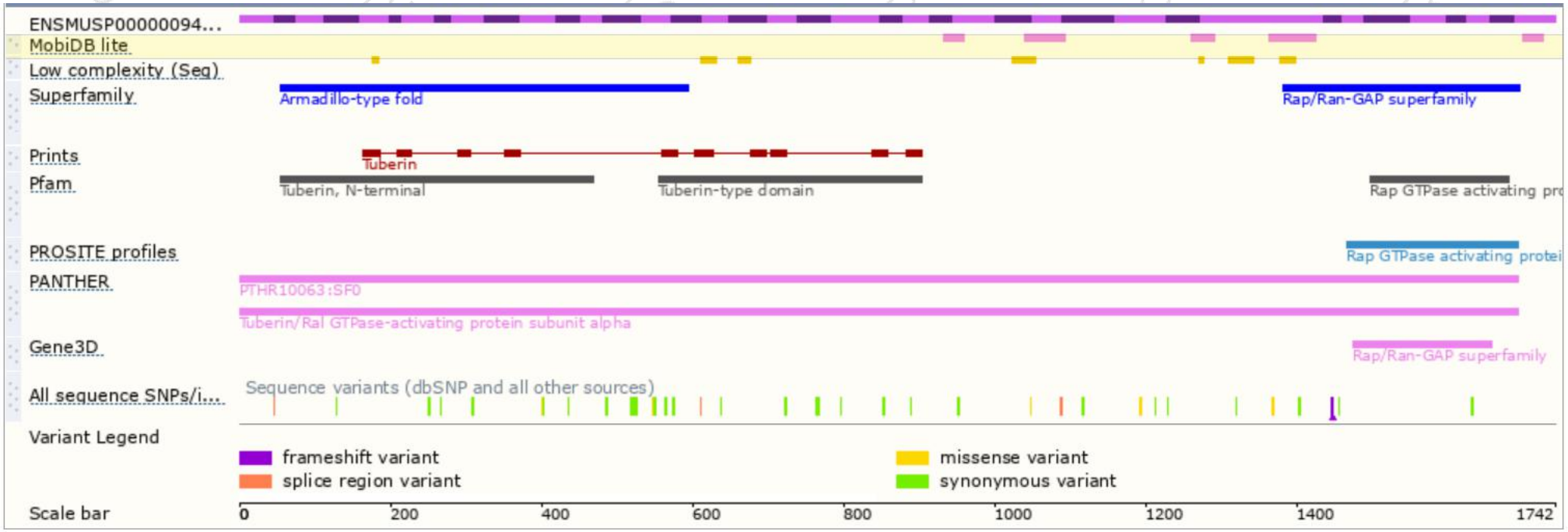
The strategy is based on the design of *Tsc2*-201 transcript, the transcription is shown below:



Genomic location distribution

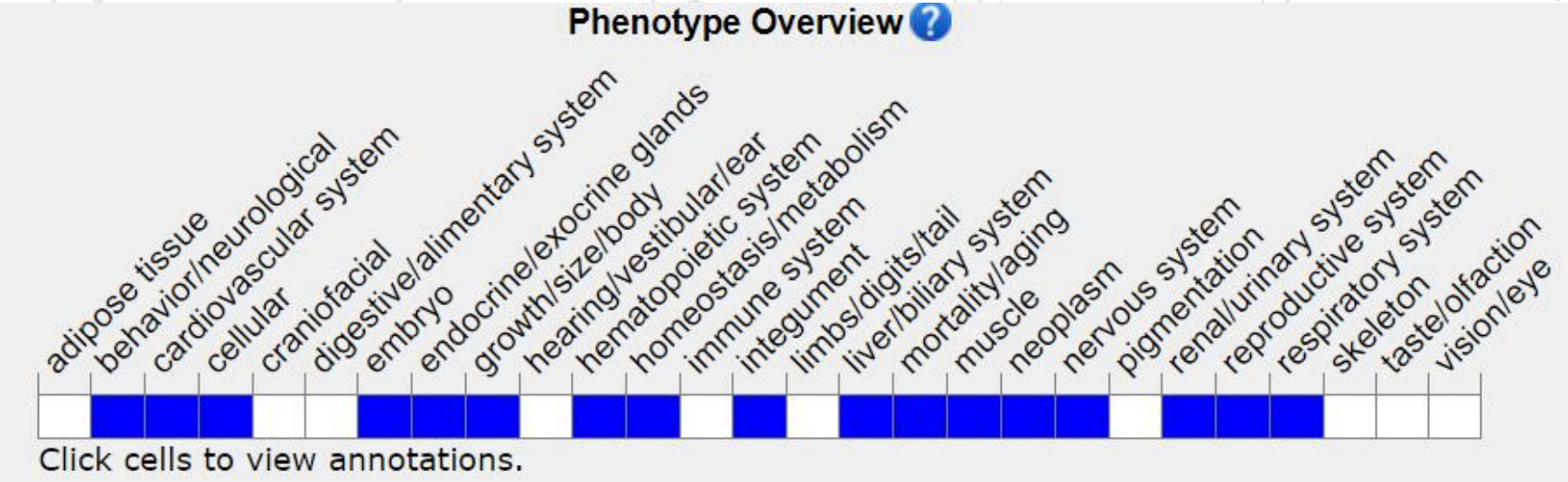


Protein domain



Mouse phenotype description(MGI)

URL link is as follows:
http://www.informatics.jax.org/marker/MGI:102548



Homozygous null mutants exhibit liver hypoplasia, open neural tube, thickened myocardium and die by embryonic day 9.5-12.5. Heterozygotes develop renal cystadenomas, liver hemangiomas (sometimes resulting in fatal bleeding) and lung adenomas.

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