

***Tns1* Cas9-CKO Strategy**

Designer:

Yang Zeng

Reviewer:

Yanhua Shen

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Project Overview

Project Name

Tns1

Project type

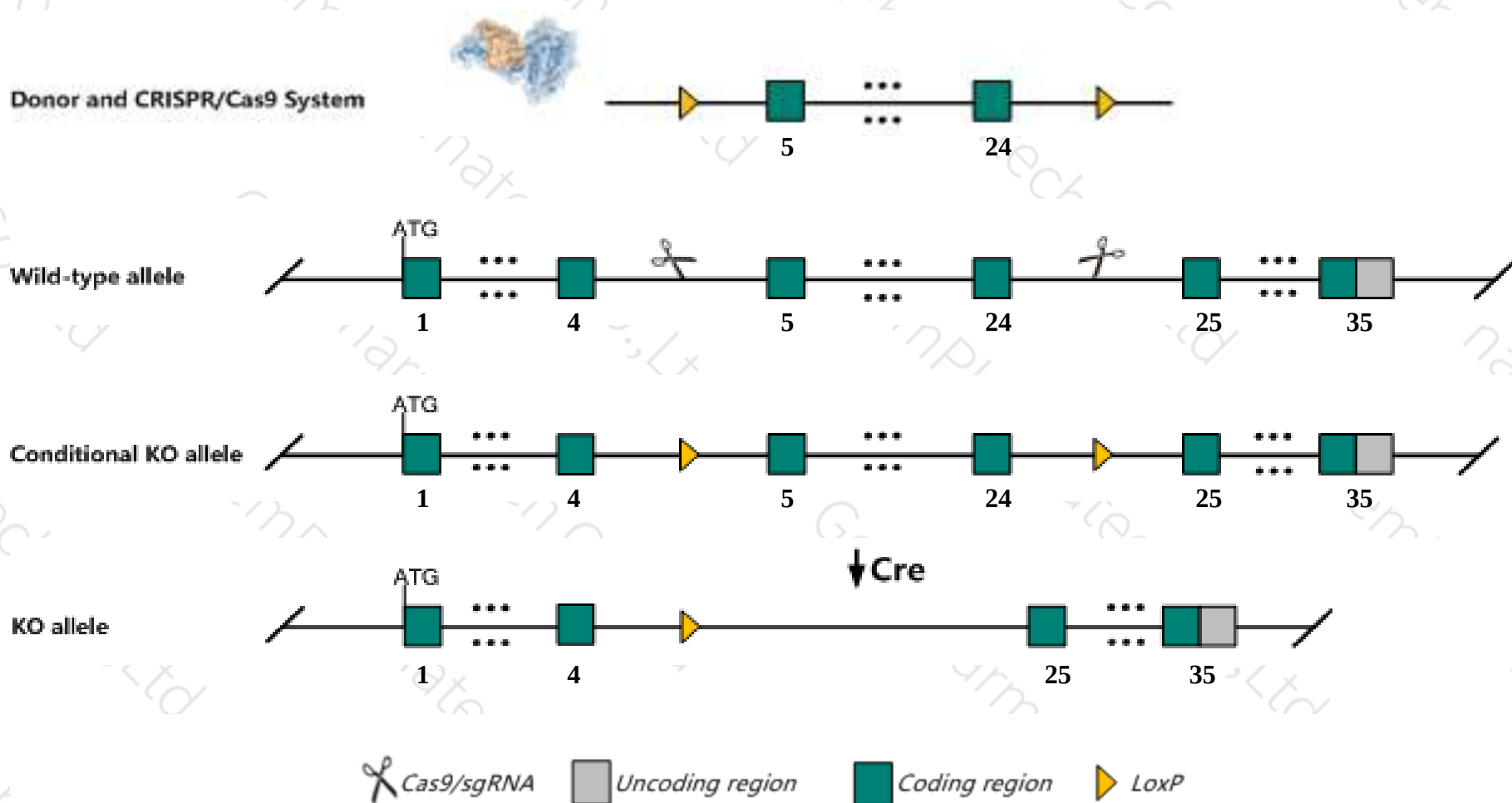
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Tns1* gene. The schematic diagram is as follows:



- The *Tns1* gene has 22 transcripts. According to the structure of *Tns1* gene, exon5-exon24 of *Tns1*-201 (ENSMUST00000169786.7) transcript is recommended as the knockout region. The region contains 3175bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Tns1* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed. Cas9, sgRNA 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.
- The flox mice was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit reduced female fertility, and develop kidney cysts and progressive kidney degeneration that may lead to death from renal failure.
- Transcript *Tns1*-202/204 lncRNA may not be affected.
- The *Tns1* gene is located on the Chr1. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

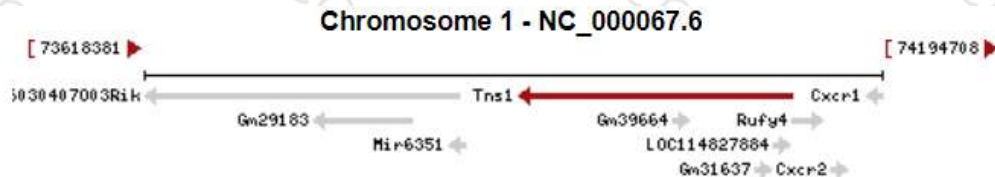
Gene information (NCBI)

Tns1 tensin 1 [*Mus musculus* (house mouse)]

Gene ID: 21961, updated on 12-Aug-2019

Summary

Official Symbol	Tns1 provided by MGI
Official Full Name	tensin 1 provided by MGI
Primary source	MGI:MGI:104552
See related	Ensembl:ENSMUSG00000055322
Gene type	protein coding
RefSeq status	VALIDATED
Organism	<i>Mus musculus</i>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Tns; A1648117; 1110018I21Rik; 1200014E20Rik; E030018G17Rik; E030037J05Rik
Expression	Broad expression in lung adult (RPKM 128.6), bladder adult (RPKM 85.1) and 18 other tissues See more
Orthologs	human all

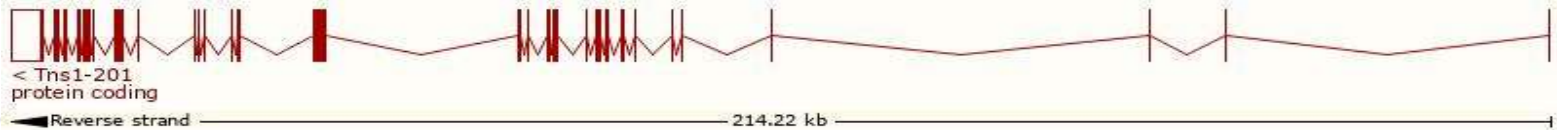


Transcript information (Ensembl)

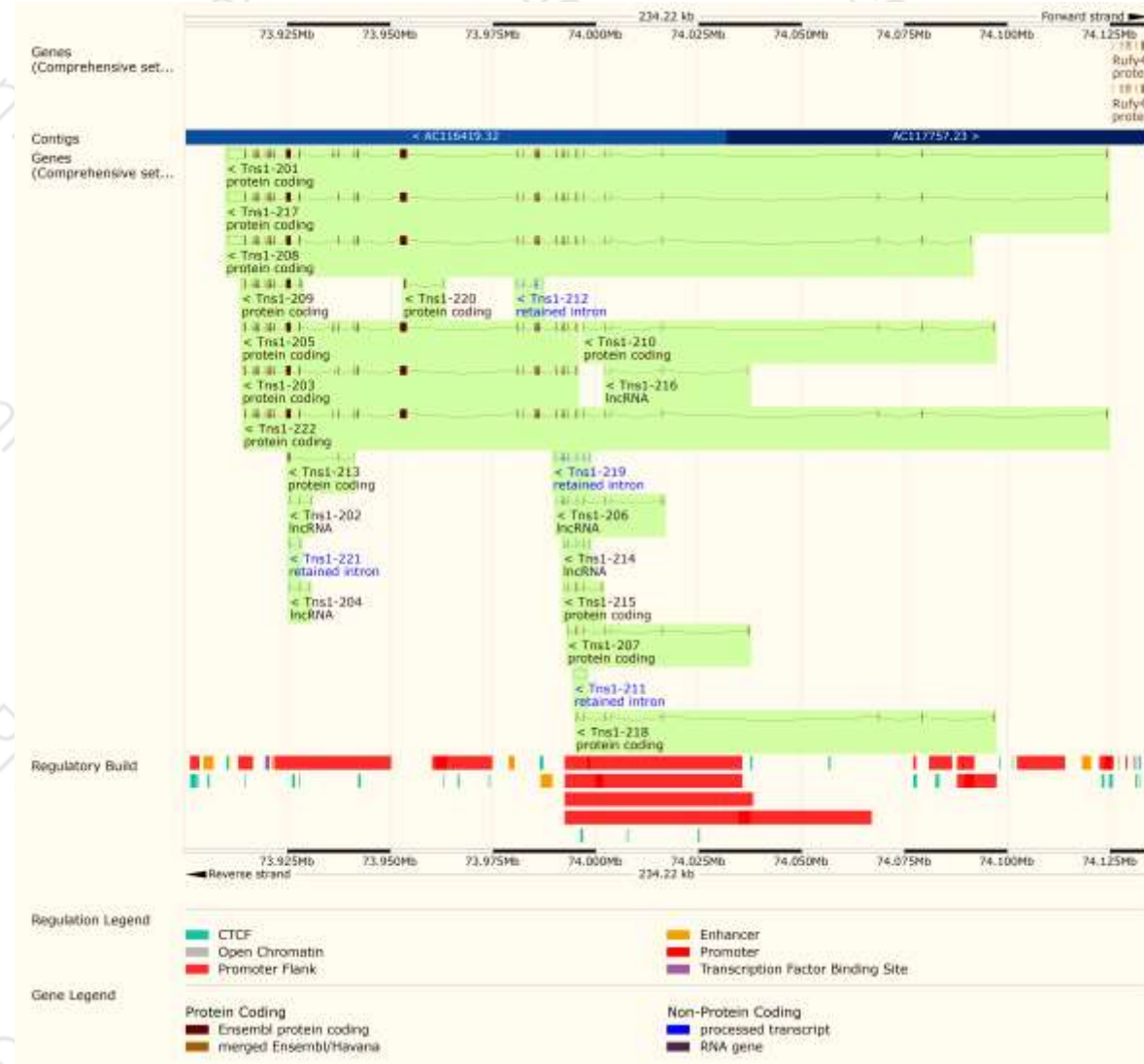
The gene has 22 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Tns1-201	ENSMUST00000169786.7	9965	1888aa	ENSMUSP00000127715.1	Protein coding	CCDS78614.2	E9Q0S8.2	TSL 5 GENCODE basic APPRIS ALT2
Tns1-217	ENSMUST00000191104.6	9904	1867aa	ENSMUSP00000140317.1	Protein coding	CCDS48288.2	A0A087WQS0.2	TSL 5 GENCODE basic APPRIS P3
Tns1-208	ENSMUST00000187584.6	9916	1823aa	ENSMUSP00000140254.1	Protein coding	-	A0A087WQM0.2	TSL 5 GENCODE basic APPRIS ALT2
Tns1-222	ENSMUST00000212888.1	5643	1880aa	ENSMUSP00000148638.1	Protein coding	-	A0A1D5RM59.2	TSL 5 GENCODE basic APPRIS ALT2
Tns1-205	ENSMUST00000185702.6	5595	1719aa	ENSMUSP00000140094.1	Protein coding	-	A0A087WQ94.2	CDS 5' incomplete TSL 5
Tns1-203	ENSMUST00000185331.6	5529	1705aa	ENSMUSP00000140771.1	Protein coding	-	A0A087WRU0.2	CDS 5' incomplete TSL 5
Tns1-209	ENSMUST00000187691.6	2813	624aa	ENSMUSP00000139844.1	Protein coding	-	Q9D8T6.2	TSL 1 GENCODE basic
Tns1-220	ENSMUST00000191367.1	711	88aa	ENSMUSP00000140991.1	Protein coding	-	A0A087WSC8.2	CDS 3' incomplete TSL 2
Tns1-207	ENSMUST00000187281.6	586	196aa	ENSMUSP00000139616.1	Protein coding	-	A0A087WP40.2	CDS 5' and 3' incomplete TSL 3
Tns1-218	ENSMUST00000191204.6	515	172aa	ENSMUSP00000140878.1	Protein coding	-	A0A087WS32.2	CDS 5' and 3' incomplete TSL 3
Tns1-213	ENSMUST00000189228.1	446	148aa	ENSMUSP00000139721.1	Protein coding	-	A0A087WPC7.2	CDS 5' and 3' incomplete TSL 3
Tns1-215	ENSMUST00000190389.6	426	142aa	ENSMUSP00000140448.1	Protein coding	-	A0A087WR39.2	CDS 5' and 3' incomplete TSL 5
Tns1-210	ENSMUST00000188208.1	412	116aa	ENSMUSP00000140837.1	Protein coding	-	A0A087VVRZ.2	CDS 3' incomplete TSL 2
Tns1-211	ENSMUST00000188436.1	2903	No protein	-	Retained intron	-	-	TSL NA
Tns1-212	ENSMUST00000186942.1	1852	No protein	-	Retained intron	-	-	TSL 1
Tns1-219	ENSMUST00000191264.6	1373	No protein	-	Retained intron	-	-	TSL 2
Tns1-221	ENSMUST00000191527.6	659	No protein	-	Retained intron	-	-	TSL 2
Tns1-206	ENSMUST00000186381.6	657	No protein	-	lncRNA	-	-	TSL 5
Tns1-214	ENSMUST00000189571.1	635	No protein	-	lncRNA	-	-	TSL 3
Tns1-202	ENSMUST00000185264.6	601	No protein	-	lncRNA	-	-	TSL 2
Tns1-204	ENSMUST00000185529.1	528	No protein	-	lncRNA	-	-	TSL 2
Tns1-216	ENSMUST00000190599.1	405	No protein	-	lncRNA	-	-	TSL 3

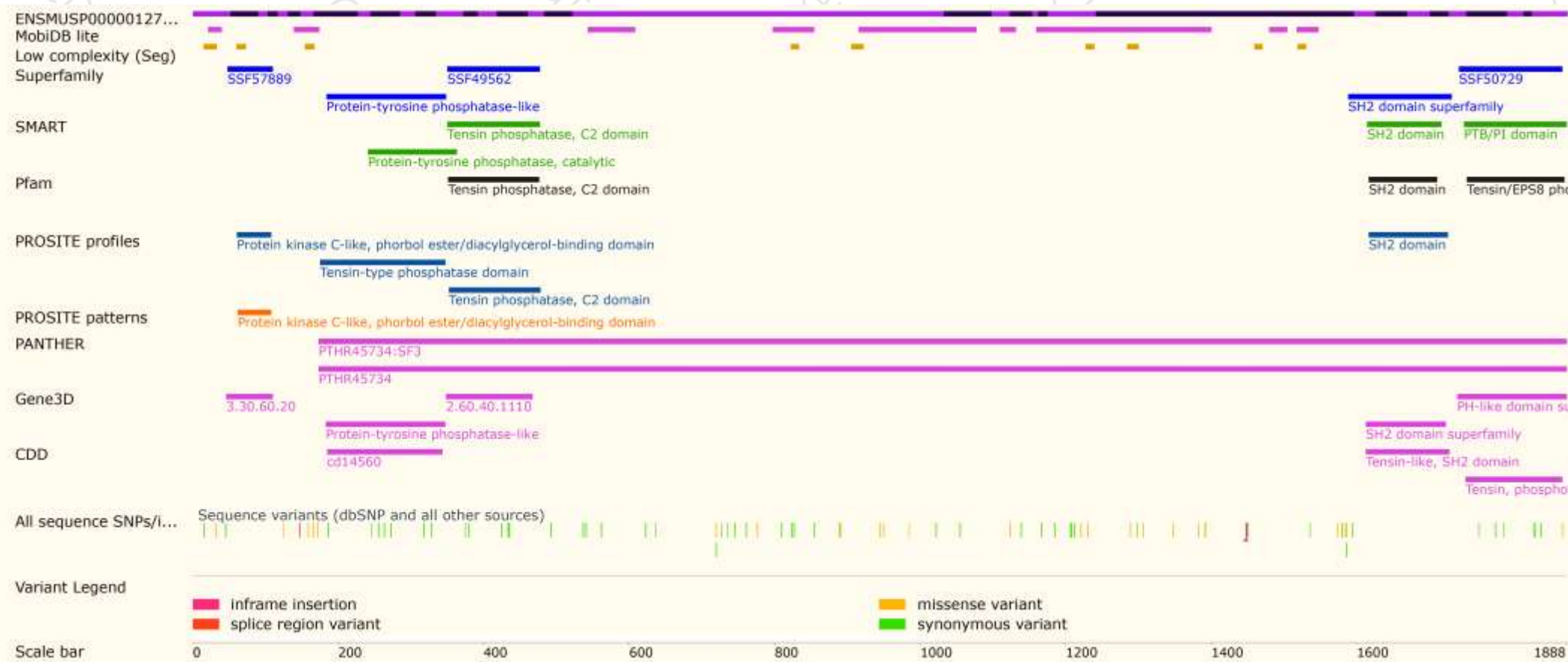
The strategy is based on the design of *Tns1-201* transcript,The transcription is shown below



Genomic location distribution

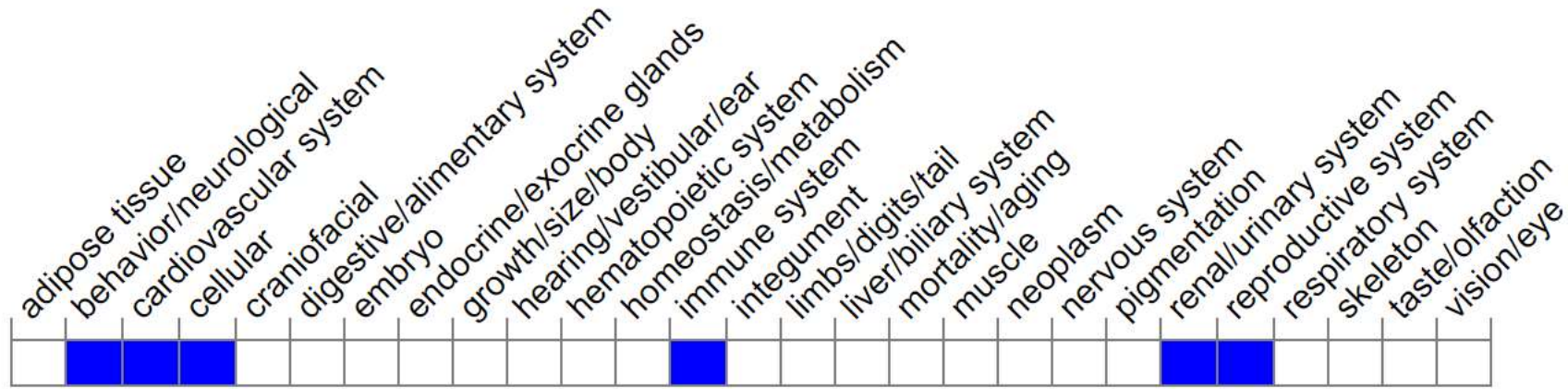


Protein domain



Mouse phenotype description(MGI)

Phenotype Overview ?



Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(<http://www.informatics.jax.org/>).

According to the existing MGI data, Mice homozygous for a knock-out allele exhibit reduced female fertility, and develop kidney cysts and progressive kidney degeneration that may lead to death from renal failure.

If you have any questions, you are welcome to inquire.

Tel: 025-5864 1534

