# *Tial1* Cas9-CKO Strategy

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**Reviewer: Wenjing Li**

**Design Date: 2020-8-1**

# Project Overview

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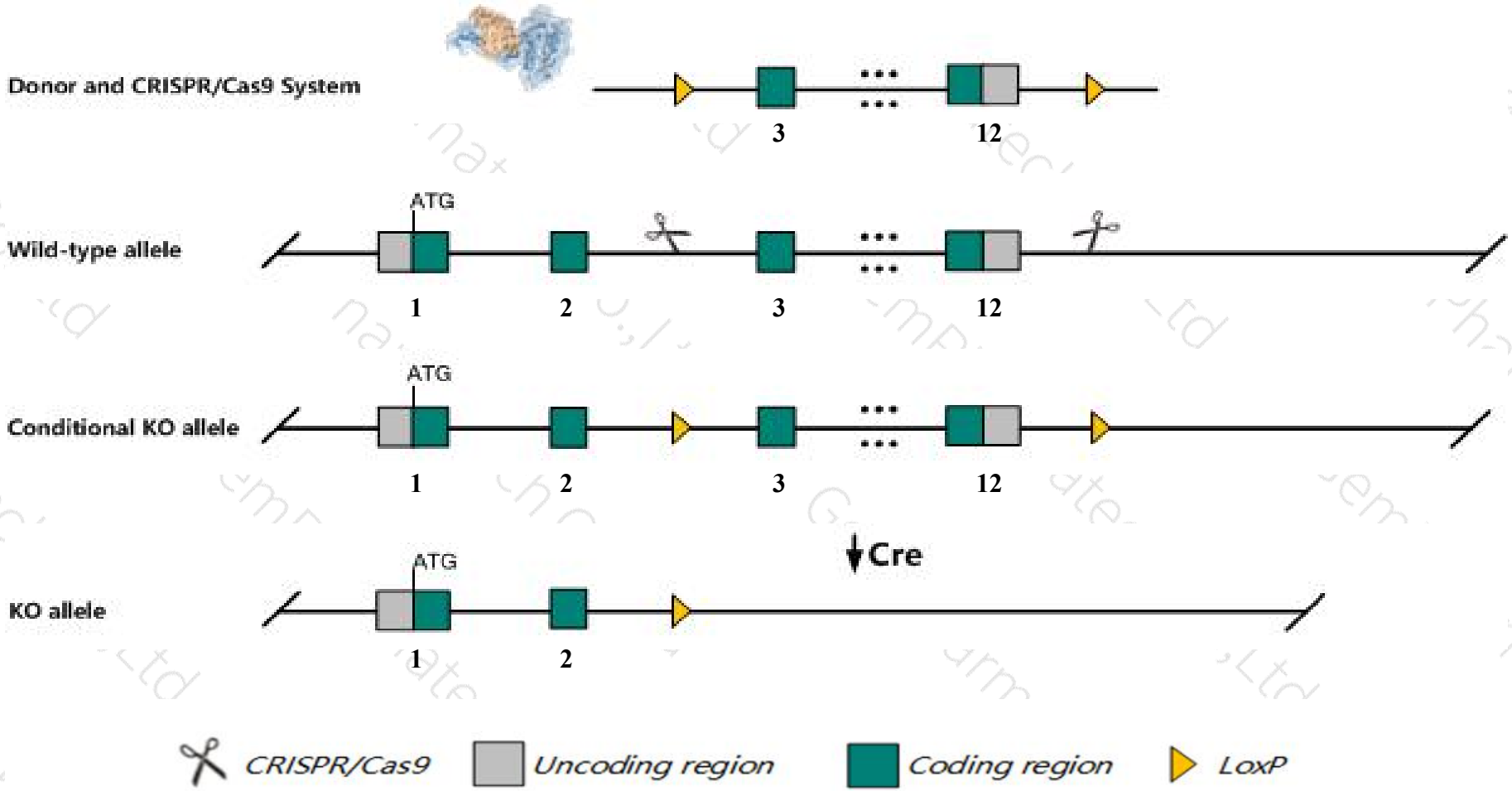
|---|---|
| **Project Name** | ***Tial1*** |
| **Project type** | **Cas9-CKO** |
| **Strain background** | **C57BL/6JGpt** |

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Tial1* gene. The schematic diagram is as follows:



# Technical routes

➢ The *Tial1* gene has 14 transcripts. According to the structure of *Tial1* gene, exon3-exon12 of *Tial1-202*(ENSMUST00000106226.8) transcript is recommended as the knockout region.The region contains most of the coding sequence.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Tial1* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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 will be 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.

# Notice

➢ According to the existing MGI data,homozygous null mice exhibit partial embryonic lethality and reduced postnatal survival, reduced embryonic and postnatal body weight, and male and female sterility. Infertility is owed to a substantial decrease in the survival of primordial germ cells atthe genital ridge.

➢ The *Tial1* gene is located on the Chr7. 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）

集萃药康 GemPharmatech

## Tial1 Tia1 cytotoxic granule-associated RNA binding protein-like 1 [Mus musculus (house mouse)]

Gene ID: 21843, updated on 20-Mar-2020

### Summary

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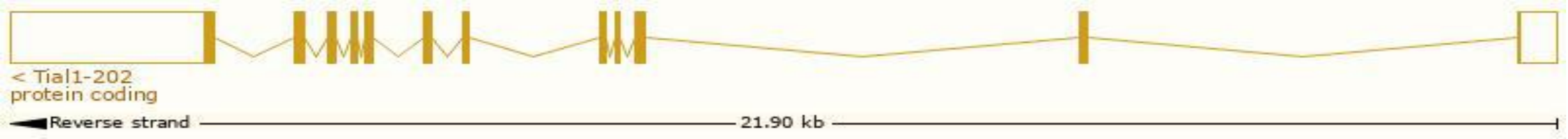
|---|---|
| **Official Symbol** | Tial1 provided by MGI |
| **Official Full Name** | Tia1 cytotoxic granule-associated RNA binding protein-like 1 provided by MGI |
| **Primary source** | MGI:MGI:107913 |
| **See related** | Ensembl:ENSMUSG00000030846 |
| **Gene type** | protein coding |
| **RefSeq status** | 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** | 5330433G13Rik, AL033329, TIAR, mTIAR |
| **Expression** | Ubiquitous expression in limb E14.5 (RPKM 23.5), CNS E14 (RPKM 22.2) and 28 other tissues See more |
| **Orthologs** | human all |

# Transcript information（Ensembl）

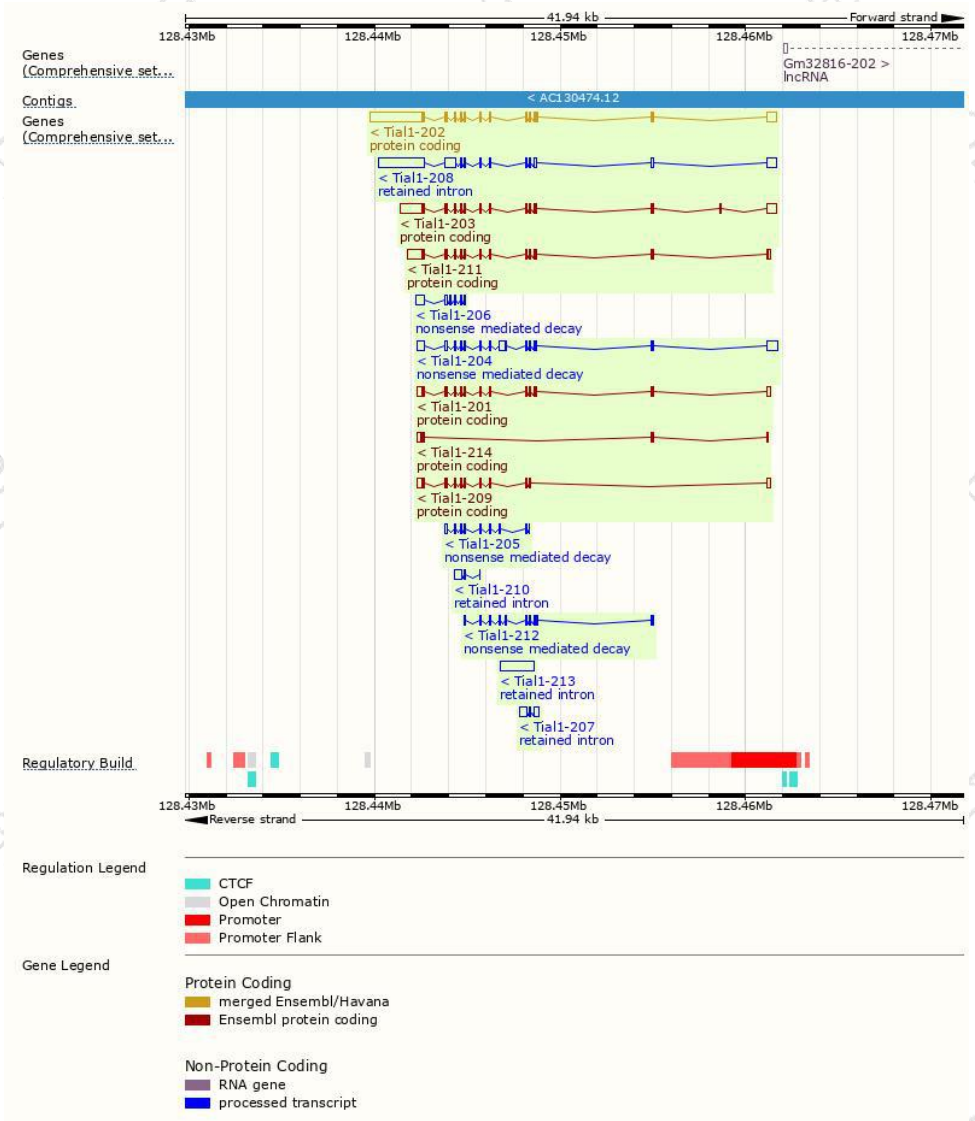
The gene has 14 transcripts,all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|---|---|---|---|---|---|---|---|
| Tial1-202 | ENSMUST00000106226.8 | 4451 | 392aa | Protein coding | CCDS21897 | P70318 Q545C1 | TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P3 |
| Tial1-203 | ENSMUST00000106228.7 | 2835 | 336aa | Protein coding | CCDS85428 | D3Z3Y4 | TSL:1 GENCODE basic |
| Tial1-211 | ENSMUST00000165023.7 | 2003 | 375aa | Protein coding | CCDS85429 | Q921W2 | TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT1 |
| Tial1-201 | ENSMUST00000033135.8 | 1580 | 375aa | Protein coding | CCDS85429 | Q921W2 | TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT1 |
| Tial1-209 | ENSMUST00000141126.7 | 1344 | 244aa | Protein coding | - | A0A0U1RPD3 | TSL:5 GENCODE basic |
| Tial1-214 | ENSMUST00000205835.1 | 562 | 107aa | Protein coding | - | A0A0U1RP00 | TSL:1 GENCODE basic |
| Tial1-204 | ENSMUST00000123666.7 | 2319 | 135aa | Nonsense mediated decay | - | D6RGU1 | TSL:5 |
| Tial1-206 | ENSMUST00000133444.7 | 953 | 106aa | Nonsense mediated decay | - | A0A0U1RPE1 | CDS 5' incomplete TSL:5 |
| Tial1-212 | ENSMUST00000205278.1 | 804 | 140aa | Nonsense mediated decay | - | A0A0U1RPB0 | CDS 5' incomplete TSL:5 |
| Tial1-205 | ENSMUST00000133089.7 | 784 | 42aa | Nonsense mediated decay | - | A0A0U1RPL4 | CDS 5' incomplete TSL:5 |
| Tial1-208 | ENSMUST00000141079.7 | 4266 | No protein | Retained intron | - | - | TSL:5 |
| Tial1-213 | ENSMUST00000205774.1 | 1808 | No protein | Retained intron | - | - | TSL:NA |
| Tial1-207 | ENSMUST00000136782.1 | 674 | No protein | Retained intron | - | - | TSL:2 |
| Tial1-210 | ENSMUST00000152328.2 | 560 | No protein | Retained intron | - | - | TSL:3 |

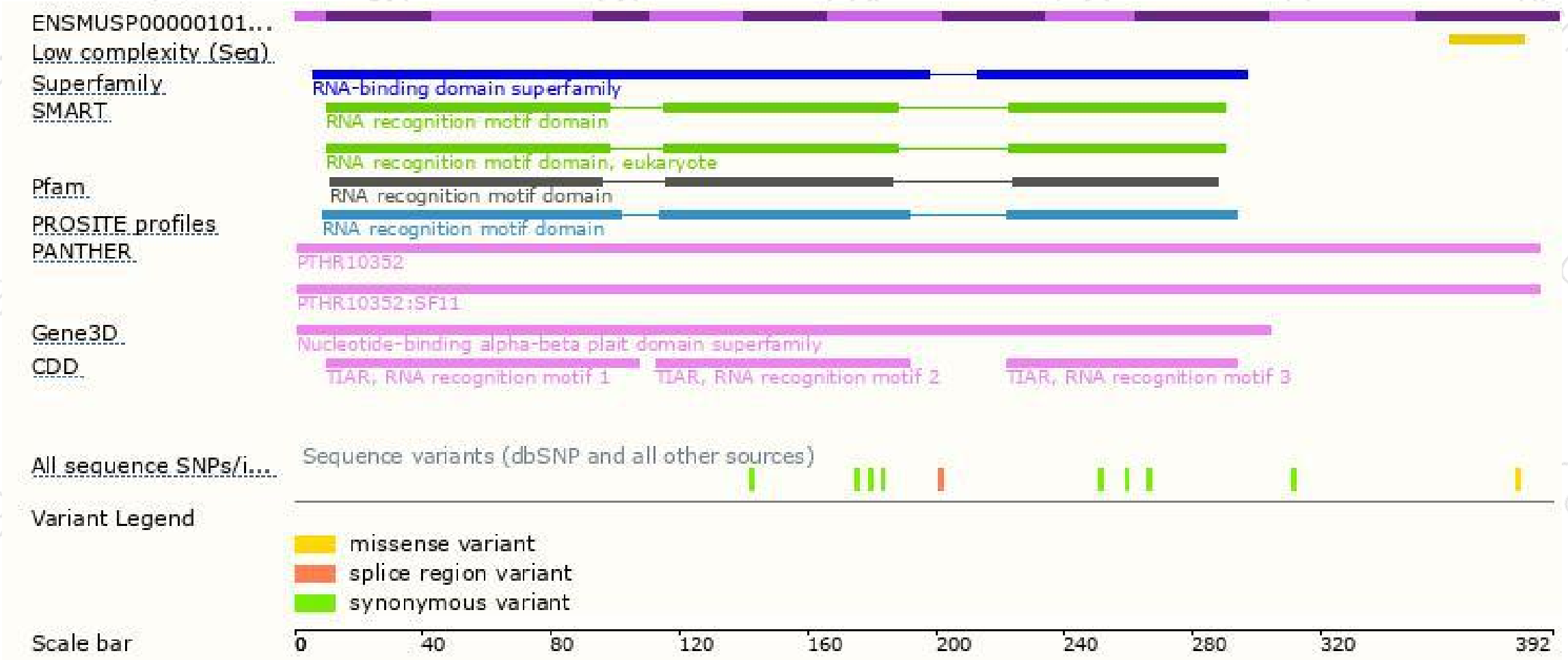
The strategy is based on the design of *Tial1-202* transcript,the transcription is shown below:



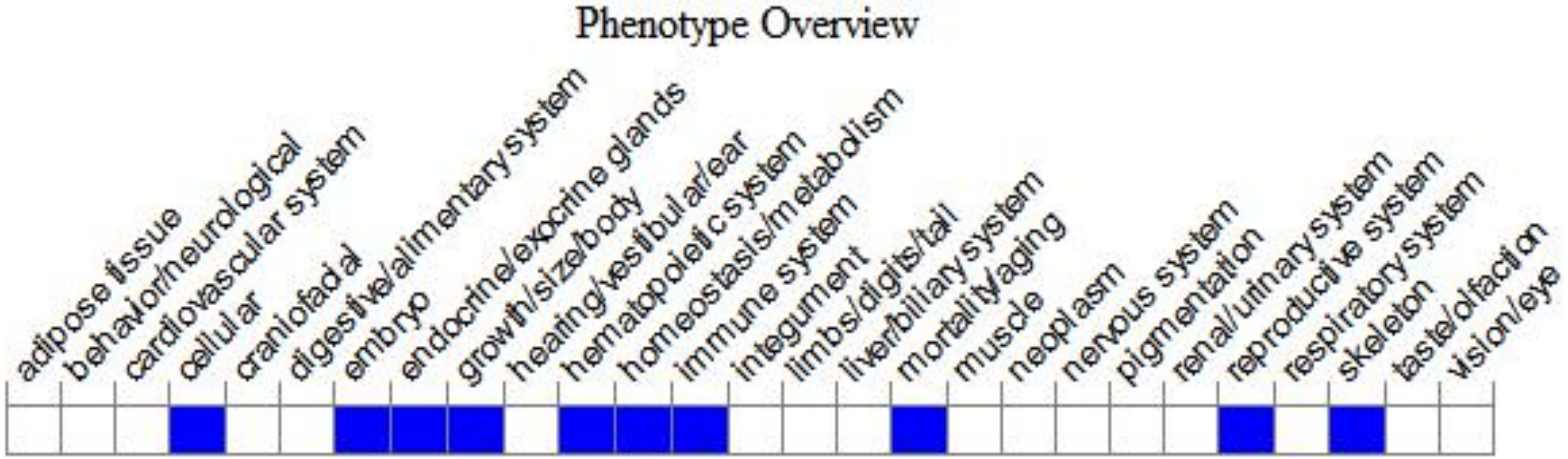
# Genomic location distribution



# Protein domain



# Mouse phenotype description(MGI )




*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,homozygous null mice exhibit partial embryonic lethality and reduced postnatal survival, reduced embryonic and postnatal body weight, and male and female sterility. Infertility is owed to a substantial decrease in the survival of primordial germ cells atthe genital ridge.

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

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