

# *Tro* Cas9-CKO Strategy

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Design Date: 2023-5-10

# Overview

## Target Gene Name

- Tro

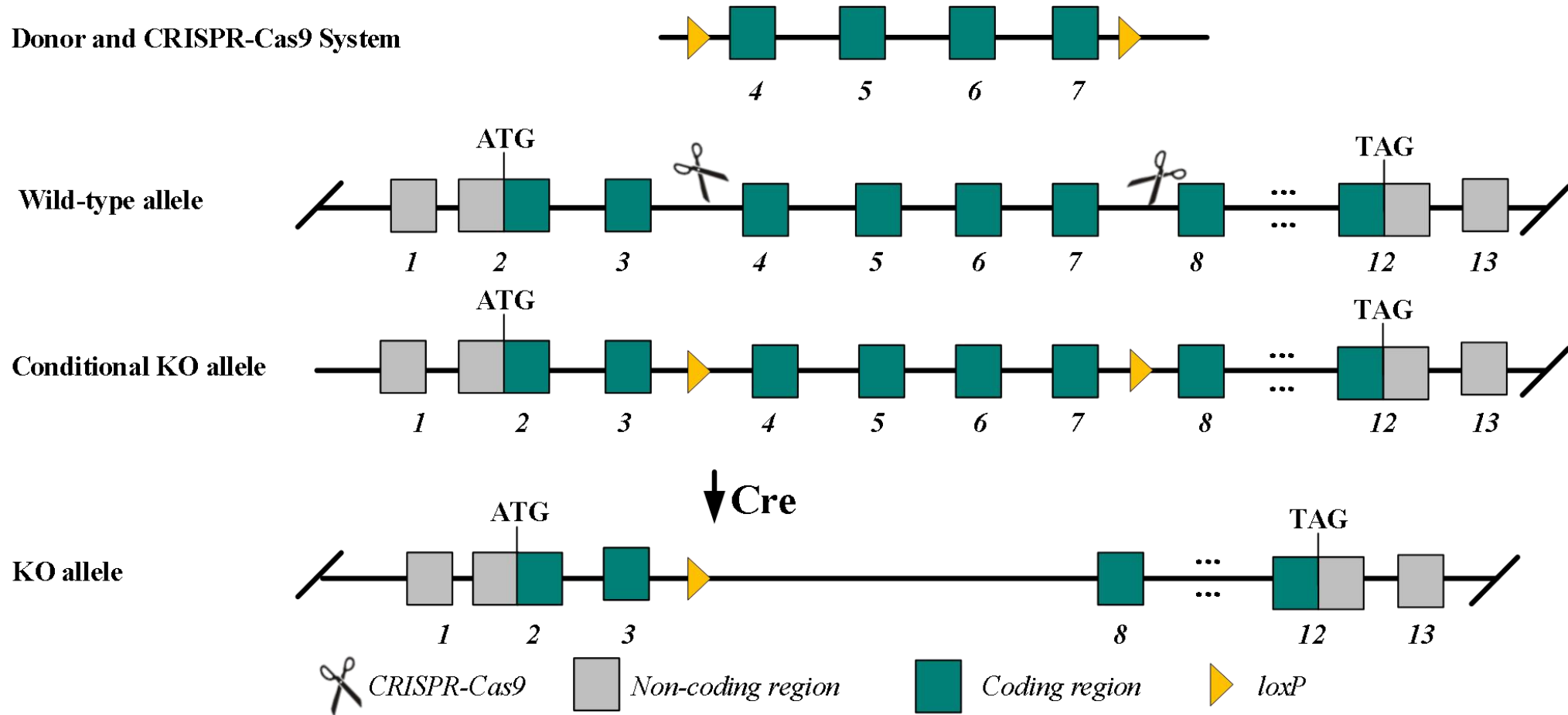
## Project Type

- Cas9-CKO

## Genetic Background

- C57BL/6JGpt

# Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Tro* gene.

# Technical Information

- The *Tro* gene has 11 transcripts. According to the structure of *Tro* gene, exon 4-7 of *Tro*-202 (ENSMUST00000087258.10) transcript is recommended as the knockout region. The region contains 341 bp of coding sequences. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Tro* gene. The brief process is as follows: CRISPR-Cas9 system 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- 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.

# Gene Information

**Tro** trophinin [ *Mus musculus* (house mouse) ]

Gene ID: 56191, updated on 12-Apr-2023

[Download Datasets](#)

## Summary

|                           |  |
|---------------------------|--|
| <b>Official Symbol</b>    | Tro provided by <a href="#">MGI</a>  |
| <b>Official Full Name</b> | trophinin provided by <a href="#">MGI</a>  |
| <b>Primary source</b>     | <a href="#">MGI:MGI:1928994</a>  |
| <b>See related</b>        | <a href="#">Ensembl:ENSMUSG00000025272</a> <a href="#">AllianceGenome:MGI:1928994</a>  |
| <b>Gene type</b>          | protein coding   |
| <b>RefSeq status</b>      | VALIDATED  |
| <b>Organism</b>           | <a href="#">Mus musculus</a>   |
| <b>Lineage</b>            | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus  |
| <b>Also known as</b>      | Tnn; Trol; Maged3; Maged3l; magphinin  |
| <b>Summary</b>            | Acts upstream of or within negative regulation of cell growth. Located in cytoplasm and nucleus. Is expressed in several structures, including branchial arch; early conceptus; limb; and secondary oocyte. Orthologous to human TRO (trophinin). [provided by Alliance of Genome Resources, Apr 2022] |
| <b>Expression</b>         | Biased expression in CNS E18 (RPKM 39.5), whole brain E14.5 (RPKM 32.7) and 10 other tissues <a href="#">See more</a>  |
| <b>Orthologs</b>          | <a href="#">human</a> <a href="#">all</a>  |
| <b>NEW</b>                | Try the new <a href="#">Gene table</a><br>Try the new <a href="#">Transcript table</a>   |

## Genomic context

**Location:** X; X F3

**Exon count:** 15

See Tro in [Genome Data Viewer](#)

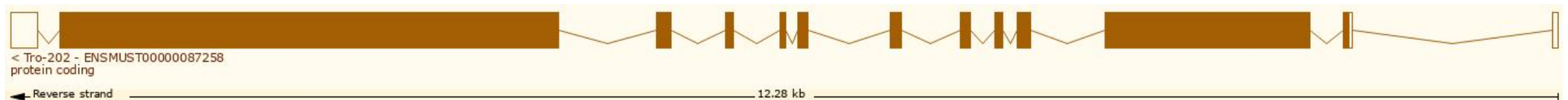
Source: <https://www.ncbi.nlm.nih.gov/>

# Transcript Information

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

| Transcript ID                         | Name    | bp   | Protein                | Biotype                 | CCDS                      | UniProt Match          | Flags   |
|---------------------------------------|---------|------|------------------------|-------------------------|---------------------------|------------------------|---|
| <a href="#">ENSMUST00000171775.2</a>  | Tro-211 | 832  | No protein             | Retained intron         |                           | -                      | TSL:5   |
| <a href="#">ENSMUST00000169450.8</a>  | Tro-210 | 684  | No protein             | Retained intron         |                           | -                      | TSL:2   |
| <a href="#">ENSMUST00000165192.2</a>  | Tro-209 | 427  | No protein             | Retained intron         |                           | -                      | TSL:5   |
| <a href="#">ENSMUST00000164071.2</a>  | Tro-208 | 891  | <a href="#">296aa</a>  | Protein coding          |                           | <a href="#">E9Q1Q4</a> | GENCODE basic APPRIS ALT2 TSL:5                 |
| <a href="#">ENSMUST00000163969.8</a>  | Tro-207 | 2644 | <a href="#">802aa</a>  | Protein coding          | <a href="#">CCDS81183</a> | <a href="#">E9Q160</a> | GENCODE basic TSL:1                             |
| <a href="#">ENSMUST00000163450.8</a>  | Tro-206 | 888  | <a href="#">35aa</a>   | Nonsense mediated decay |                           | <a href="#">E9Q1E1</a> | TSL:5   |
| <a href="#">ENSMUST00000151403.9</a>  | Tro-205 | 2715 | <a href="#">838aa</a>  | Protein coding          | <a href="#">CCDS72448</a> | <a href="#">E9Q098</a> | GENCODE basic APPRIS ALT2 TSL:1                 |
| <a href="#">ENSMUST00000148604.2</a>  | Tro-204 | 1822 | <a href="#">560aa</a>  | Protein coding          |                           | <a href="#">A2AFN3</a> | TSL:1 CDS 3' incomplete                         |
| <a href="#">ENSMUST00000112709.8</a>  | Tro-203 | 6610 | <a href="#">2087aa</a> | Protein coding          | <a href="#">CCDS30465</a> | <a href="#">Q6DIC6</a> | Ensembl Canonical GENCODE basic APPRIS P2 TSL:5 |
| <a href="#">ENSMUST00000087258.10</a> | Tro-202 | 6559 | <a href="#">2087aa</a> | Protein coding          | <a href="#">CCDS30465</a> | <a href="#">Q6DIC6</a> | GENCODE basic APPRIS P2 TSL:1                   |
| <a href="#">ENSMUST00000087253.11</a> | Tro-201 | 3224 | <a href="#">972aa</a>  | Protein coding          | <a href="#">CCDS53221</a> | <a href="#">E9Q886</a> | GENCODE basic APPRIS ALT2 TSL:1                 |

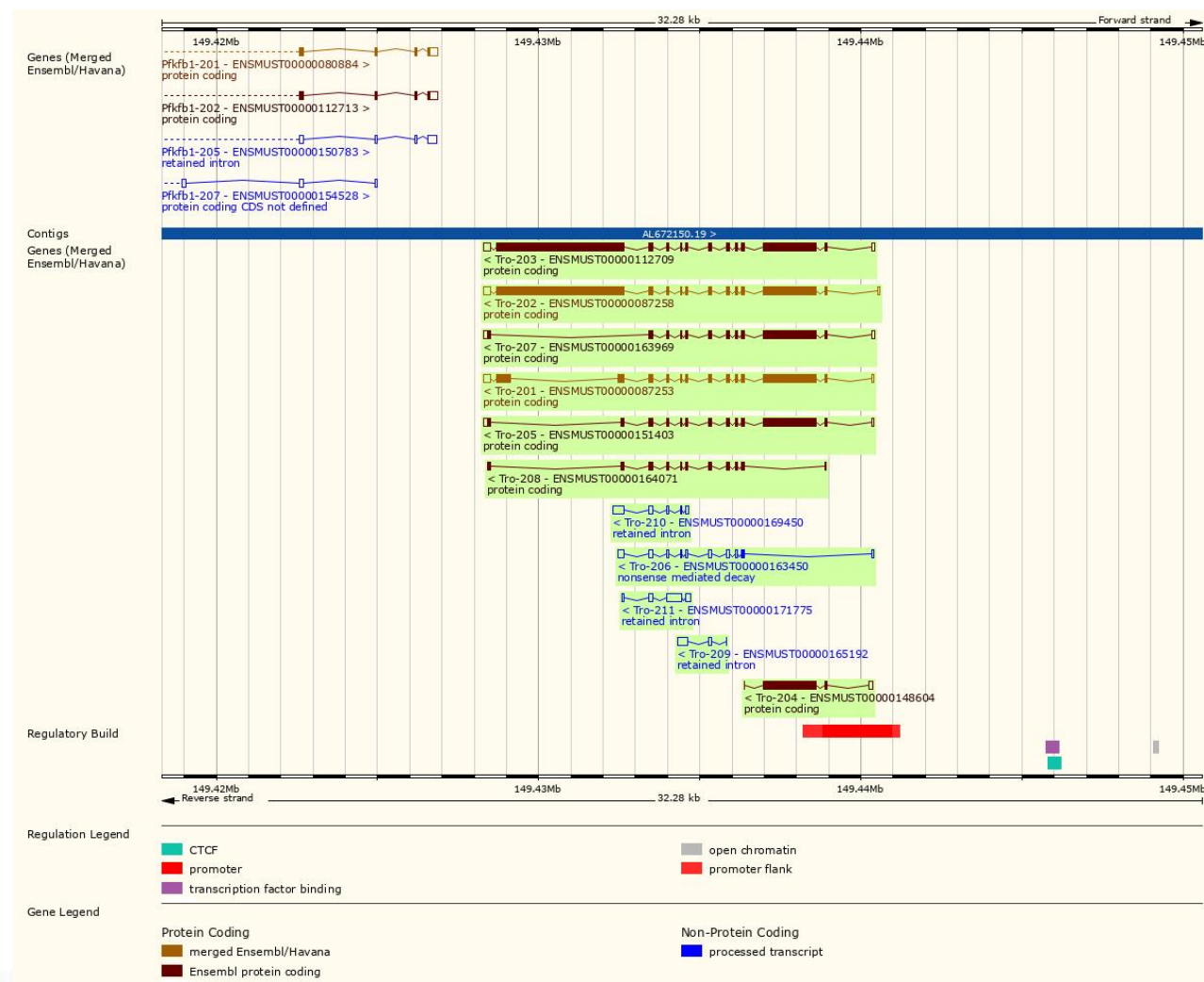
The strategy is based on the design of *Tro-202* transcript, the transcription is shown below:



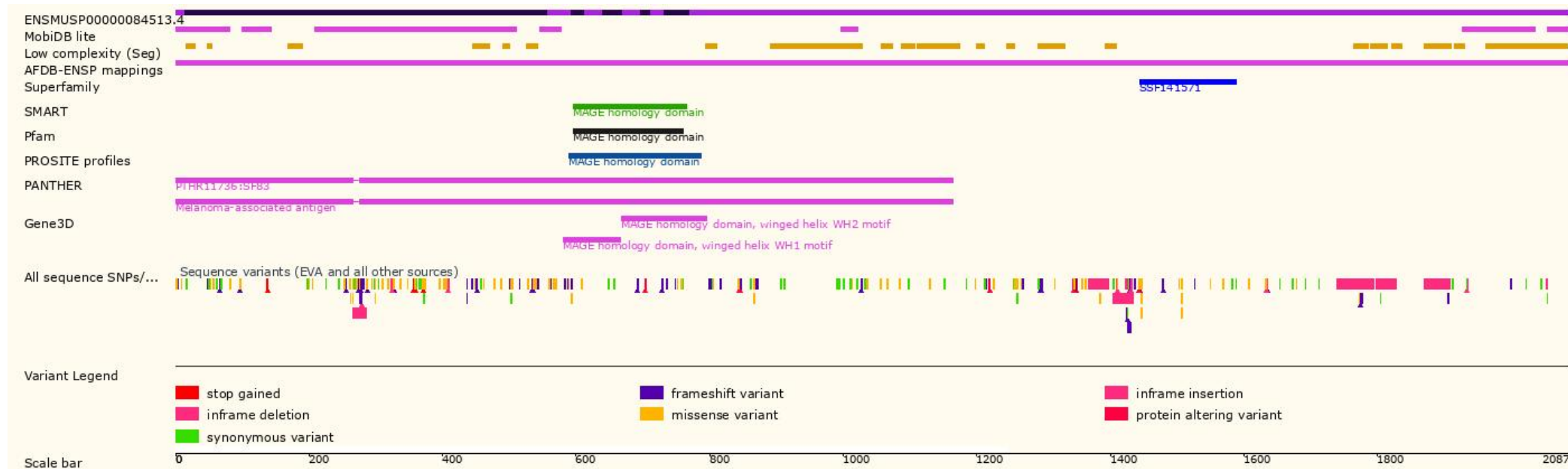
Source: <https://www.ensembl.org>



# Genomic Information

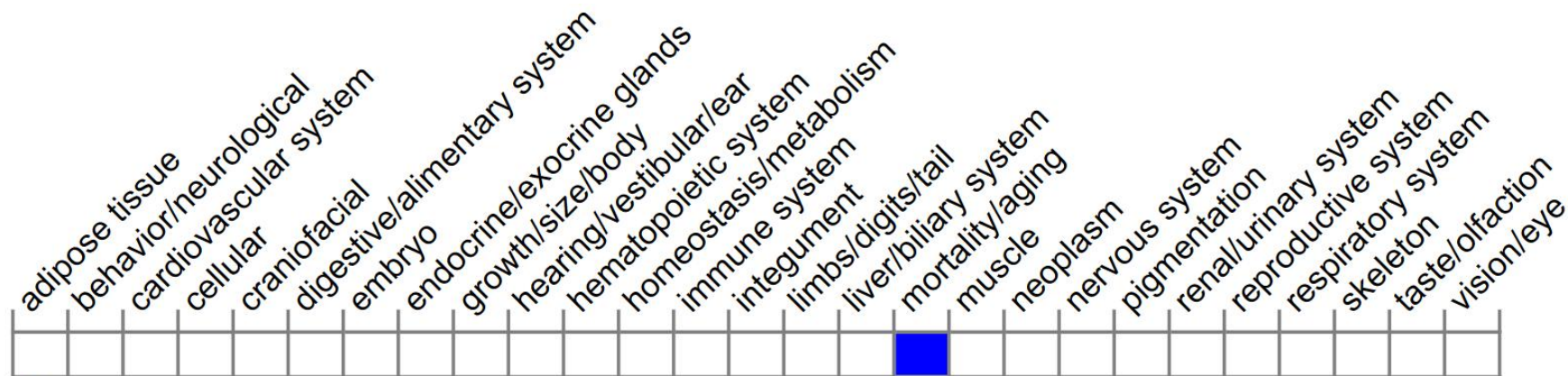


# Protein Information





# Mouse Phenotype Information (MGI)



Homozygous female or hemizygous male mice on a mixed 129 and NIH black Swiss background are viable, fertile and elderly mice exhibit no disorders. On an inbred 129 background, homozygous female or hemizygous male mice show partial embryonic lethality between E3.5 and E8.5.

# Important Information

- Homozygous female or hemizygous male mice on a mixed 129 and NIH black Swiss background are viable, fertile and elderly mice exhibit no disorders. On an inbred 129 background, homozygous female or hemizygous male mice show partial embryonic lethality between E3.5 and E8.5.
- The N-terminal of *Tro* gene will remain 557 aa, it may remain the partial function of *Tro* gene.
- Intron 3-4 (592 bp) and intron 7-8 (651 bp) is small and loxp insertion may interfere with normal splicing.
- The effect of this strategy on the uncoded transcript *Tro*-210, *Tro*-211 is unknown.
- *Tro* is located on Chr X. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is 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 the existing technology level.