GemPharmatech™

Trim65 Cas9-CKO Strategy

Designer: Xiangli Bian

Reviewer: Xiaojing Li

Design Date: 2023-3-27

Overview

Target Gene Name

- *Trim65*

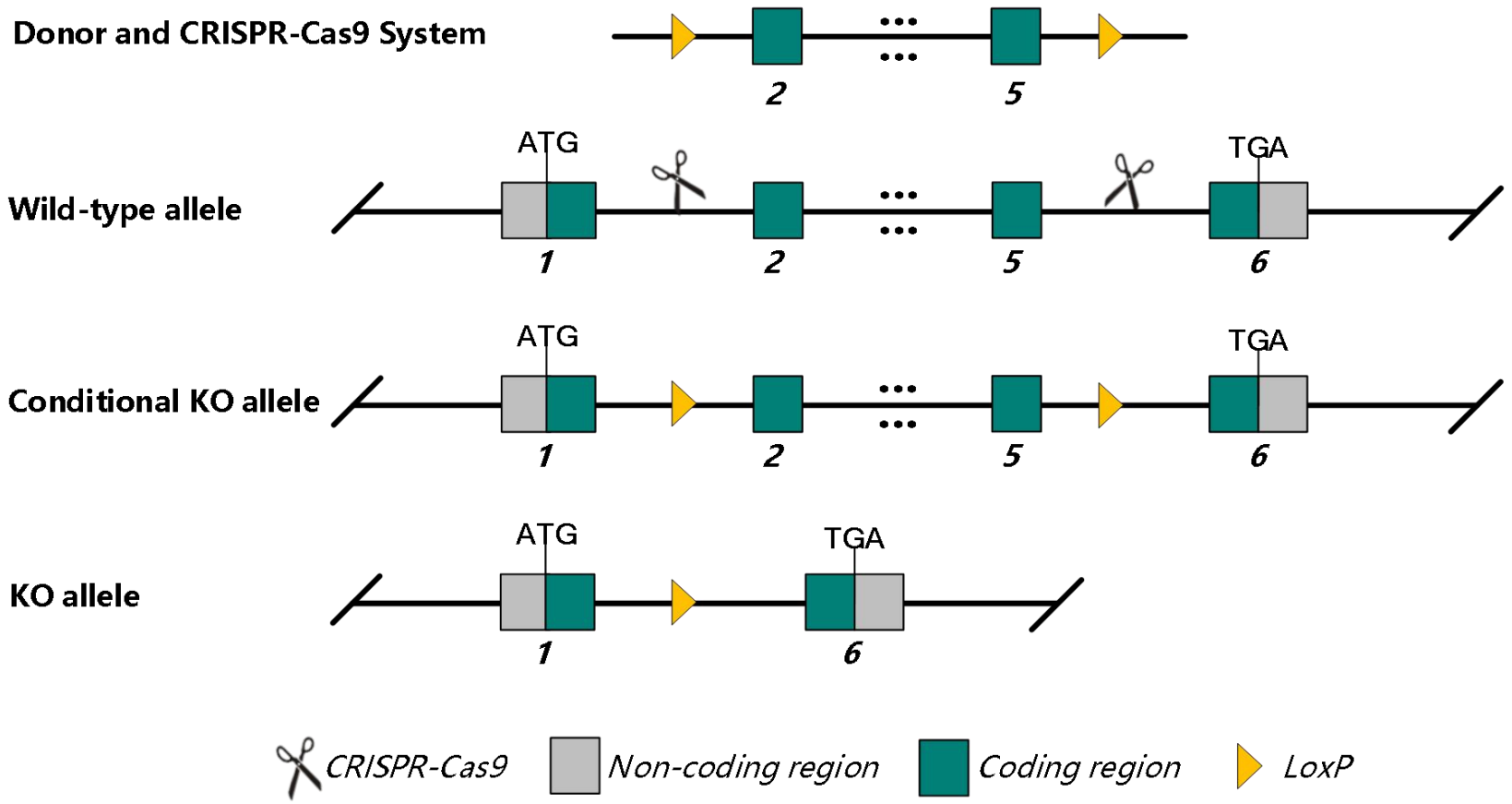
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Trim65* gene.

Technical Information

- The *Trim65* gene has 3 transcripts. According to the structure of *Trim65* gene, exon 2-5 of *Trim65*-201 (ENSMUST00000067632.4) is recommended as the knockout region. The region contains 574 bp of coding sequence. Knocking out the region will result in disruption of gene function.
- In this project we use CRISPR-Cas9 technology to modify *Trim65* 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

Trim65 tripartite motif-containing 65 [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 338364, updated on 9-Mar-2023

Summary

Official Symbol	Trim65 provided by MGI
Official Full Name	tripartite motif-containing 65 provided by MGI
Primary source	MGI:MGI:2442815
See related	Ensembl:ENSMUSG00000054517 AllianceGenome:MGI:2442815
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	4732463G12Rik
Summary	Predicted to enable metal ion binding activity. Predicted to be involved in several processes, including defense response to other organism; negative regulation of viral transcription; and regulation of viral entry into host cell. Predicted to be located in cytosol and nucleoplasm. Orthologous to several human genes including TRIM65 (tripartite motif containing 65). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Ubiquitous expression in spleen adult (RPKM 8.3), ovary adult (RPKM 6.0) and 27 other tissues See more
Orthologs	human all

NEW **Try the new Gene table**

Try the new Transcript table

Genomic context

Location: 11; 11 E2

See Trim65 in Genome Data Viewer

Exon count: 9

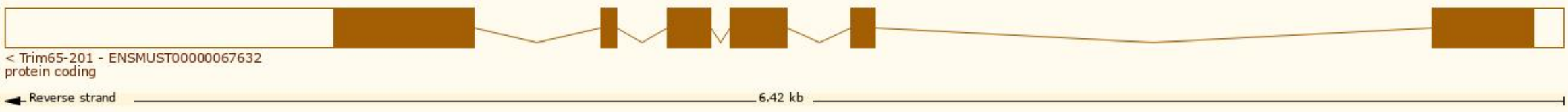
https://www.ncbi.nlm.nih.gov/gene/338364

Transcript Information

The gene has 3 transcripts, all transcripts are shown below:

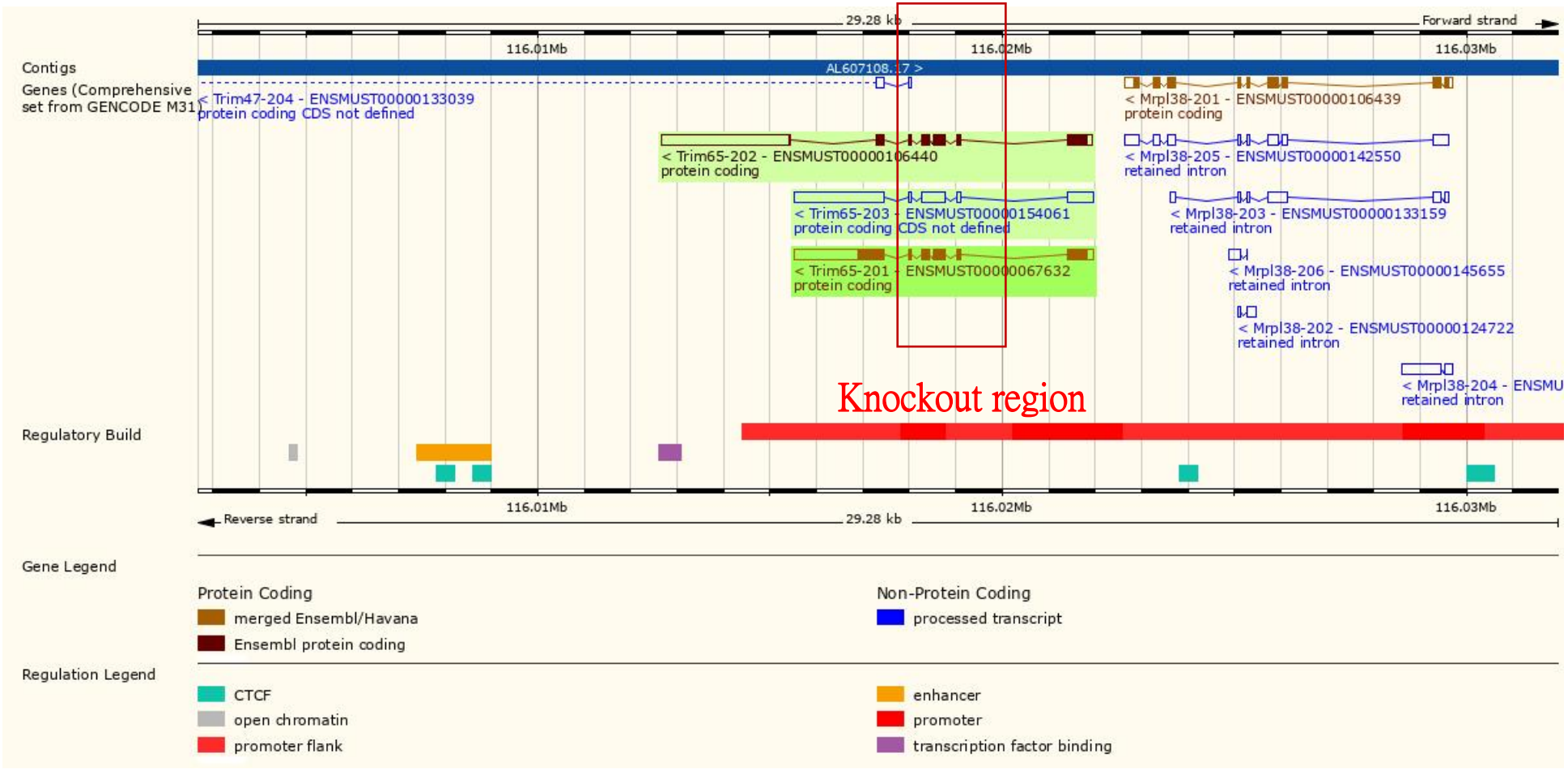
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000106440.9	Trim65-202	4040	396aa	Protein coding		Q8BFW4-2	GENCODE basic TSL:5
ENSMUST00000154061.2	Trim65-203	3132	No protein	Protein coding CDS not defined		-	TSL:2
ENSMUST00000067632.4	Trim65-201	3048	522aa	Protein coding	CCDS48981	Q8BFW4-1	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1

The strategy is based on the design of *Trim65*-201 transcript, the transcription is shown below:



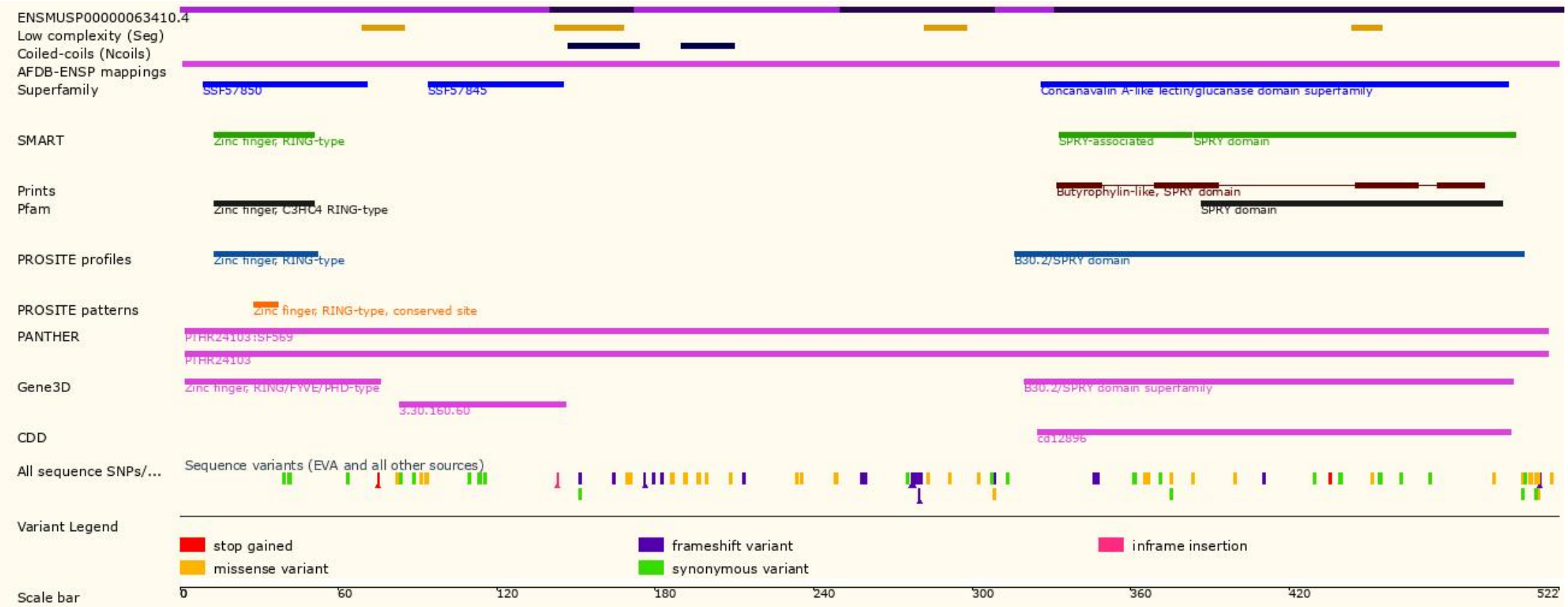
Source: http://asia.ensembl.org/

Genomic Information



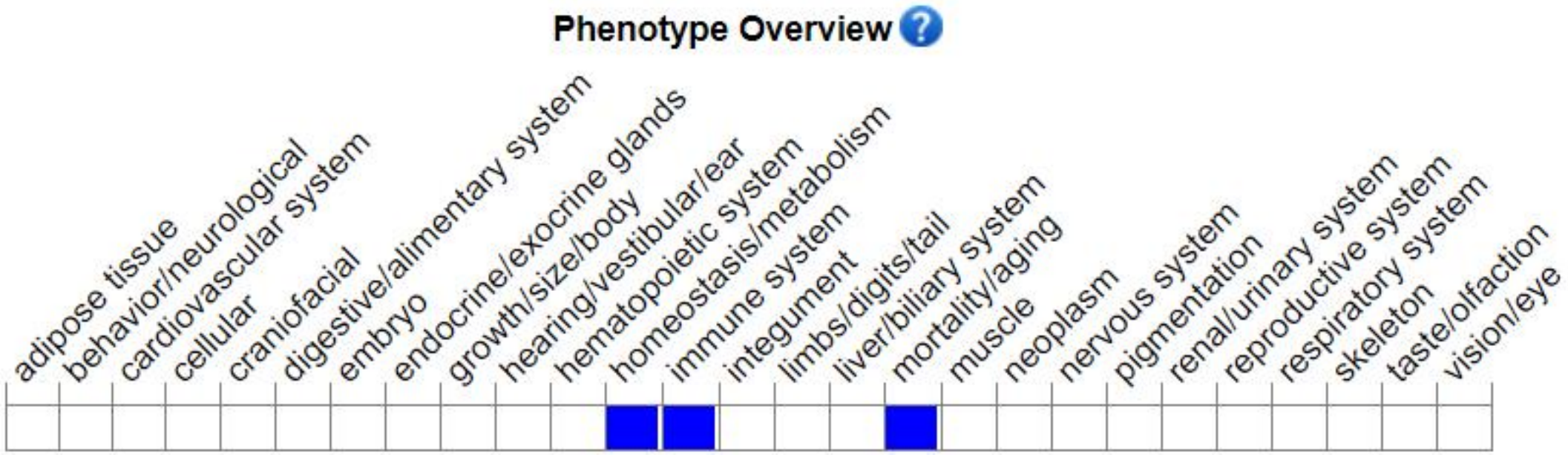
Source:http://asia.ensembl.org/

Protein Information



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

Mouse Phenotype Information (MGI)



- Mice homozygous for a knock-out allele exhibit increased susceptibility to Picornaviridae infection and associated morbidity/ mortality, with markedly decreased circulating type I interferon levels following infection with encephalomyocarditis virus.

Source: https://www.informatics.jax.org/marker/MGI:2442815

Important Information

- The intron 5-6 is 521 bp, the loxp insertion may affect the regulation of this gene.
- The knockout region overlaps with *Trim47*-204 transcript, which will knockout this transcript.
- *Trim65* is located on Chr 11. 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.