

Gzma Cas9-CKO Strategy

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Project Overview

Project Name

Gzma

Project type

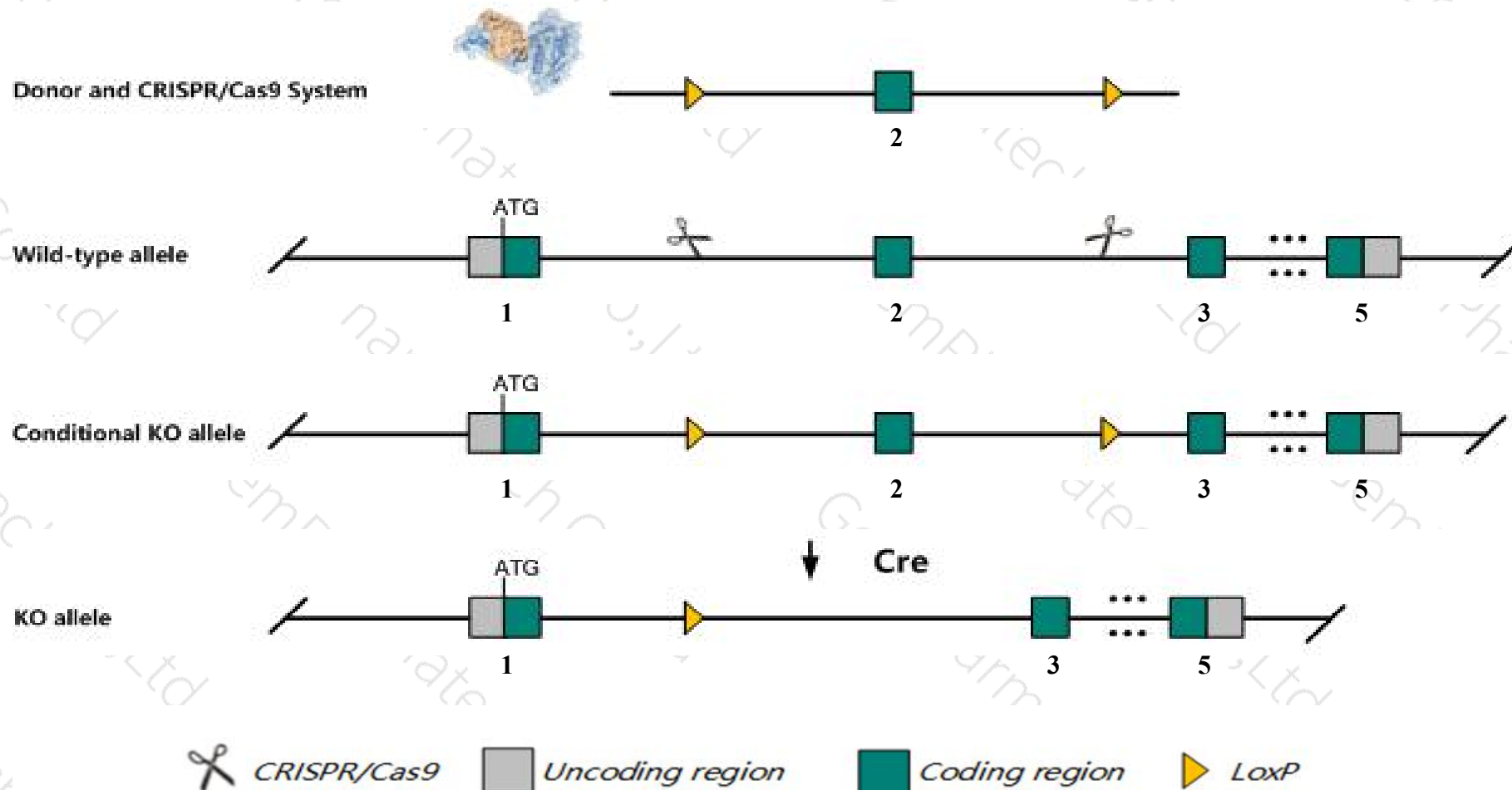
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



Technical routes

- The *Gzma* gene has 2 transcripts. According to the structure of *Gzma* gene, exon2 of *Gzma-201*(ENSMUST00000023897.6) transcript is recommended as the knockout region. The region contains 145bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Gzma* 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 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.

- According to the existing MGI data, homozygotes for a null allele show normal T/NK cell-mediated cytotoxicity, recovery from LCM virus or L. monocytogenes infection, and control of syngeneic tumor growth. Homozygotes for a different null allele show defective CTL cytolysis and increased tumor burden upon challenge with RMAS cells.
- The KO region contains functional region of the *Cdc20b* gene. Knockout the region may affect the function of *Cdc20b* gene.
- The *Gzma* gene is located on the Chr13. 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)

Gzma granzyme A [Mus musculus (house mouse)]

Gene ID: 14938, updated on 13-Mar-2020

Summary

Official Symbol Gzma provided by [MGI](#)

Official Full Name granzyme A provided by [MGI](#)

Primary source [MGI:MGI:109266](#)

See related [Ensembl:ENSMUSG00000023132](#)

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 AW494114, Ctla-3, Ctla3, Hf, Hf1, SE1, TSP-1, TSP1

Expression Biased expression in large intestine adult (RPKM 10.7), thymus adult (RPKM 4.7) and 9 other tissues [See more](#)

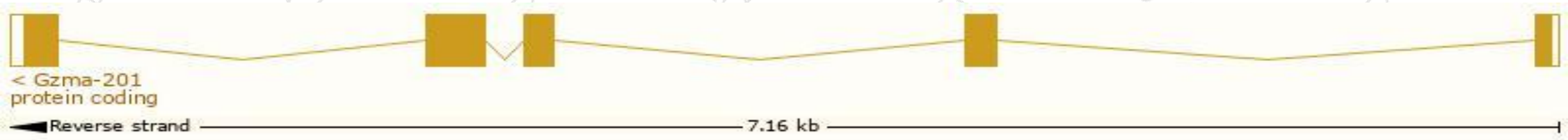
Orthologs [human](#) [all](#)

Transcript information (Ensembl)

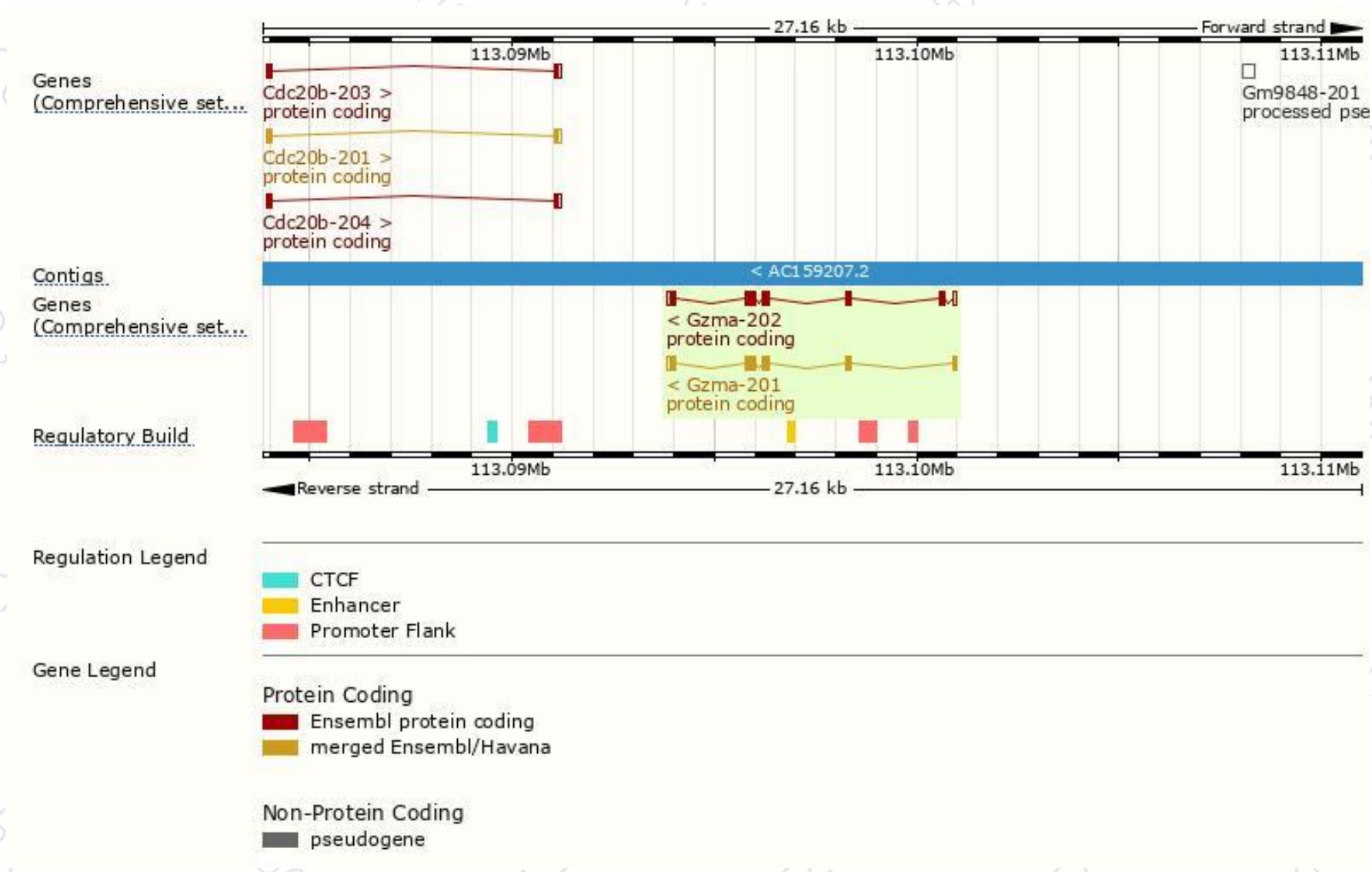
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Gzma-201	ENSMUST00000023897.6	878	260aa	Protein coding	CCDS26782	P11032_Q3U0N0	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 P2
Gzma-202	ENSMUST000000224282.1	996	257aa	Protein coding	-	P11032	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 ALT2

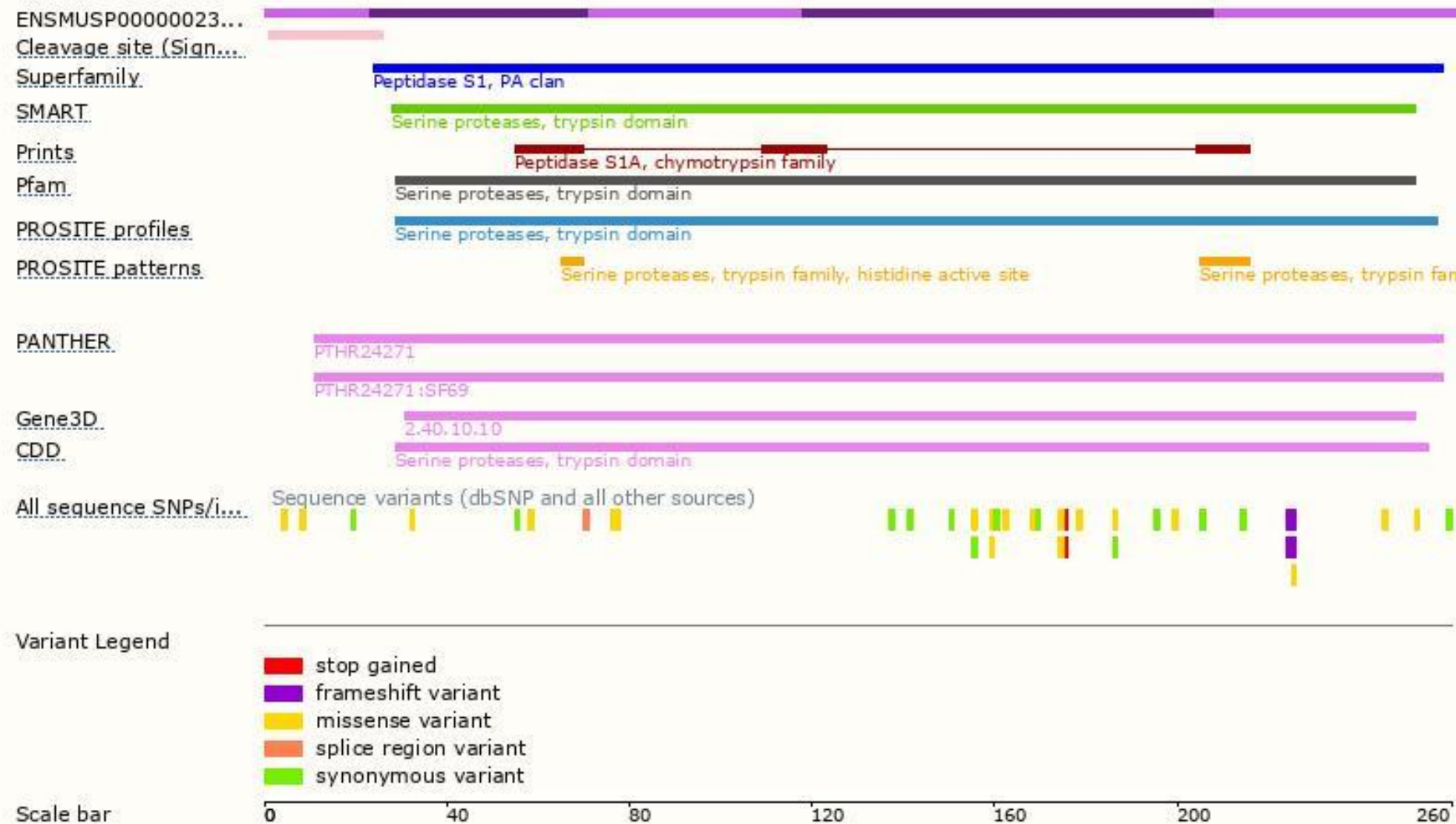
The strategy is based on the design of *Gzma-201* transcript,the transcription is shown below:



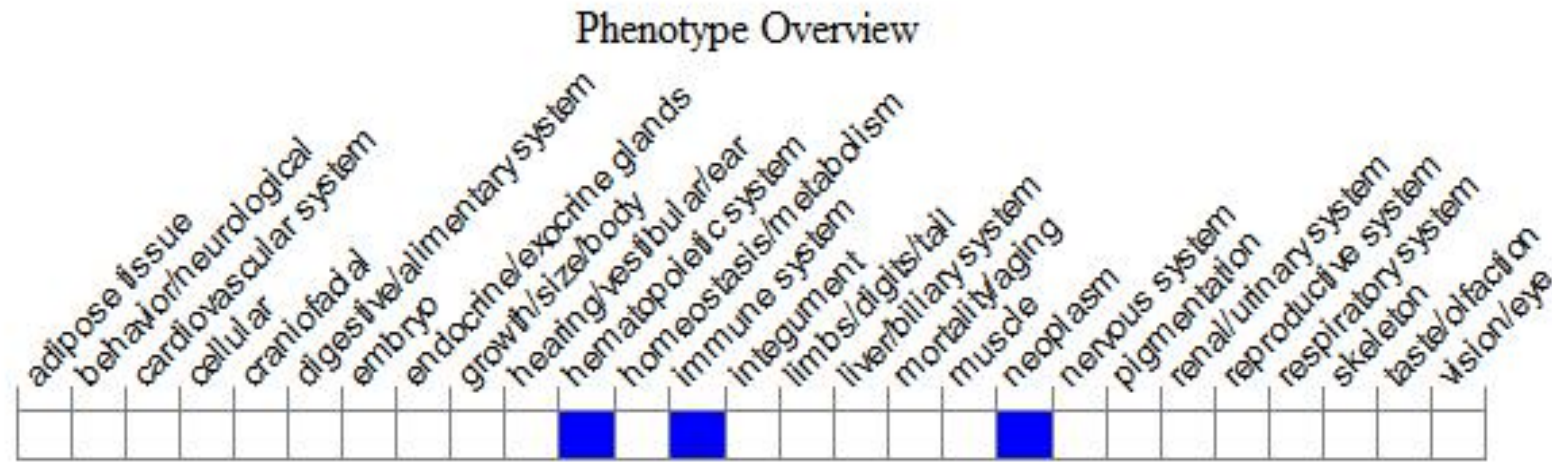
Genomic location distribution



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, homozygotes for a null allele show normal T/NK cell-mediated cytotoxicity, recovery from LCM virus or L. monocytogenes infection, and control of syngeneic tumor growth. Homozygotes for a different null allele show defective CTL cytotoxicity and increased tumor burden upon challenge with RMA-S cells.

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

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