

***Milr1* Cas9-CKO Strategy**

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Design Date: 2020-7-29

Project Overview

Project Name

Milr1

Project type

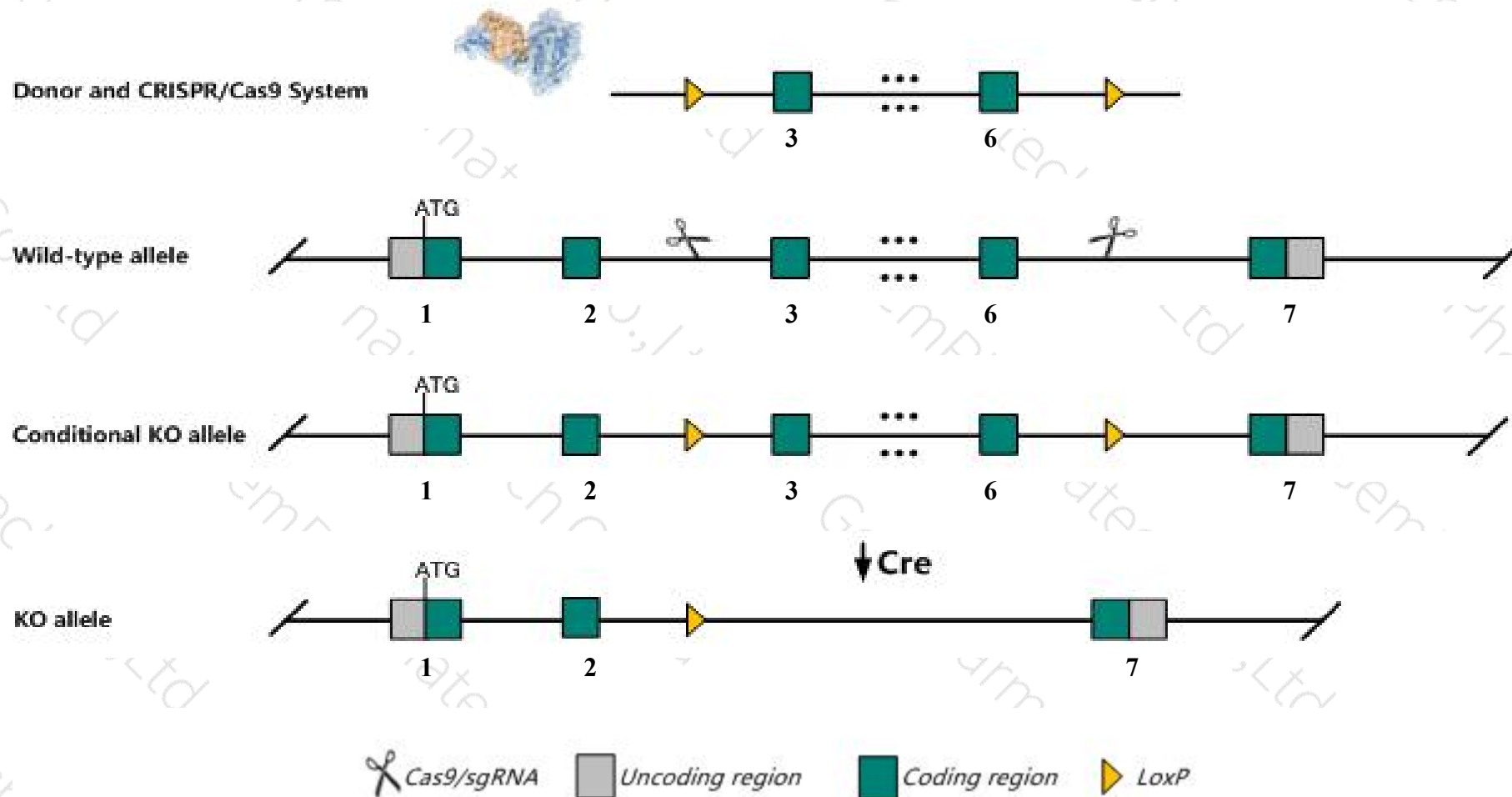
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Milr1* gene. The schematic diagram is as follows:



Technical routes

- The *Milr1* gene has 8 transcripts. According to the structure of *Milr1* gene, exon3-exon6 of *Milr1*-206(ENSMUST00000182896.7) transcript is recommended as the knockout region. The region contains 533bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Milr1* 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 null allele have decreased mast cell degranulation and an increased susceptibility to type I hypersensitivity reaction.
- The effect on transcript *Milr1*-205 is unknown.
- *Gm25889* gene will be deleted.
- The floxed region is near to the N-terminal of *Pecam1* gene, this strategy may influence the regulatory function of the N-terminal of *Pecam1* gene.
- The *Milr1* gene is located on the Chr11. 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)

Milr1 mast cell immunoglobulin like receptor 1 [Mus musculus (house mouse)]

Gene ID: 380732, updated on 13-Mar-2020

Summary



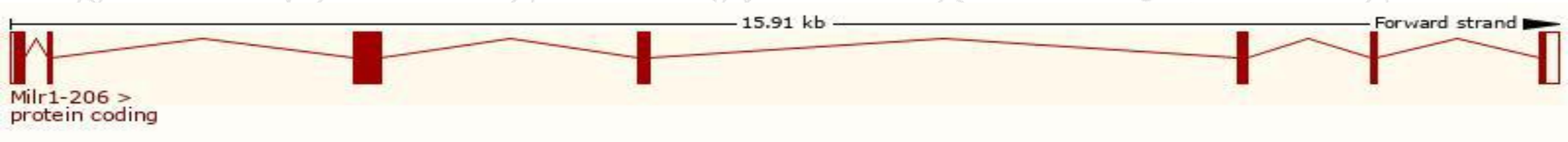
Official Symbol	Milr1 provided by MGI
Official Full Name	mast cell immunoglobulin like receptor 1 provided by MGI
Primary source	MGI:MGI:2685731
See related	Ensembl:ENSMUSG00000040528
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Allergin-1, Gm885, Mca32
Expression	Low expression observed in reference dataset See more
Orthologs	human all

Transcript information (Ensembl)

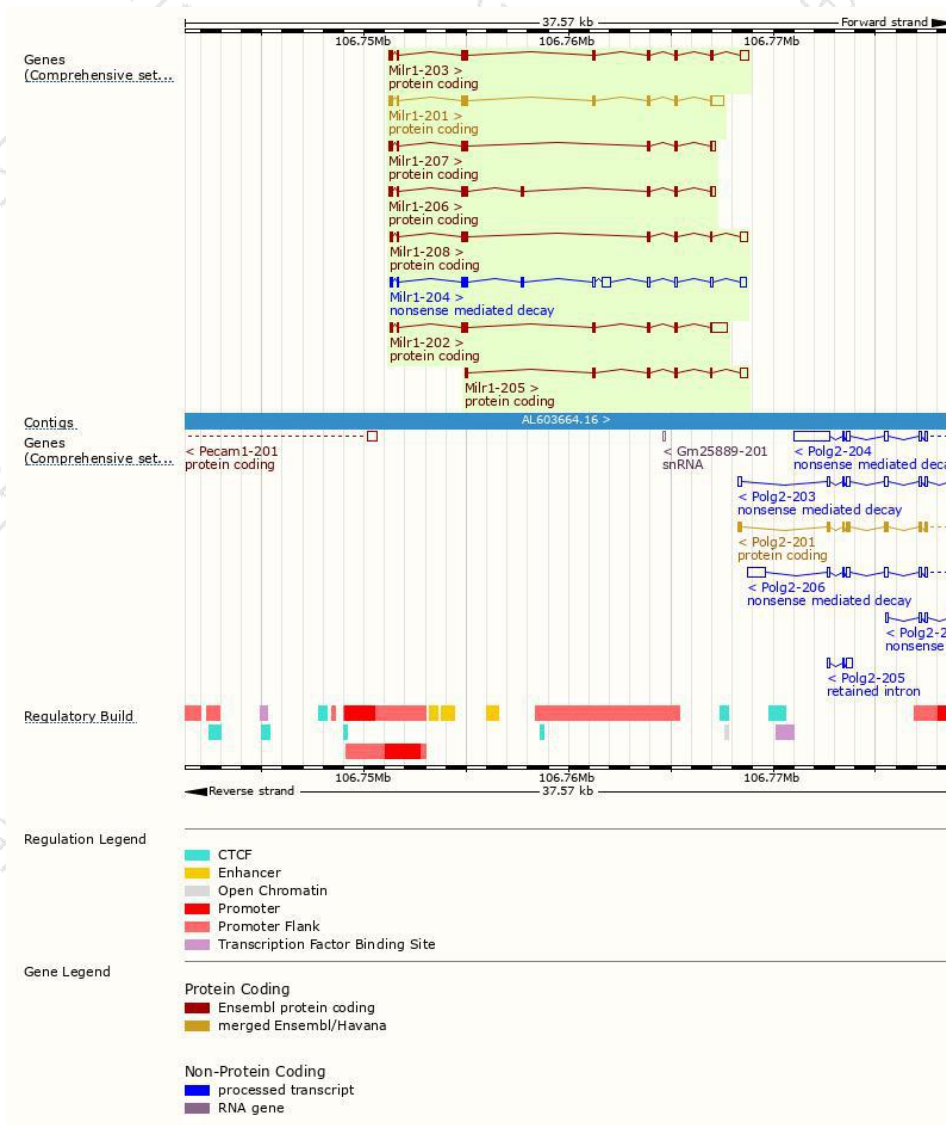
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Milr1-202	ENSMUST00000106794.8	1514	245aa	Protein coding	CCDS70334	A0A140T8Q6	TSL:1 GENCODE basic APPRIS ALT2
Milr1-201	ENSMUST00000086353.10	1348	246aa	Protein coding	CCDS25560	Q3TB92	TSL:1 GENCODE basic APPRIS P3
Milr1-203	ENSMUST00000147326.8	1234	246aa	Protein coding	CCDS25560	Q3TB92	TSL:5 GENCODE basic APPRIS P3
Milr1-208	ENSMUST00000183111.7	982	208aa	Protein coding	CCDS70335	Q3TB92	TSL:1 GENCODE basic APPRIS ALT2
Milr1-206	ENSMUST00000182896.7	918	246aa	Protein coding	CCDS70333	B7ZP19	TSL:1 GENCODE basic APPRIS ALT2
Milr1-207	ENSMUST00000182908.7	811	208aa	Protein coding	CCDS70335	Q3TB92	TSL:1 GENCODE basic APPRIS ALT2
Milr1-205	ENSMUST00000182479.1	769	143aa	Protein coding	-	S4R1V2	CDS 5' incomplete TSL:5
Milr1-204	ENSMUST00000182023.1	1637	178aa	Nonsense mediated decay	-	A0A0B4J1N6	TSL:2

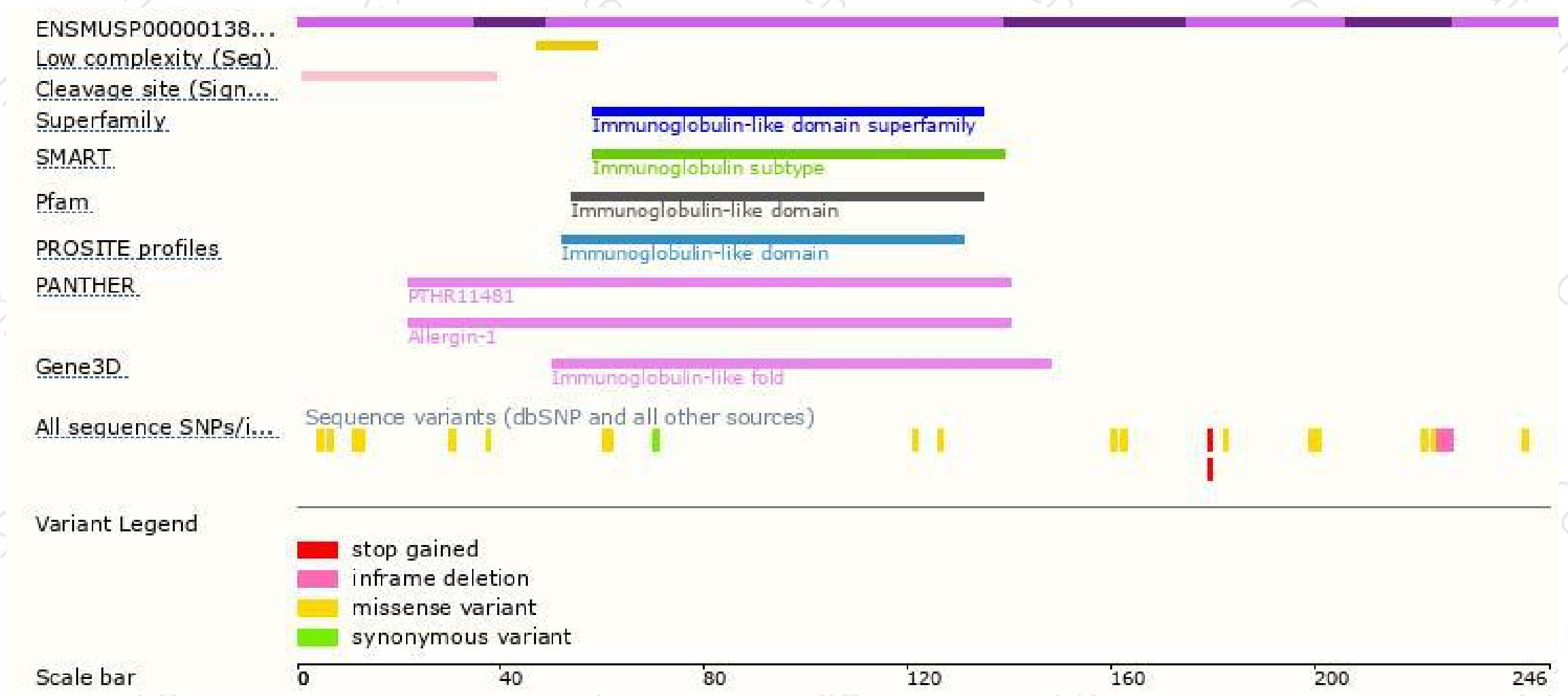
The strategy is based on the design of *Milr1-206* transcript,the transcription is shown below:



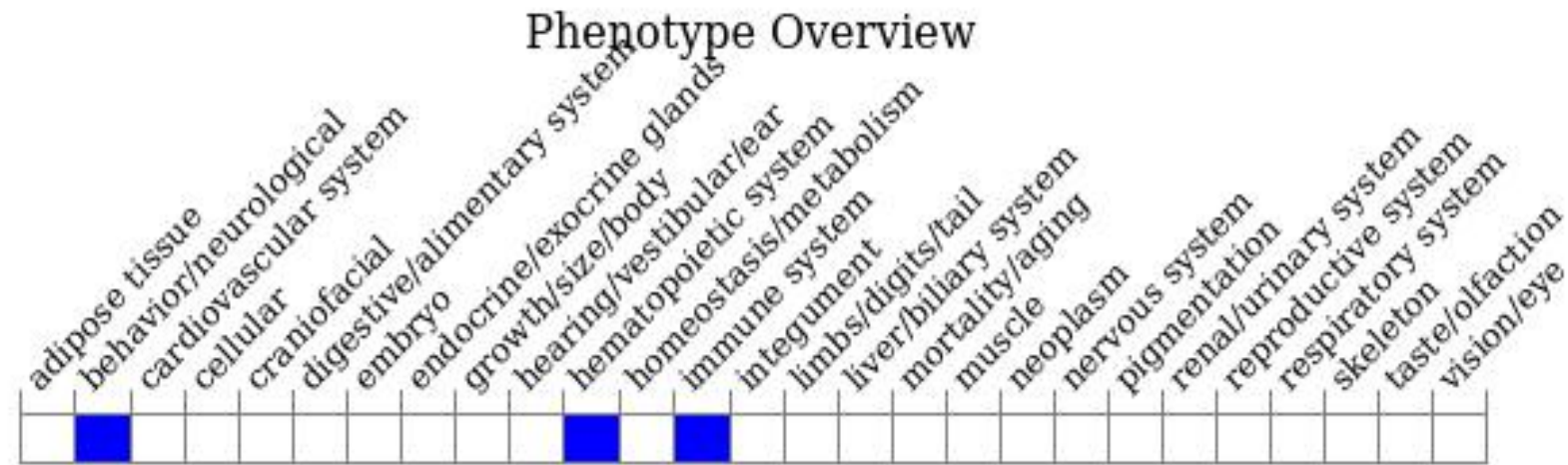
Genomic location distribution



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, mice homozygous for a null allele have decreased mast cell degranulation and an increased susceptibility to type I hypersensitivity reaction.

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

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