Psmb8 Cas9-CKO Strategy

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Reviewer: JiaYu

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Project Overview

Project Name	***Psmb8***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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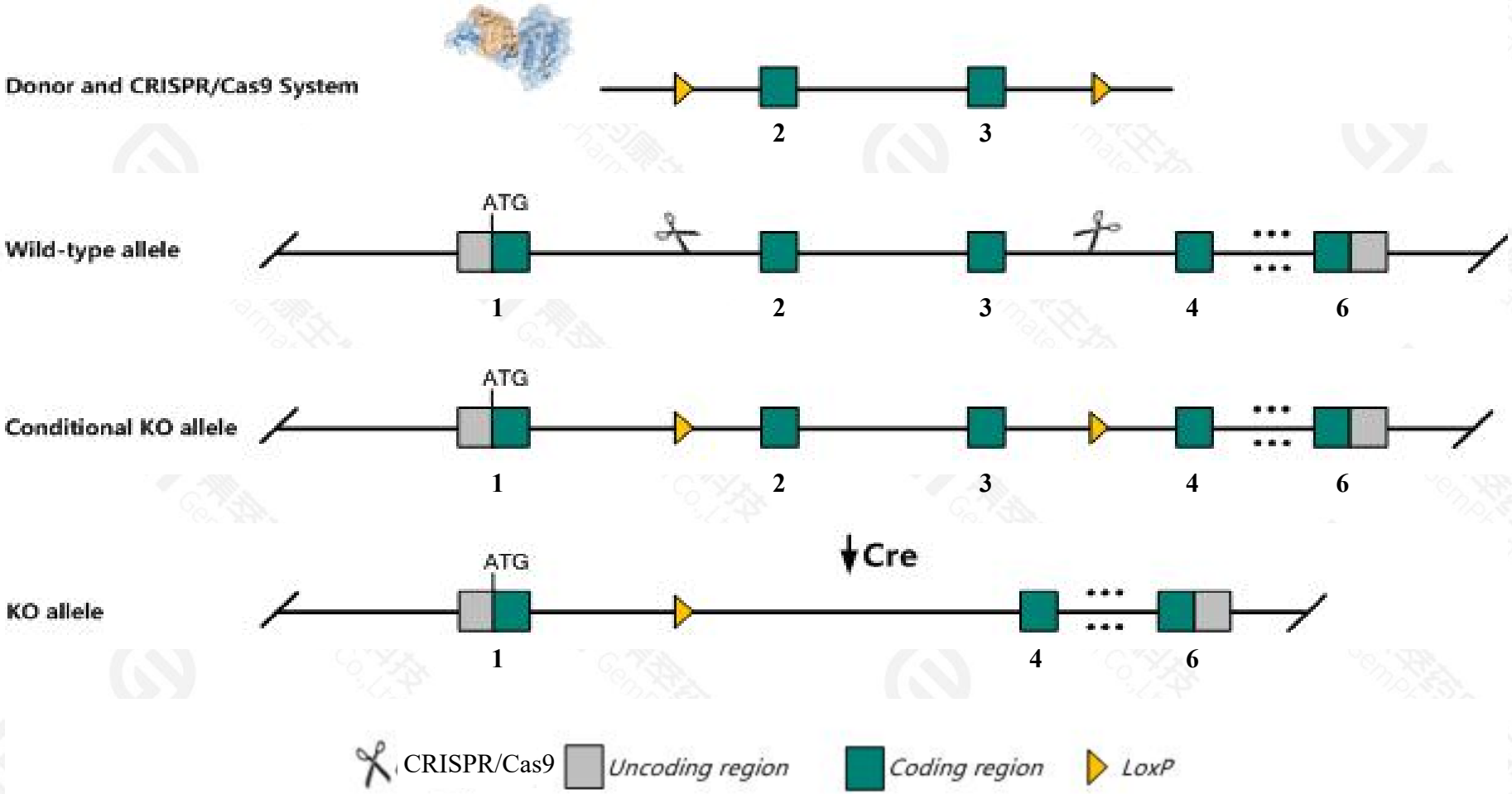
This model will use CRISPR/Cas9 technology to edit the *Psmb8* gene. The schematic diagram is as follows:



Technical routes

➢ The *Psmb8* gene has 7 transcripts. According to the structure of *Psmb8* gene, exon2-exon3 of *Psmb8-201*(ENSMUST00000025196.9) transcript is recommended as the knockout region.The region contains 260bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Psmb8* 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 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.

Notice

➢ According to the existing MGI data,mice homozygous for disruptions in this gene display an essentially normal phenotype. However they have a reduced ability to process MHC class I restricted antigens.

➢ The effect of this strategy on *Psmb8*-204 is unknown because the 5-terminus of the *Psmb8*-204 is incomplete.

➢ The knockout region is about 3.6 kb in the 5-terminal of *Tap2*, which may affect the 5-terminal regulation function of *Tap2*.

➢ This strategy also knocked out *Gm20496* gene.

➢ The intron3 is only 458bp,loxp insertion may affect mRNA splicing.

➢ The *Psmb8* gene is located on the Chr17. 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）

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Psmb8 proteasome (prosome, macropain) subunit, beta type 8 (large multifunctional peptidase 7) [Mus musculus (house mouse)]

Gene ID: 16913, updated on 13-Mar-2020

Summary

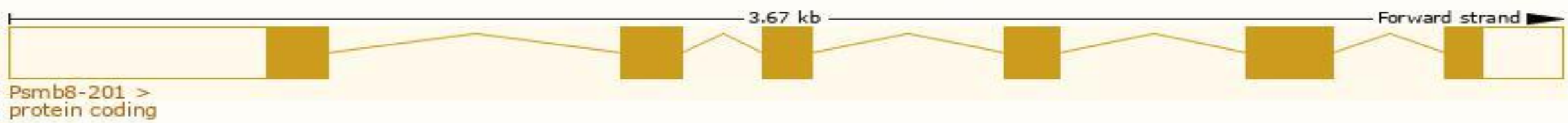
Official Symbol	Psmb8 provided by MGI
Official Full Name	proteasome (prosome, macropain) subunit, beta type 8 (large multifunctional peptidase 7) provided by MGI
Primary source	MGI:MGI:1346527
See related	Ensembl:ENSMUSG00000024338
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Lmp-7, Lmp7
Expression	Broad expression in thymus adult (RPKM 141.3), large intestine adult (RPKM 117.3) and 15 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

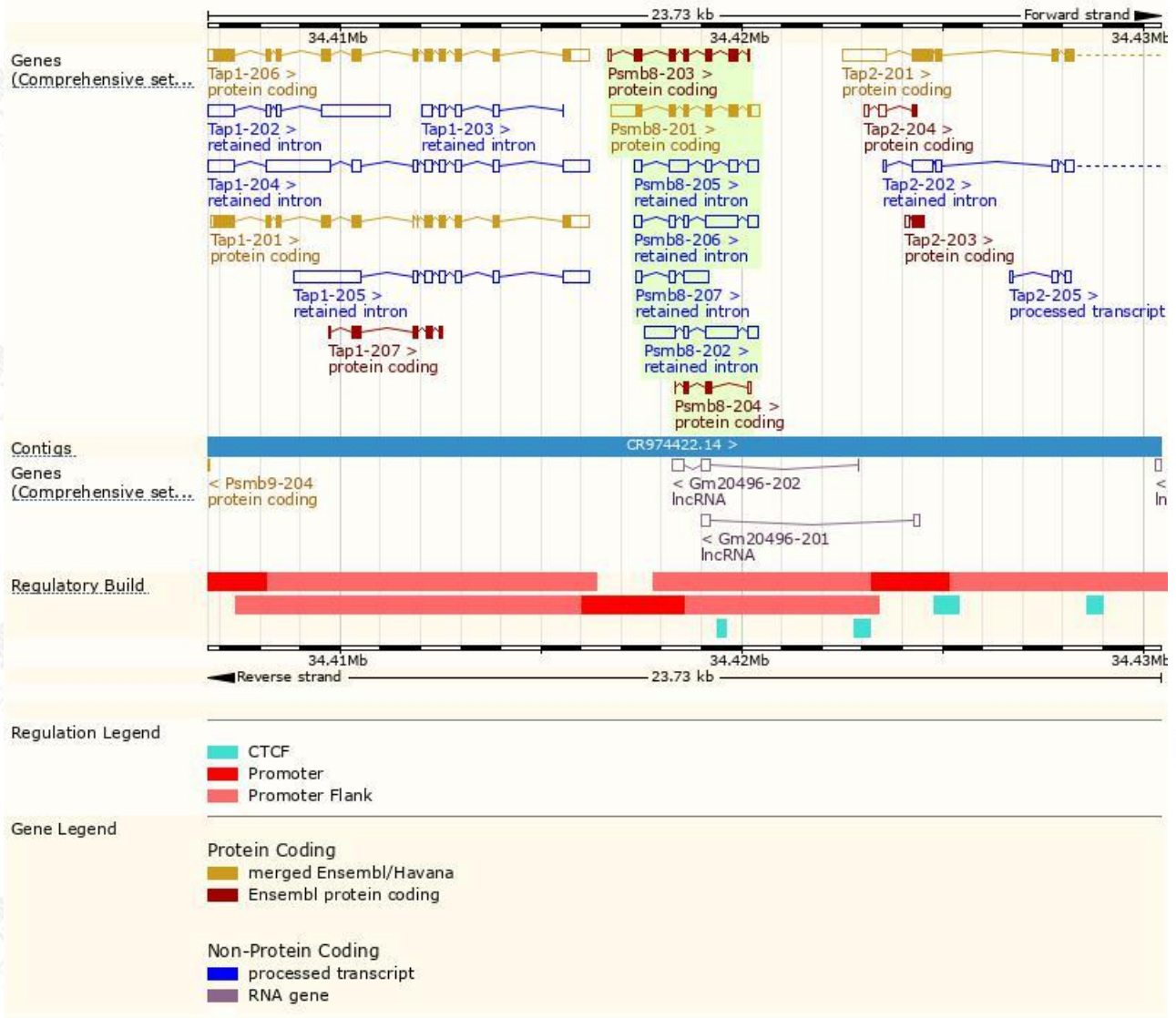
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Psmb8-201	ENSMUST00000025196.9	1628	276aa	Protein coding	CCDS50073	P28063	GENCODE basic APPRIS P1 TSL:1
Psmb8-202	ENSMUST00000172960.2	1907	No protein	Retained intron	-	-	TSL:2
Psmb8-203	ENSMUST00000173441.9	840	256aa	Protein coding	-	G3UZW8	TSL:5 CDS 3' incomplete
Psmb8-204	ENSMUST00000236331.2	358	93aa	Protein coding	-	A0A494BAB6	CDS 5' incomplete
Psmb8-205	ENSMUST00000236557.2	1234	No protein	Retained intron	-	-	-
Psmb8-206	ENSMUST00000237106.2	1484	No protein	Retained intron	-	-	-
Psmb8-207	ENSMUST00000238071.2	968	No protein	Retained intron	-	-	-

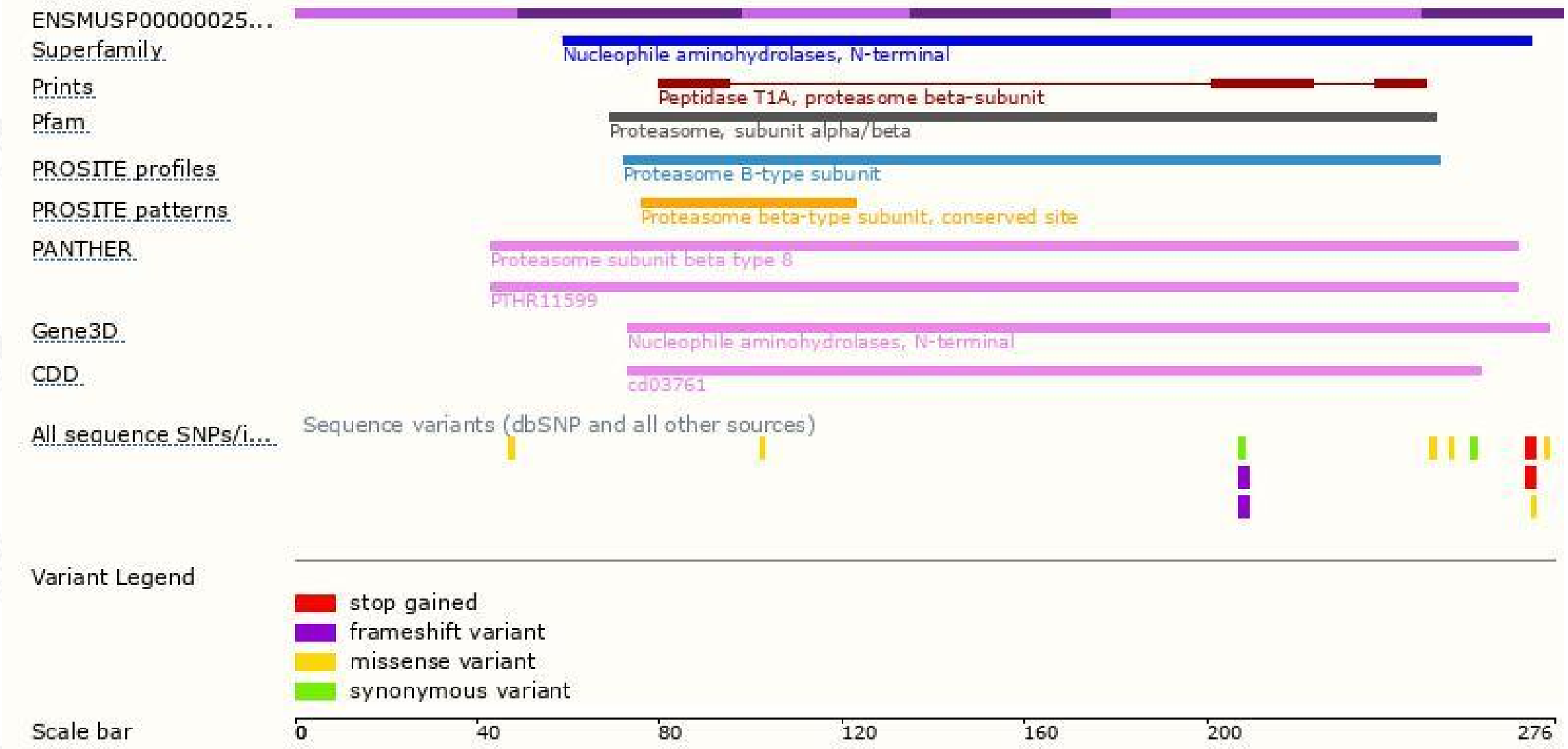
The strategy is based on the design of *Psmb8-201* transcript,the transcription is shown below:



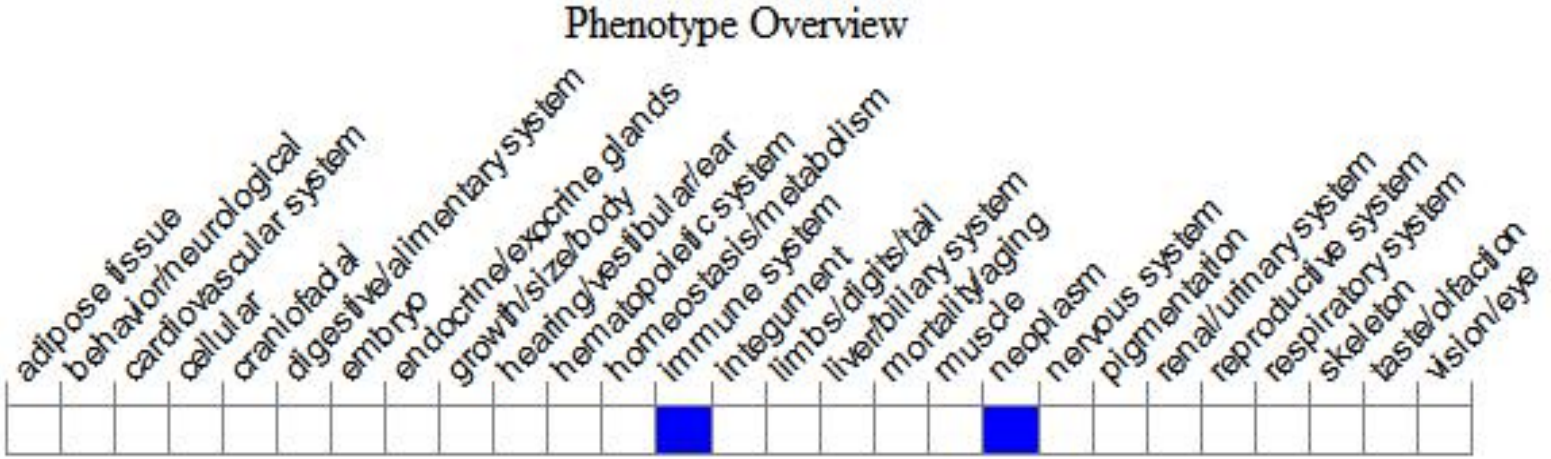
Genomic location distribution



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,mice homozygous for disruptions in this gene display an essentially normal phenotype. However they have a reduced ability to process MHC class I restricted antigens.

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

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