

***Grap2* Cas9-CKO Strategy**

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Project Overview

Project Name

Grap2

Project type

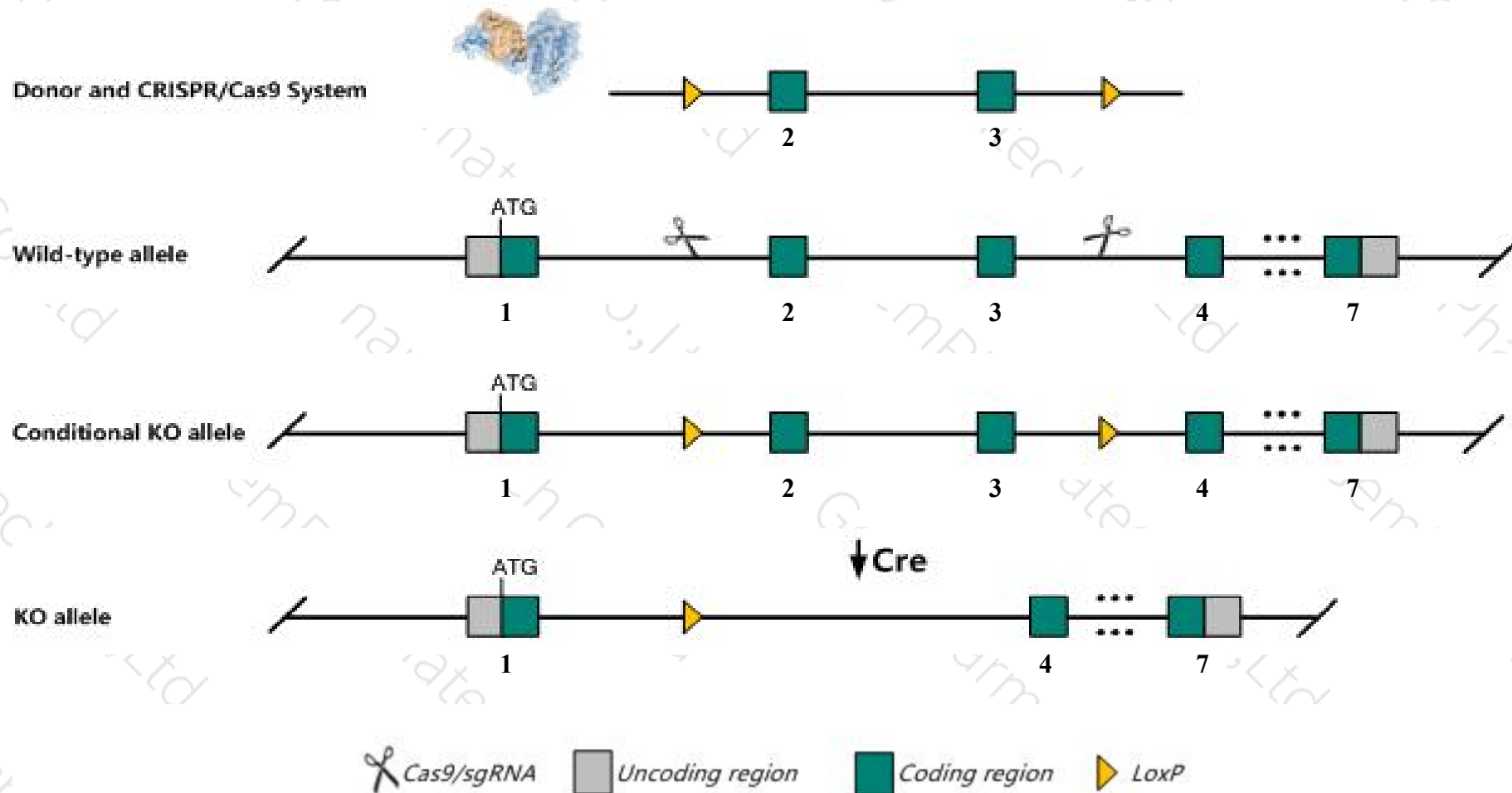
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Grp2* gene. The schematic diagram is as follows:



Technical routes

- The *Grap2* gene has 5 transcripts. According to the structure of *Grap2* gene, exon2-exon3 of *Grap2*-201(ENSMUST00000043149.8) transcript is recommended as the knockout region. The region contains 212bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Grap2* 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 disruptions in this gene are grossly healthy but display abnormal T cell development.
- The *Grap2* gene is located on the Chr15. 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)

Grap2 GRB2-related adaptor protein 2 [Mus musculus (house mouse)]

Gene ID: 17444, updated on 13-Mar-2020

Summary



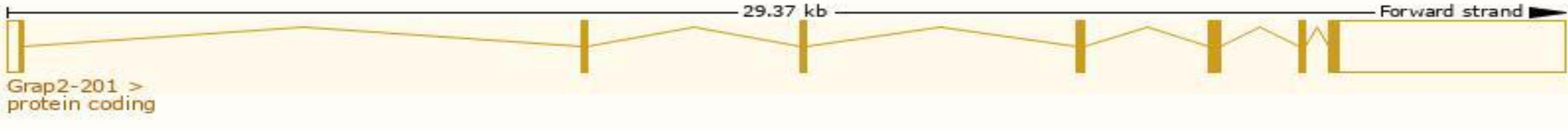
Official Symbol	Grap2 provided by MGI
Official Full Name	GRB2-related adaptor protein 2 provided by MGI
Primary source	MGI:MGI:1333842
See related	Ensembl:ENSMUSG00000042351
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	GRAP-2, GRB2L, GRBLG, GRID, Gads, GrbX, Grf40, GrpL, Mona, P38
Expression	Biased expression in thymus adult (RPKM 26.9), spleen adult (RPKM 12.9) and 5 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

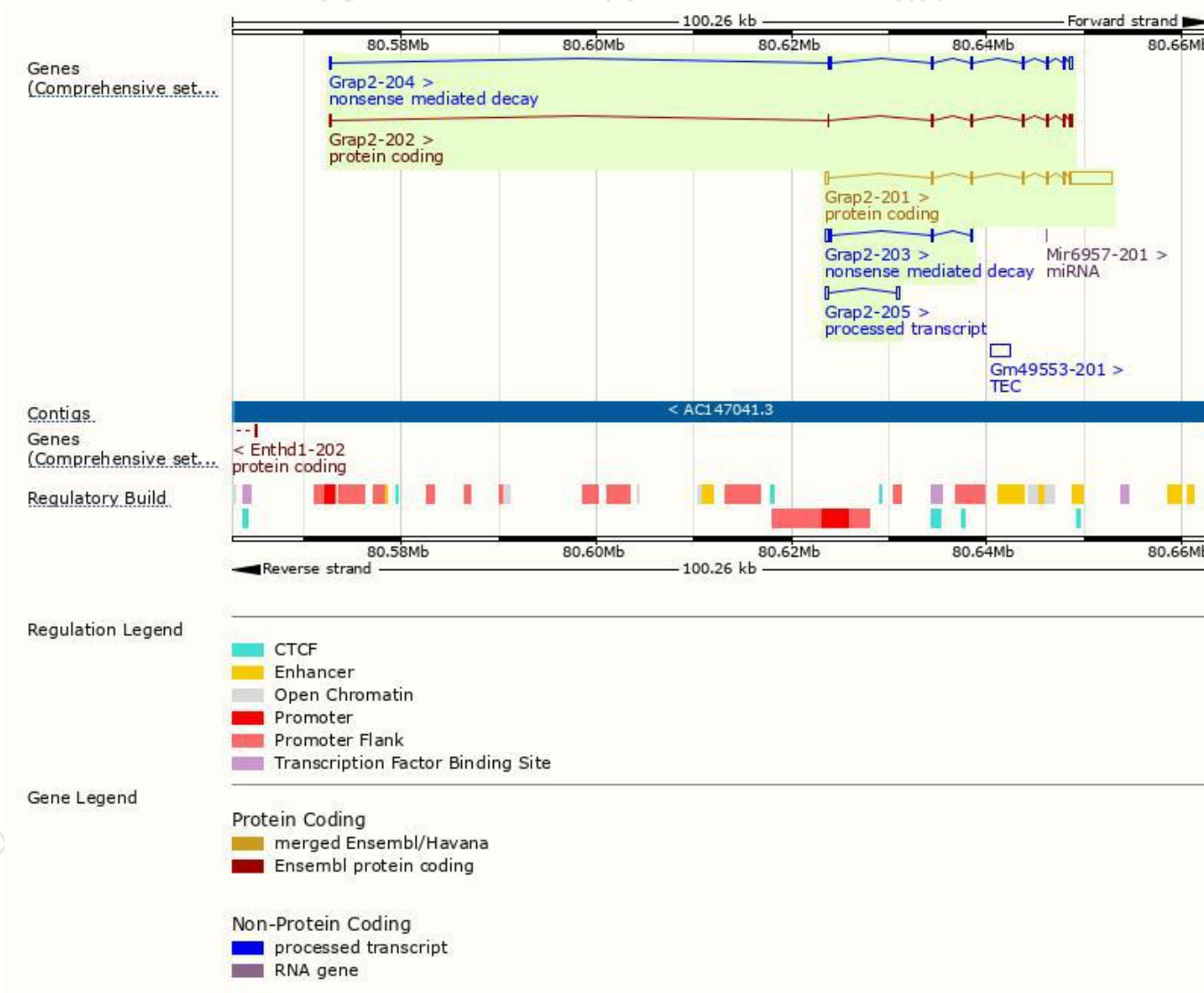
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Grap2-201	ENSMUST00000043149.8	5475	322aa	Protein coding	CCDS27663	O89100 Q3U0E8	TSL:1 GENCODE basic APPRIS P1
Grap2-202	ENSMUST00000229980.1	1294	322aa	Protein coding	CCDS27663	O89100 Q3U0E8	GENCODE basic APPRIS P1
Grap2-204	ENSMUST00000230856.1	1556	85aa	Nonsense mediated decay	-	A0A2R8VH91	
Grap2-203	ENSMUST00000230573.1	540	53aa	Nonsense mediated decay	-	A0A2R8VHI5	
Grap2-205	ENSMUST00000230910.1	679	No protein	Processed transcript	-	-	

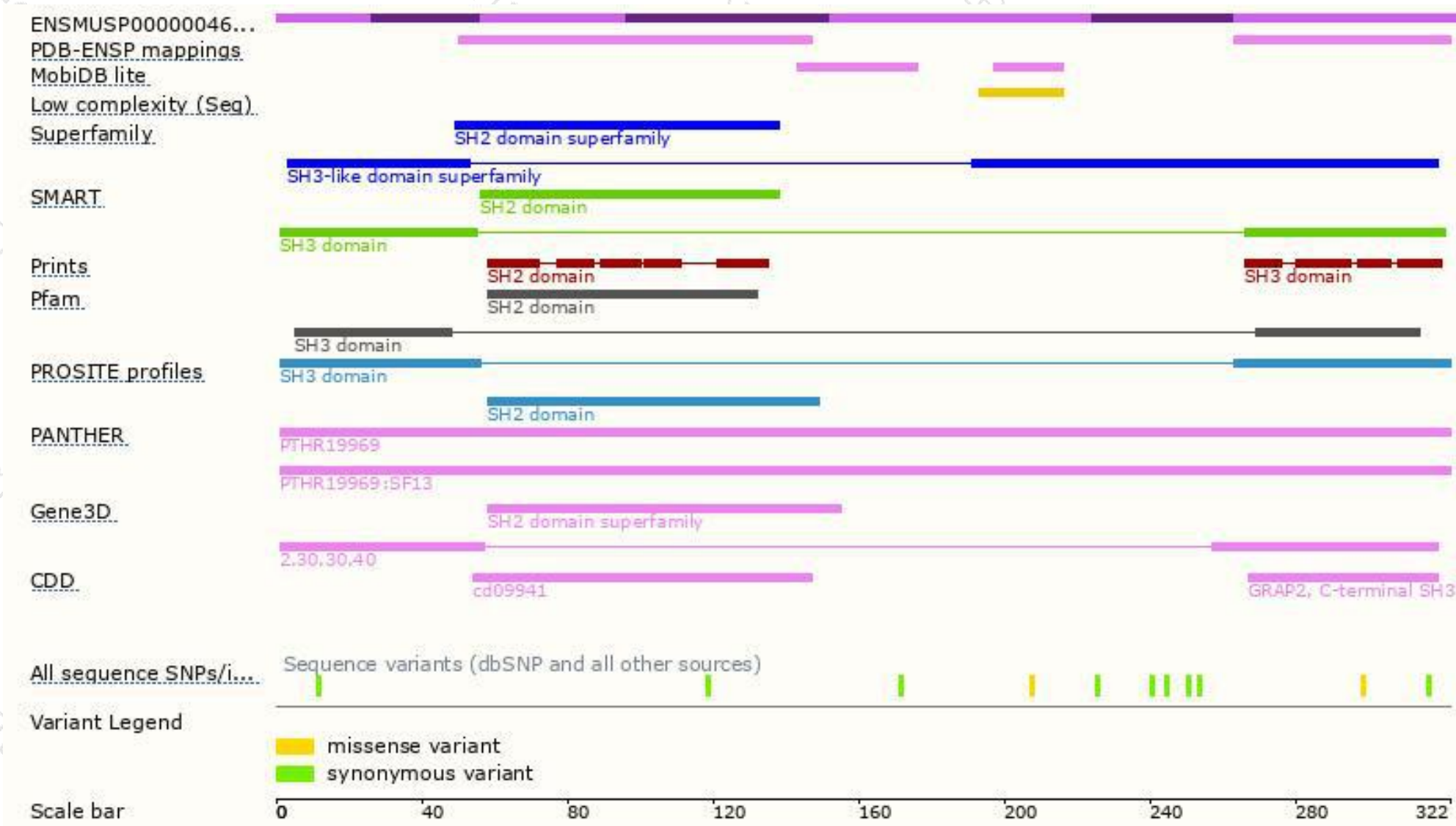
The strategy is based on the design of *Grap2-201* transcript,the transcription is shown below:



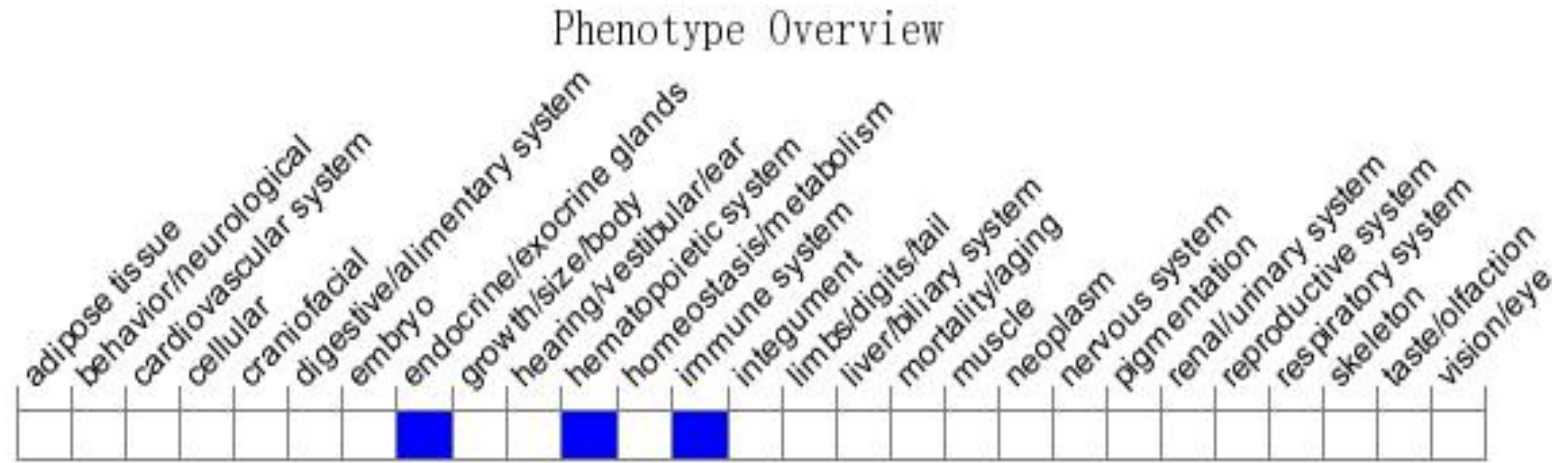
Genomic location distribution



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 are grossly healthy but display abnormal T cell development.

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

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