Rhbdf2 Cas9-CKO Strategy

Designer: Daohua Xu

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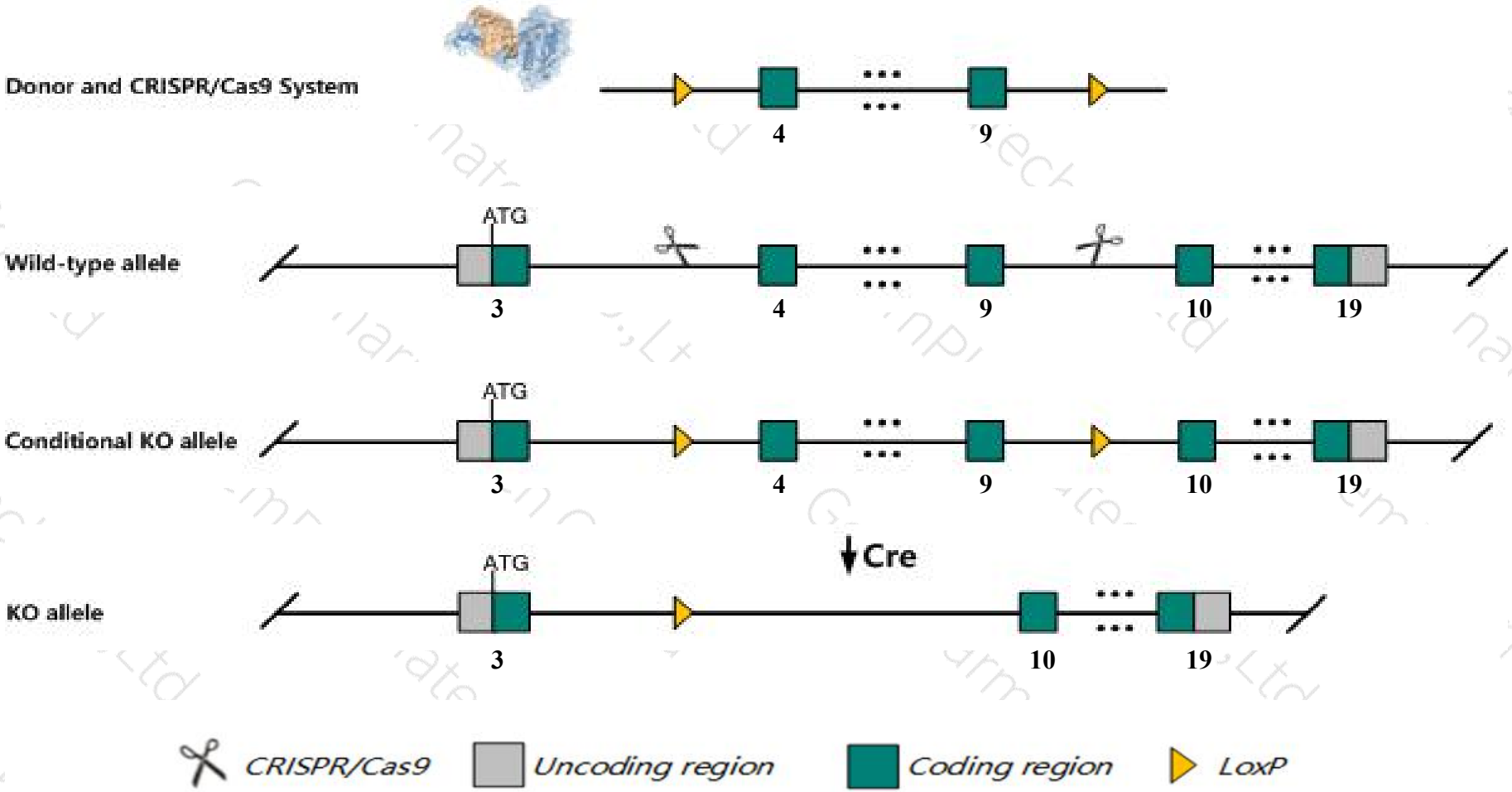
Project Overview

Project Name	***Rhbdf2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rhbdf2* gene. The schematic diagram is as follows:



Technical routes

- The *Rhbdf2* gene has 4 transcripts. According to the structure of *Rhbdf2* gene, exon4-exon9 of *Rhbdf2-201* (ENSMUST00000103028.7) transcript is recommended as the knockout region.The region contains 974bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Rhbdf2* 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.

Notice

➢ According to the existing MGI data,Mice homozygous for a null mutation display impaired TNF secretion and increased sensitivity to bacterial infection induced mortality.

➢ The *Rhbdf2* 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）

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Rhbdf2 rhomboid 5 homolog 2 [Mus musculus (house mouse)]

Gene ID: 217344, updated on 9-Apr-2019

Summary

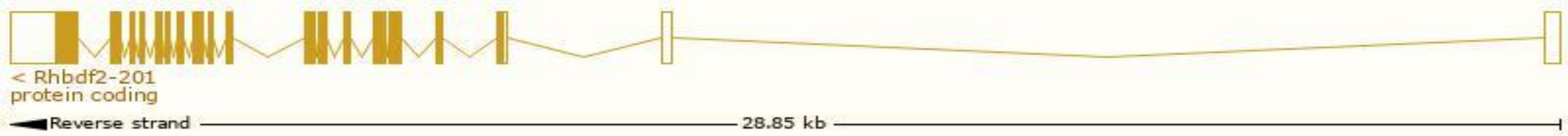
Official Symbol	Rhbdf2 provided by MGI
Official Full Name	rhomboid 5 homolog 2 provided by MGI
Primary source	MGI:MGI:2442473
See related	Ensembl:ENSMUSG00000020806
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	4732465I17Rik, Rhbdl6, cub
Expression	Broad expression in ovary adult (RPKM 35.3), spleen adult (RPKM 33.7) and 21 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

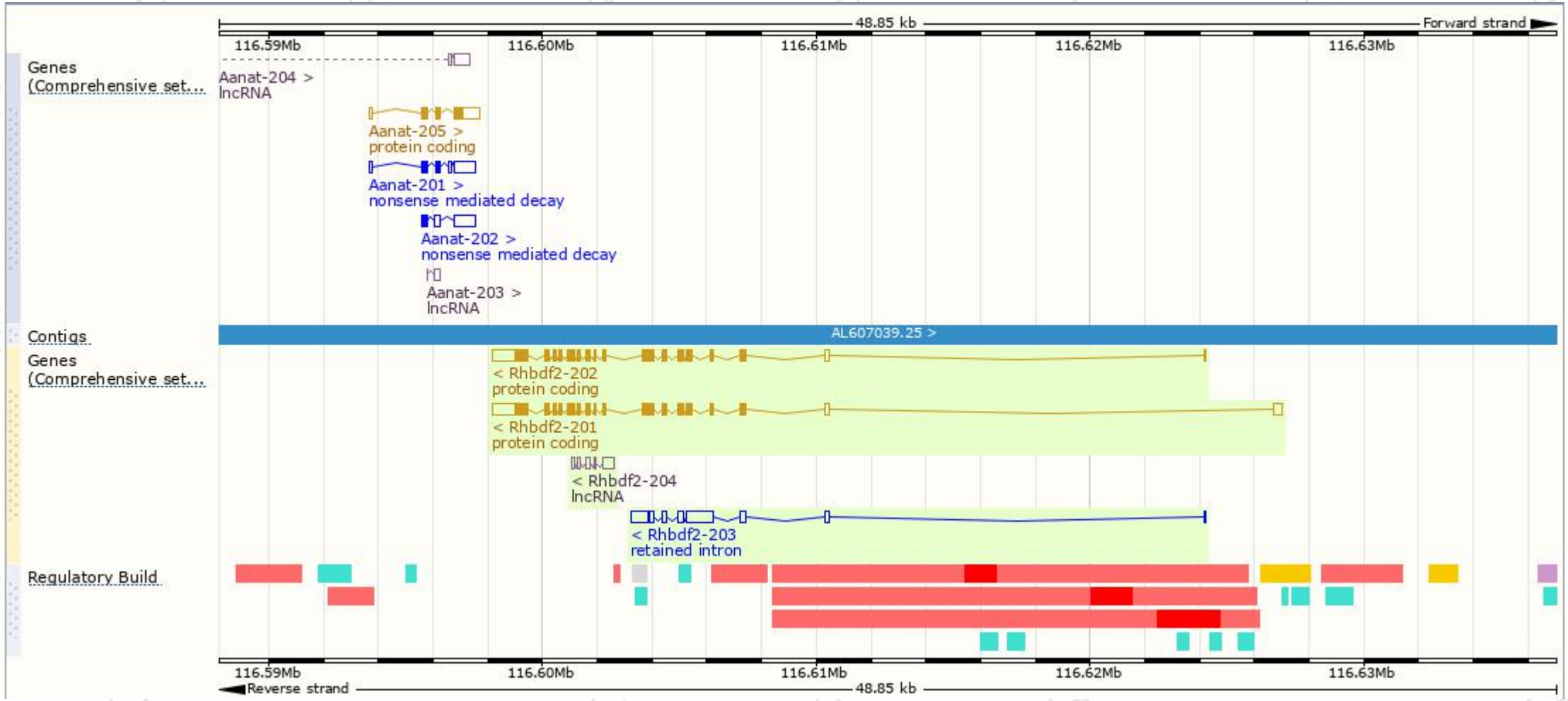
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rhbdf2-201	ENSMUST00000103028.7	3855	827aa	Protein coding	CCDS25672	Q80WQ6	TSL:1 GENCODE basic APPRIS P1
Rhbdf2-202	ENSMUST00000103029.9	3593	827aa	Protein coding	CCDS25672	Q80WQ6	TSL:1 GENCODE basic APPRIS P1
Rhbdf2-203	ENSMUST00000126819.1	2489	No protein	Retained intron	-	-	TSL:2
Rhbdf2-204	ENSMUST00000138125.1	815	No protein	lncRNA	-	-	TSL:5

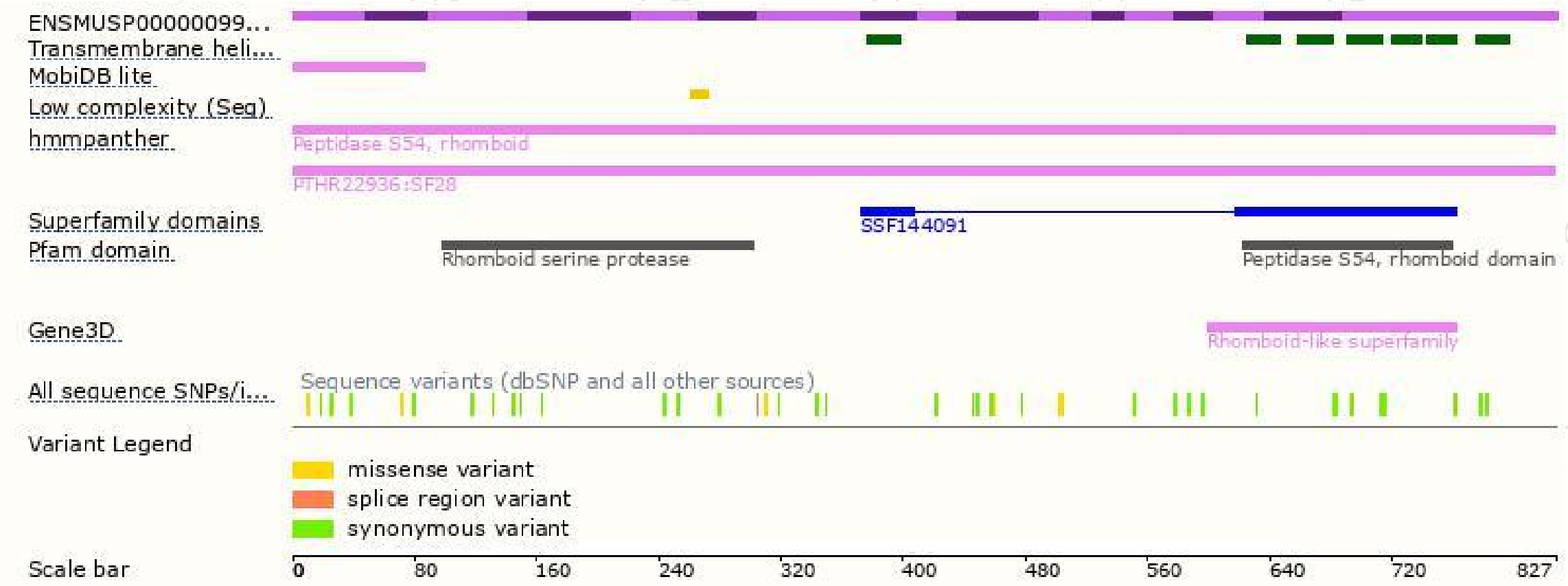
The strategy is based on the design of *Rhbdf2-201* transcript,The transcription is shown below



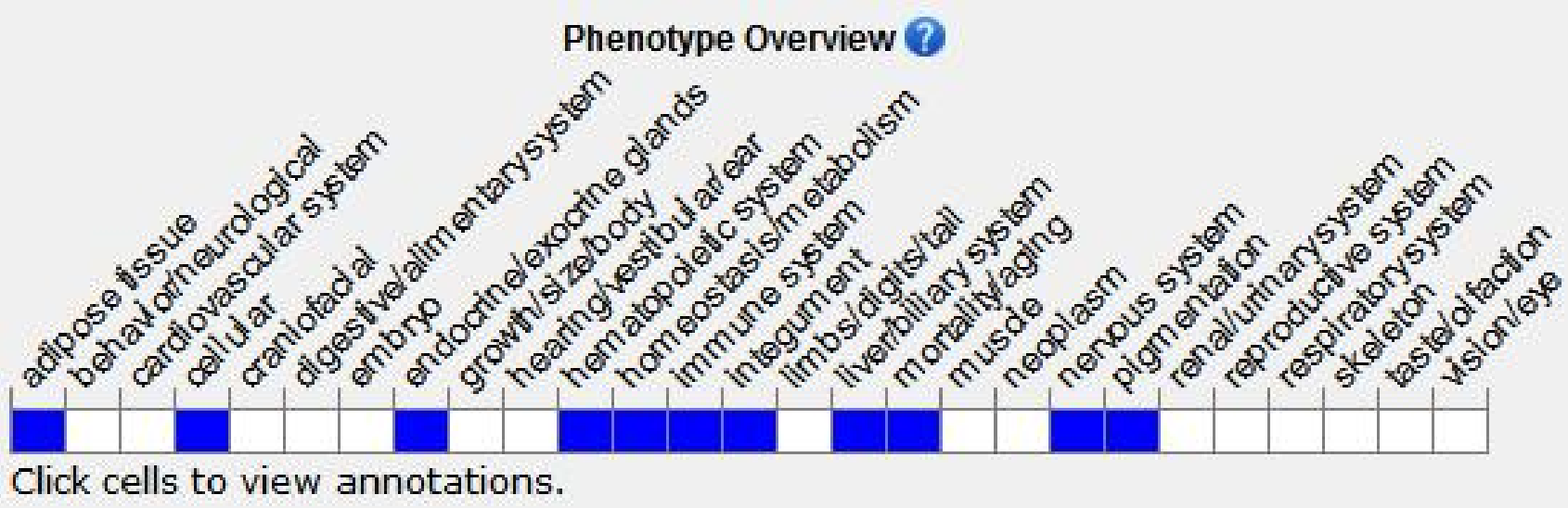
Genomic location distribution



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 a null mutation display impaired TNF secretion and increased sensitivity to bacterial infection induced mortality.

集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd