Itk Cas9-CKO Strategy

Designer: Huan Wang

Reviewer: Huan Fan

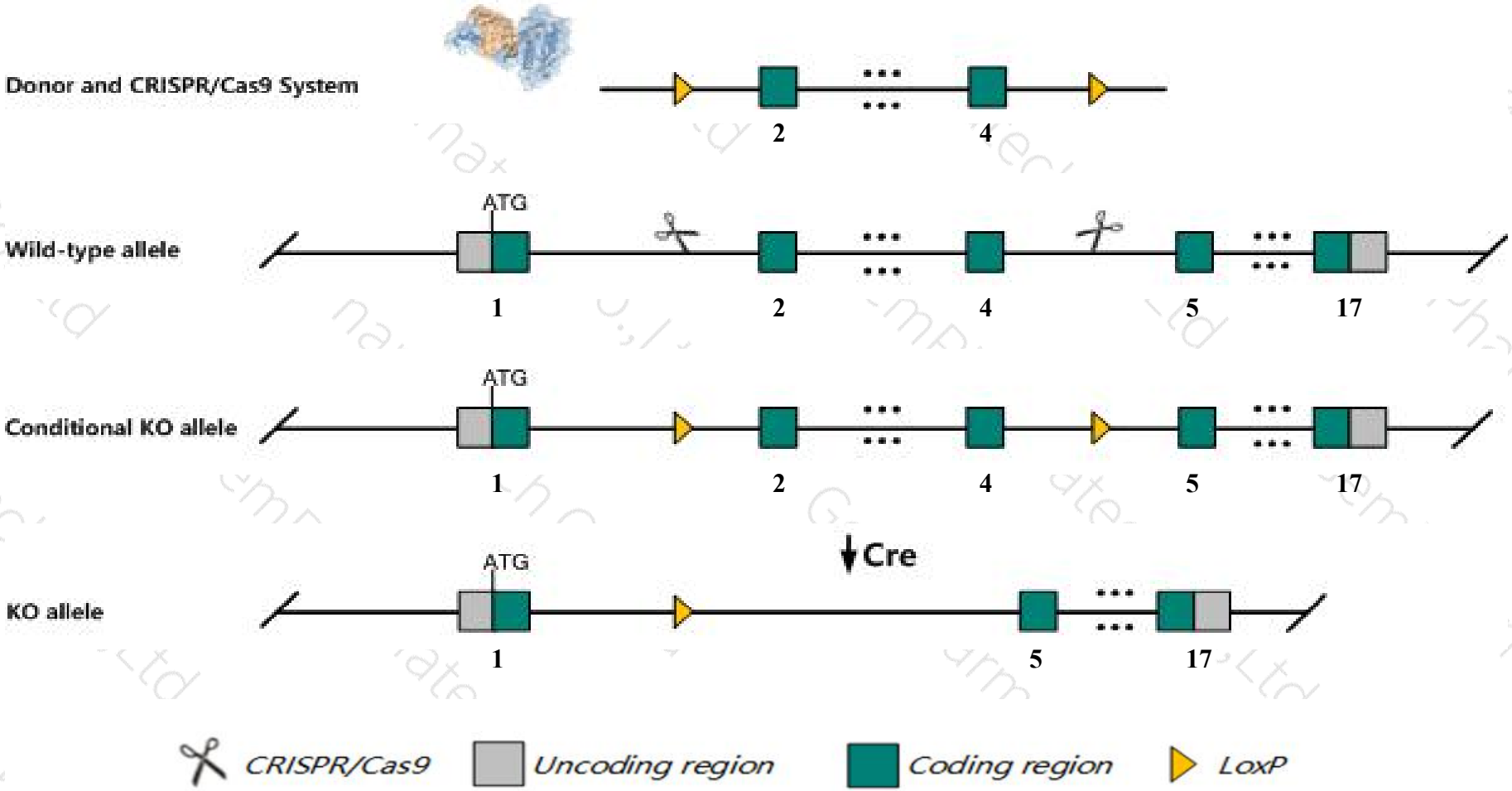
Design Date: 2020-4-10

Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Itk* gene has 4 transcripts. According to the structure of *Itk* gene, exon2-exon4 of *Itk-203* (ENSMUST00000109237.8) transcript is recommended as the knockout region.The region contains 334bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Itk* 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 disruptions in this gene display decreased percentages of CD4 and CD8 cells, increased percentage of B cells, impaired T cell receptor signaling, and increased susceptibility to Toxoplasma gondii infection.
- The *Itk* 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）

集萃药康 GemPharmatech

Itk IL2 inducible T cell kinase [Mus musculus (house mouse)]

Gene ID: 16428, updated on 13-Mar-2020

Summary

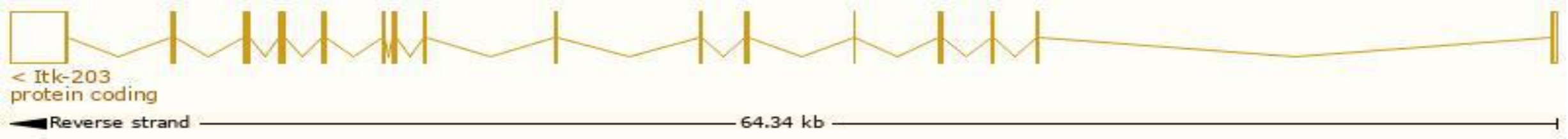
Official Symbol	Itk provided by MGI
Official Full Name	IL2 inducible T cell kinase provided byMGI
Primary source	MGI:MGI:96621
See related	Ensembl:ENSMUSG00000020395
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Emt, Tcsk, Tsk
Expression	Biased expression in thymus adult (RPKM 35.8), spleen adult (RPKM 4.7) and 1 other tissueSee more
Orthologs	human all

Transcript information（Ensembl）

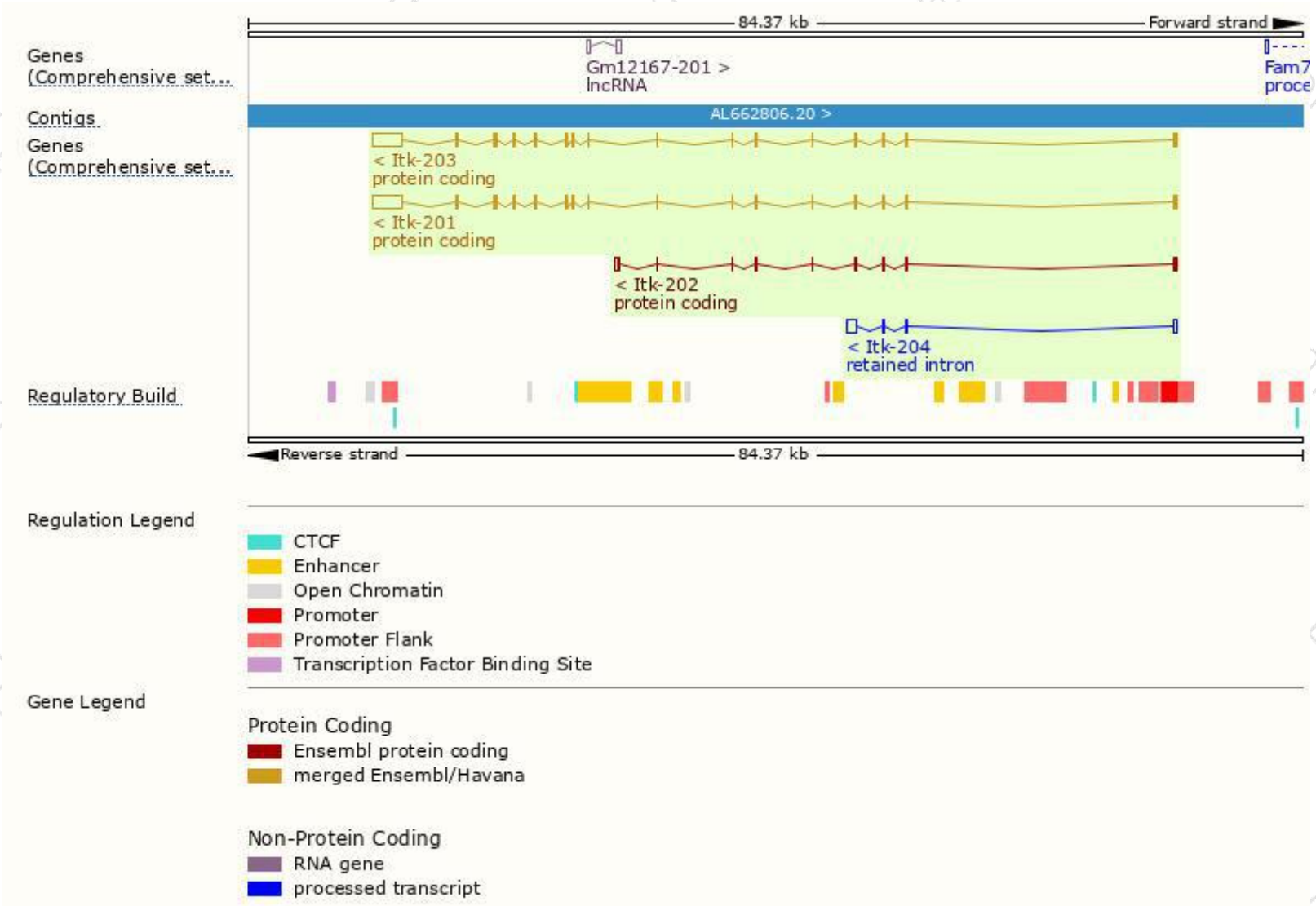
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Itk-201	ENSMUST00000020664.12	4272	619aa	Protein coding	CCDS24575	Q5STT8	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 P1
Itk-203	ENSMUST00000109237.8	4268	625aa	Protein coding	CCDS70169	Q03526	TSL:1 GENCODE basic
Itk-202	ENSMUST00000101306.3	1185	264aa	Protein coding	CCDS70168	Q5STT7	TSL:1 GENCODE basic
Itk-204	ENSMUST00000155991.1	1199	No protein	Retained intron	-	-	TSL:1

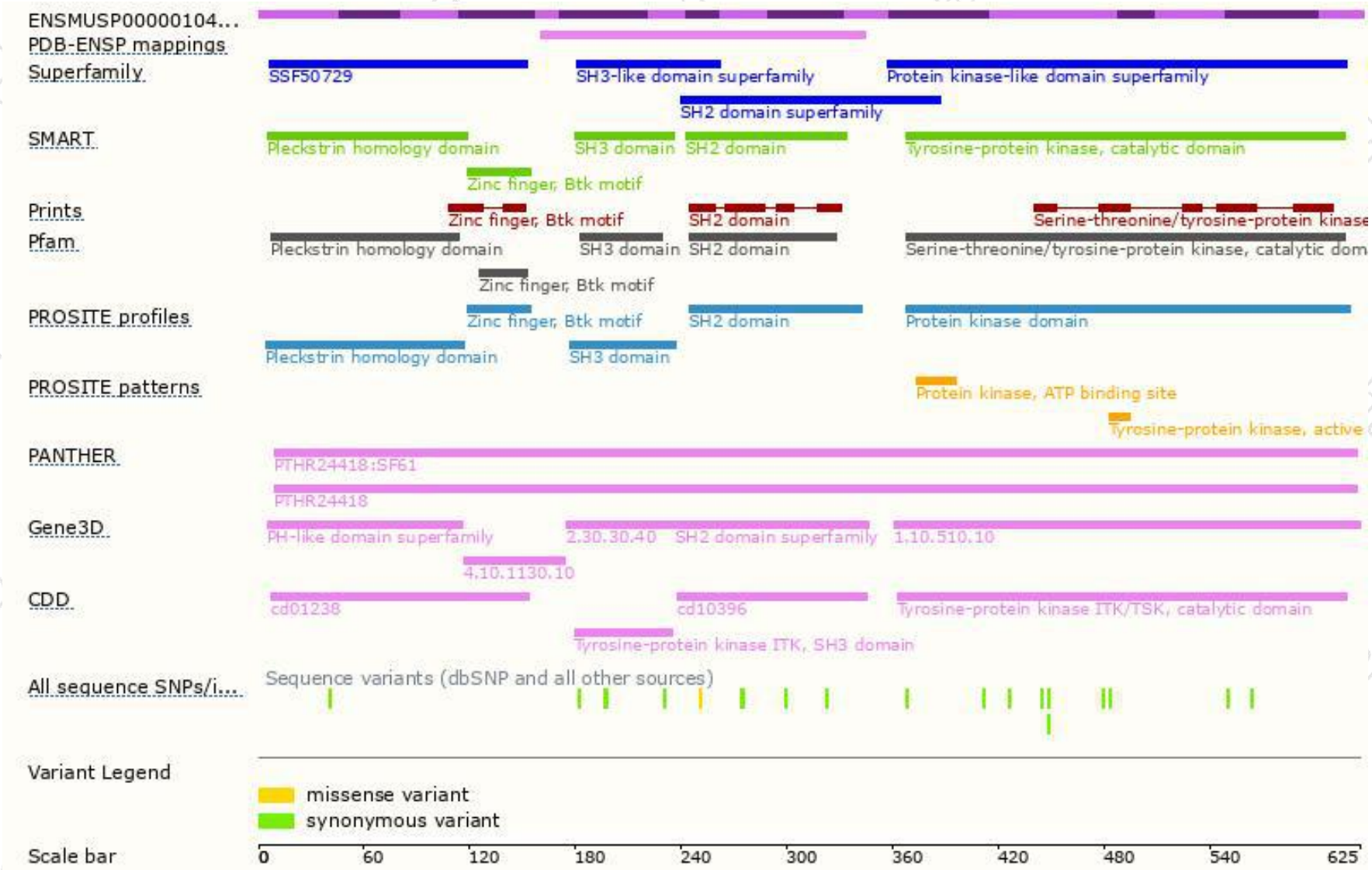
The strategy is based on the design of *Itk-203* transcript,the transcription is shown below



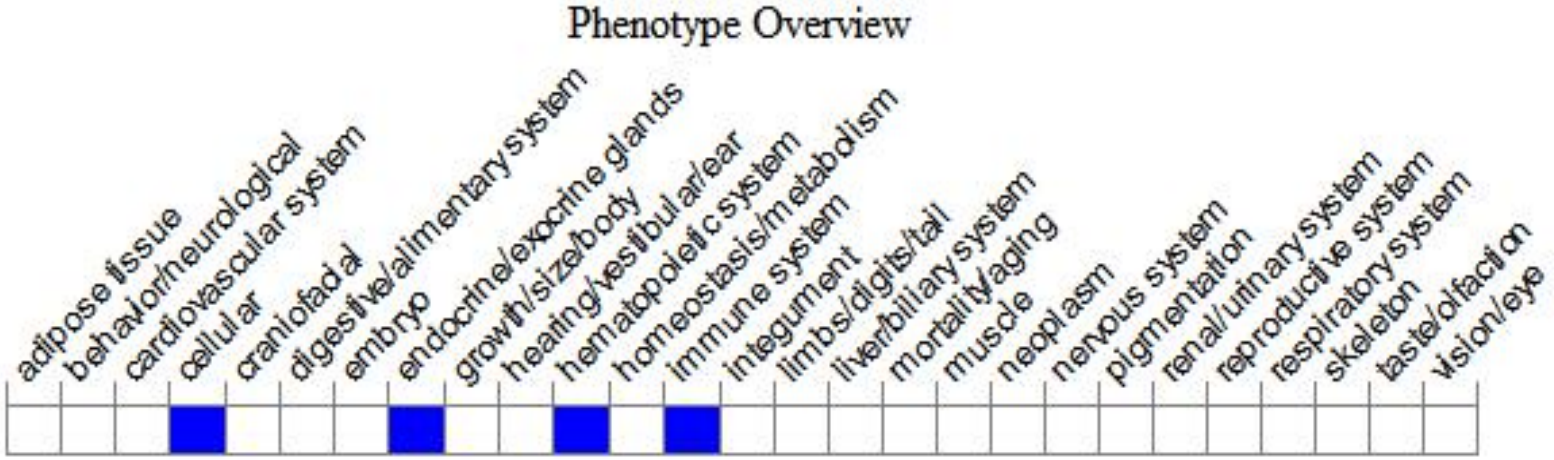
Genomic location distribution



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 decreased percentages of CD4 and CD8 cells, increased percentage of B cells, impaired T cell receptor signaling, and increased susceptibility to Toxoplasma gondii infection.

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