Cxcl9 Cas9-CKO Strategy

Designer: Jinling Wang

Reviewer: Yun Li

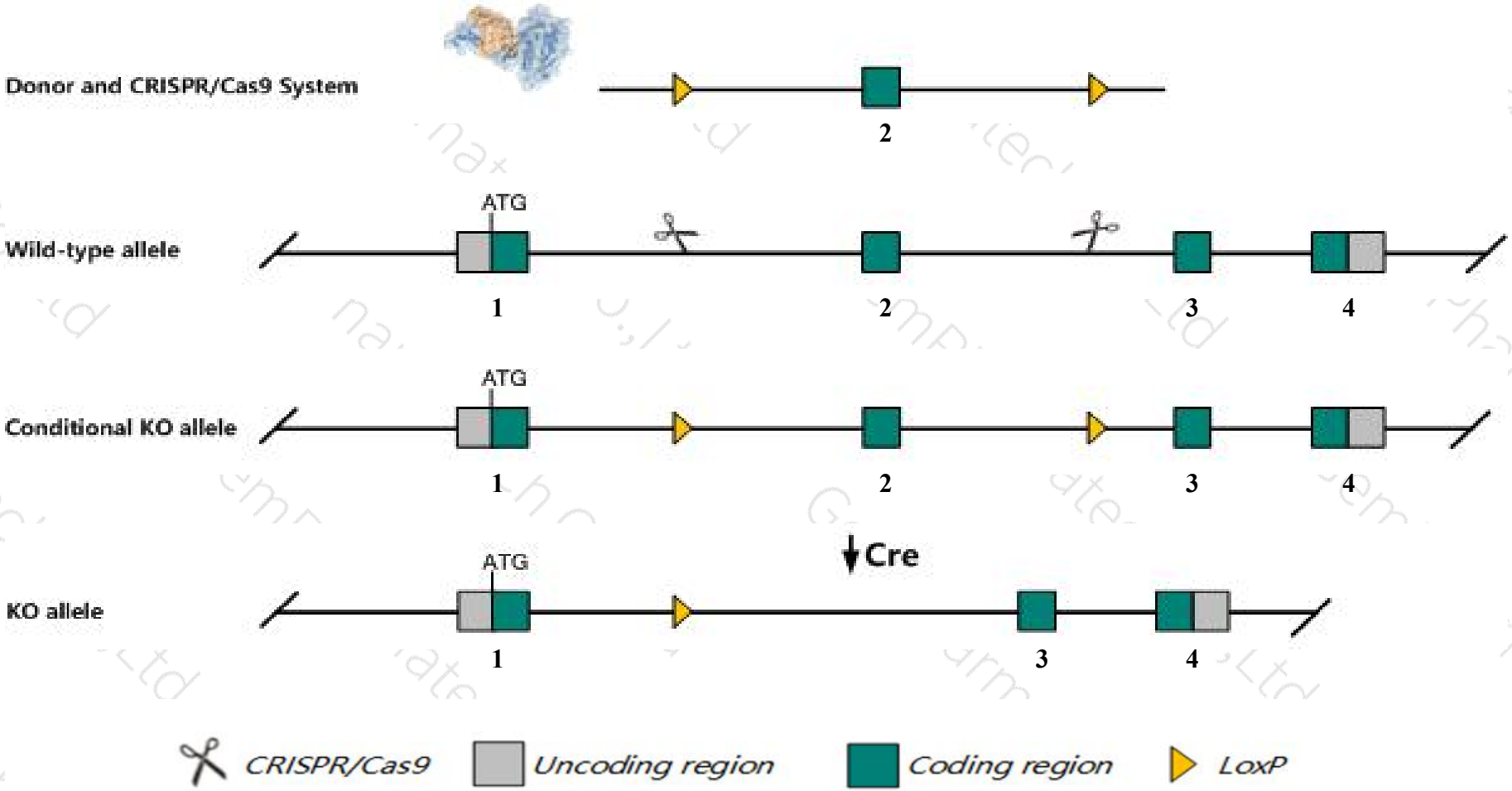
Design Date: 2018/9/7

Project Overview

Project Name	***Cxcl9***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cxcl9* gene. The schematic diagram is as follows:



Technical routes

➢ The *Cxcl9* gene has 2 transcripts. According to the structure of *Cxcl9* gene, exon2 of *Cxcl9-201*(ENSMUST00000113093.4) transcript is recommended as the knockout region.The region contains 127bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Cxcl9* 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 knock-out allele show a significant reduction in CD4+ T cell infiltration into the cornea in response to ocular HSV-1 infection, and produce lower titers of antibodies in response to primary infection with the intracellular bacterium Francisella tularensis live vaccine strain.

➢ The *Cxcl9* gene is located on the Chr5. 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

Cxcl9 chemokine (C-X-C motif) ligand 9 [Mus musculus (house mouse)]

Gene ID: 17329, updated on 13-Mar-2020

Summary

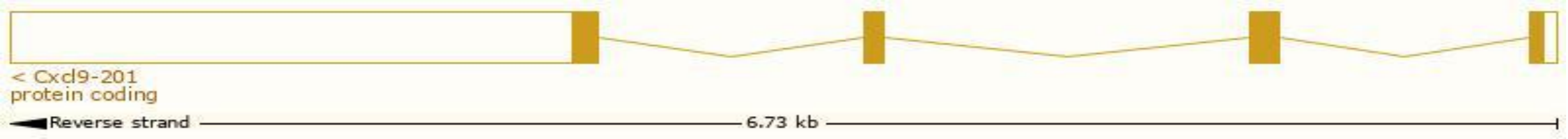
Official Symbol	Cxcl9 provided by MGI
Official Full Name	chemokine (C-X-C motif) ligand 9 provided by MGI
Primary source	MGI:MGI:1352449
See related	Ensembl:ENSMUSG00000029417
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	BB139920, CMK, Mig, MuMIG, Scyb9, crg-10
Expression	Biased expression in subcutaneous fat pad adult (RPKM 7.5), mammary gland adult (RPKM 6.2) and 12 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

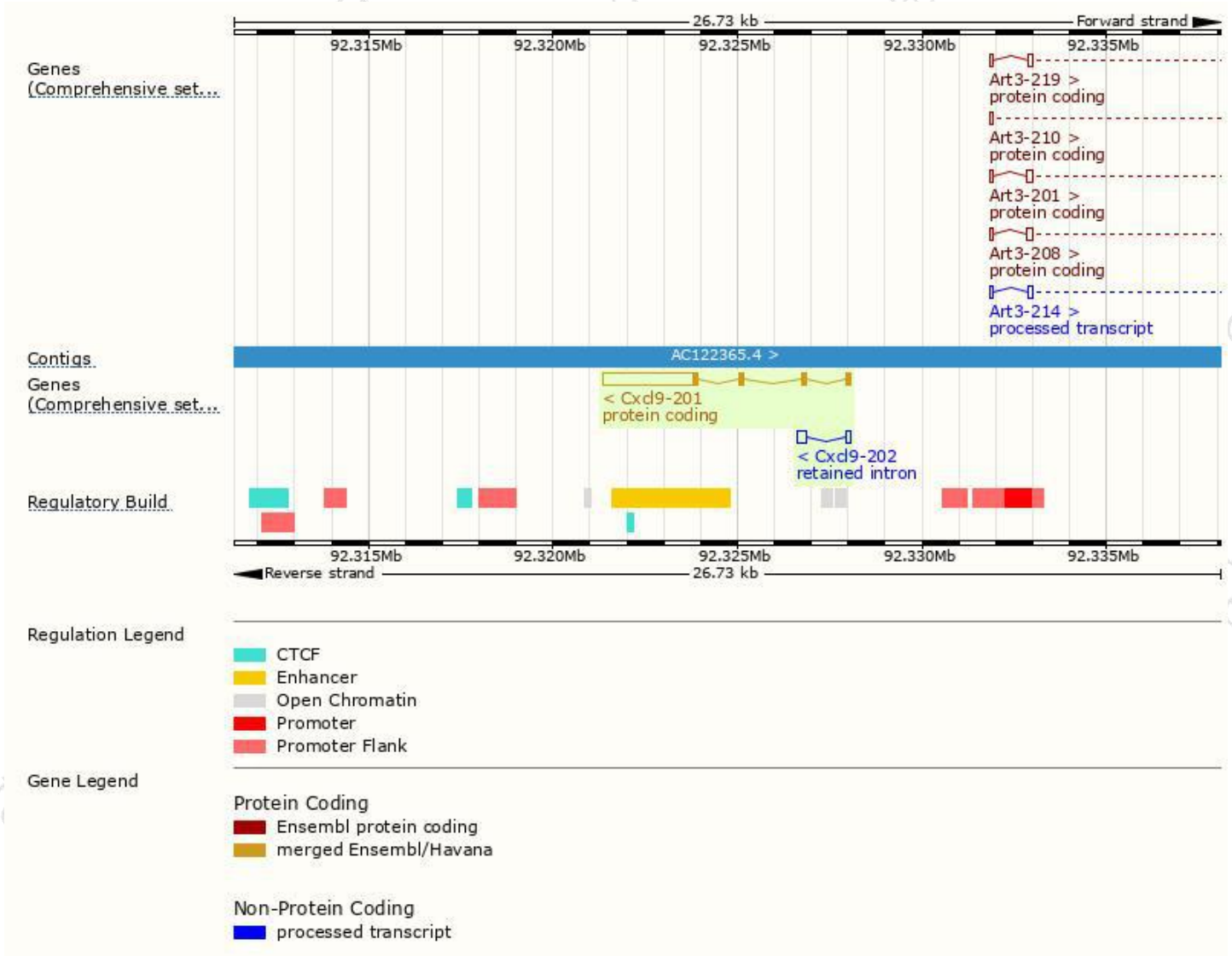
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cxcl9-201	ENSMUST00000113093.4	2889	126aa	Protein coding	CCDS39152	P18340	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
Cxcl9-202	ENSMUST00000202404.1	370	No protein	Retained intron	-	-	TSL:2

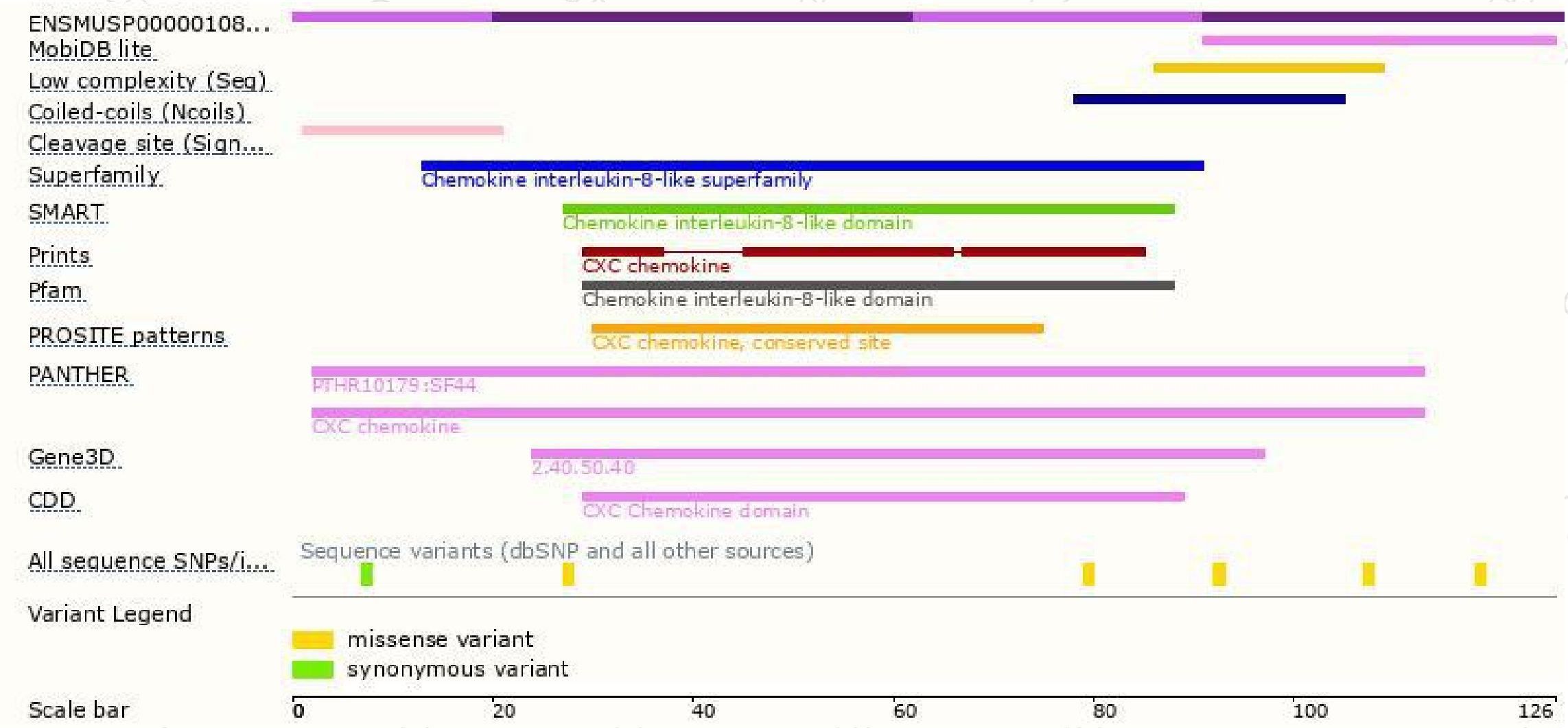
The strategy is based on the design of *Cxcl9-201* transcript,the transcription is shown below:



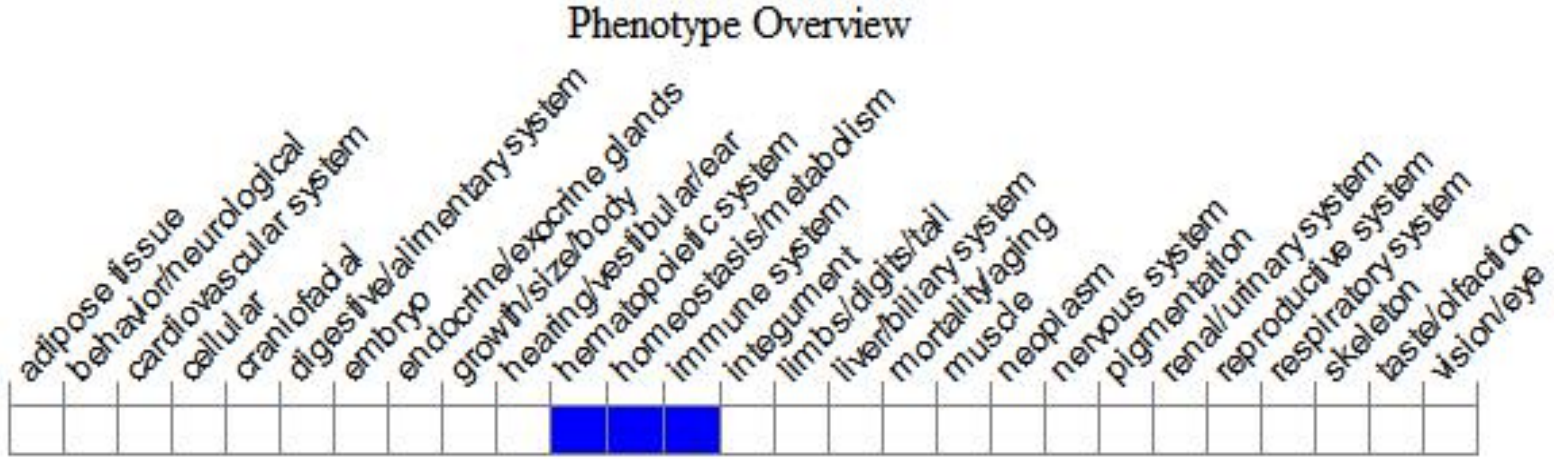
Genomic location distribution



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 knock-out allele show a significant reduction in CD4+ T cell infiltration into the cornea in response to ocular HSV-1 infection, and produce lower titers of antibodies in response to primary infection with the intracellular bacterium Francisella tularensis live vaccine strain.

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd