

Irf3 Cas9-CKO Strategy

Designer:

Yanhua Shen

Reviewer:

Jia Yu

Design Date:

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Project Overview

Project Name

Irf3

Project type

Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

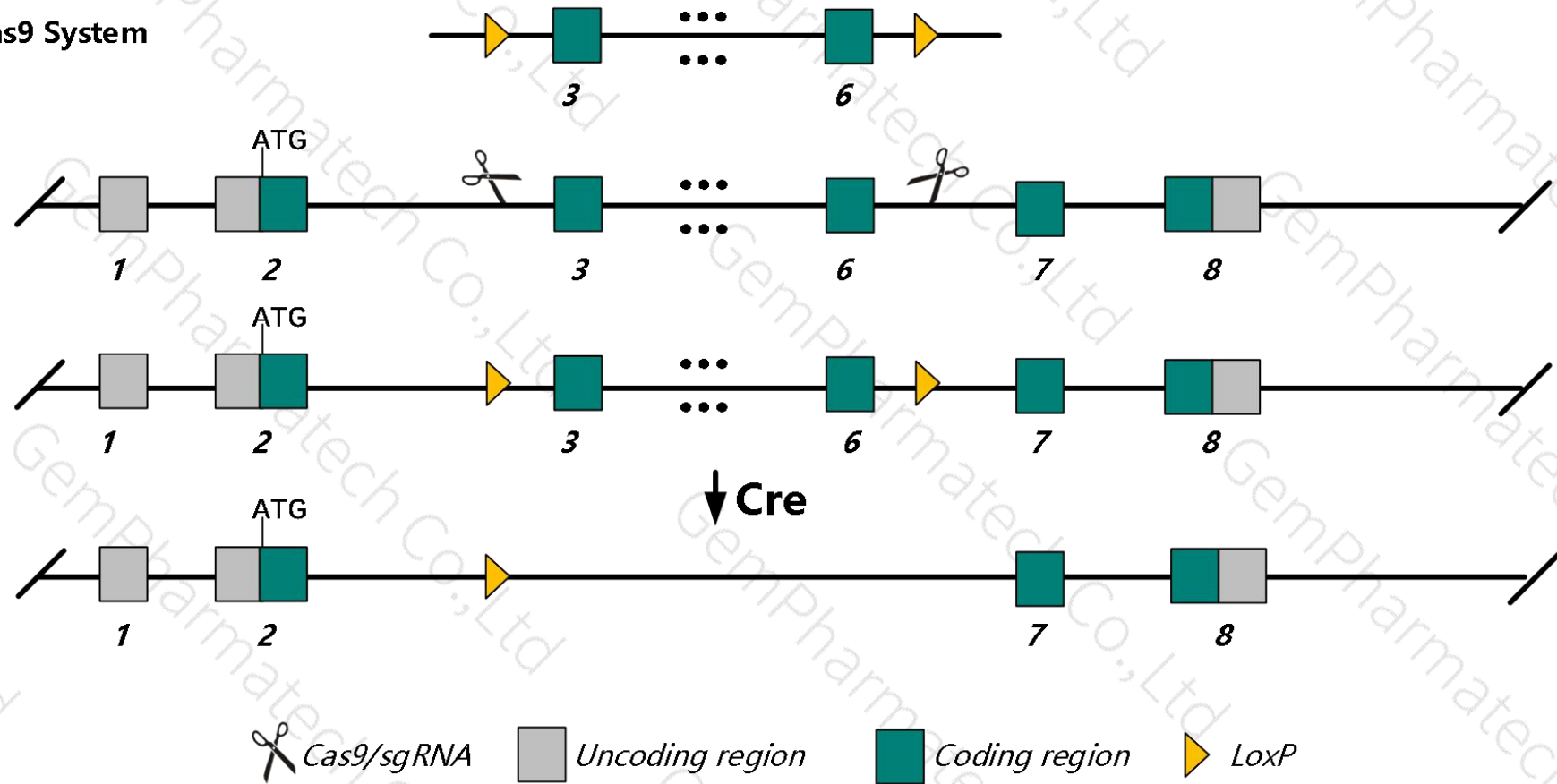
This model will use CRISPR/Cas9 technology to edit the *Irf3* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System

Wild-type allele

Conditional KO allele

KO allele



Technical routes

- The *Irf3* gene has 13 transcripts. According to the structure of *Irf3* gene, exon3-6 of *Irf3*-201 (ENSMUST00000003284.15) transcript is recommended as the knockout region. The region contains 796bp of coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Irf3* 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.

- According to the existing MGI data, homozygous null mice are more susceptible to viral infection and exhibit lower serum interferon levels in response to viral infection.
- Transcripts 210,212 may not be affected, and the effect on transcripts 203,204,206,211 are unknown.
- The floxed region is near to the N-terminal of *Bcl2l12* gene, this strategy may influence the regulatory function of the N-terminal of *Bcl2l12* gene.
- The *Irf3* gene is located on the Chr7. 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)

Irf3 interferon regulatory factor 3 [*Mus musculus* (house mouse)]

Gene ID: 54131, updated on 26-Nov-2019

Summary

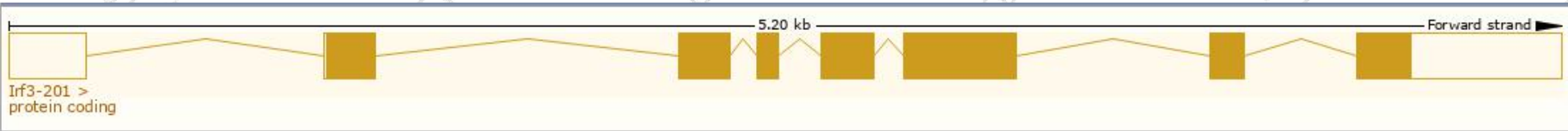
| | |
|--------------------|---|
| Official Symbol | Irf3 provided by MGI |
| Official Full Name | interferon regulatory factor 3 provided by MGI |
| Primary source | MGI:MGI:1859179 |
| See related | Ensembl:ENSMUSG000000003184 |
| Gene type | protein coding |
| RefSeq status | VALIDATED |
| Organism | Mus musculus |
| Lineage | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| Also known as | IRF-3; C920001K05Rik |
| Expression | Ubiquitous expression in thymus adult (RPKM 26.3), spleen adult (RPKM 21.3) and 28 other tissues See more |
| Orthologs | human all |

Transcript information (Ensembl)

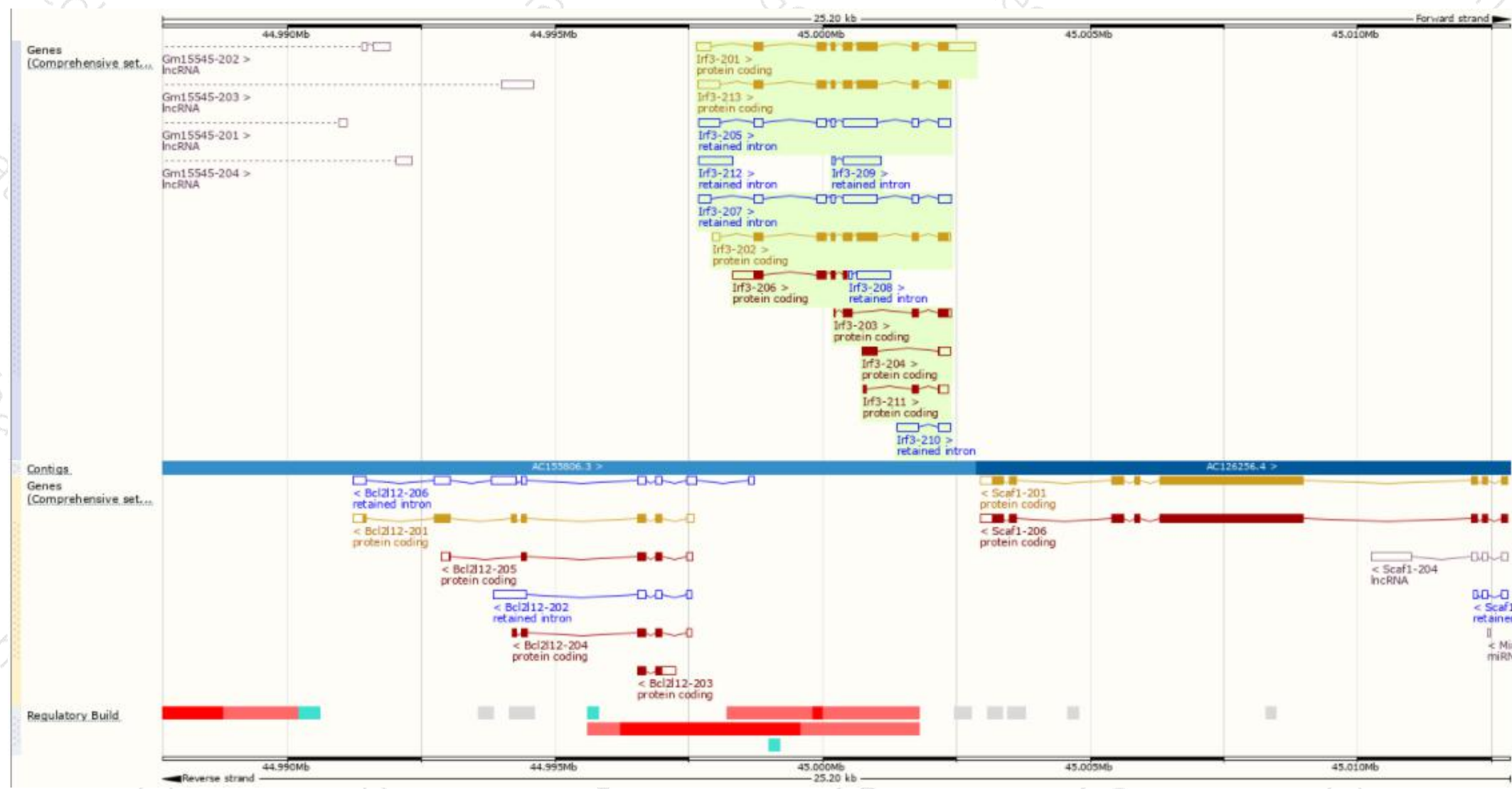
The gene has 13 transcripts,all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|----------|---------------------------------------|------|-----------------------|-----------------|---------------------------|---|-------------------------------|
| Irf3-201 | ENSMUST00000003284.15 | 2031 | 419aa | Protein coding | CCDS21224 | P70671 Q3U9K6 | TSL:1 GENCODE basic APPRIS P1 |
| Irf3-213 | ENSMUST000000209066.1 | 1712 | 419aa | Protein coding | CCDS21224 | P70671 Q3U9K6 | TSL:1 GENCODE basic APPRIS P1 |
| Irf3-202 | ENSMUST000000107834.1 | 1429 | 419aa | Protein coding | CCDS21224 | P70671 Q3U9K6 | TSL:1 GENCODE basic APPRIS P1 |
| Irf3-206 | ENSMUST000000207521.1 | 869 | 160aa | Protein coding | - | A0A140LHE6 | CDS 3' incomplete TSL:5 |
| Irf3-203 | ENSMUST000000207128.1 | 537 | 161aa | Protein coding | - | A0A140LJE8 | CDS 5' incomplete TSL:5 |
| Irf3-204 | ENSMUST000000207129.1 | 517 | 103aa | Protein coding | - | A0A140LJD9 | CDS 5' incomplete TSL:2 |
| Irf3-211 | ENSMUST000000208958.1 | 369 | 68aa | Protein coding | - | A0A140LI96 | CDS 5' incomplete TSL:3 |
| Irf3-205 | ENSMUST000000207476.1 | 1797 | No protein | Retained intron | - | - | TSL:2 |
| Irf3-207 | ENSMUST000000207555.1 | 1641 | No protein | Retained intron | - | - | TSL:2 |
| Irf3-209 | ENSMUST000000208767.1 | 774 | No protein | Retained intron | - | - | TSL:3 |
| Irf3-208 | ENSMUST000000208143.1 | 659 | No protein | Retained intron | - | - | TSL:2 |
| Irf3-212 | ENSMUST000000209006.1 | 635 | No protein | Retained intron | - | - | TSL:NA |
| Irf3-210 | ENSMUST000000208906.1 | 629 | No protein | Retained intron | - | - | TSL:2 |

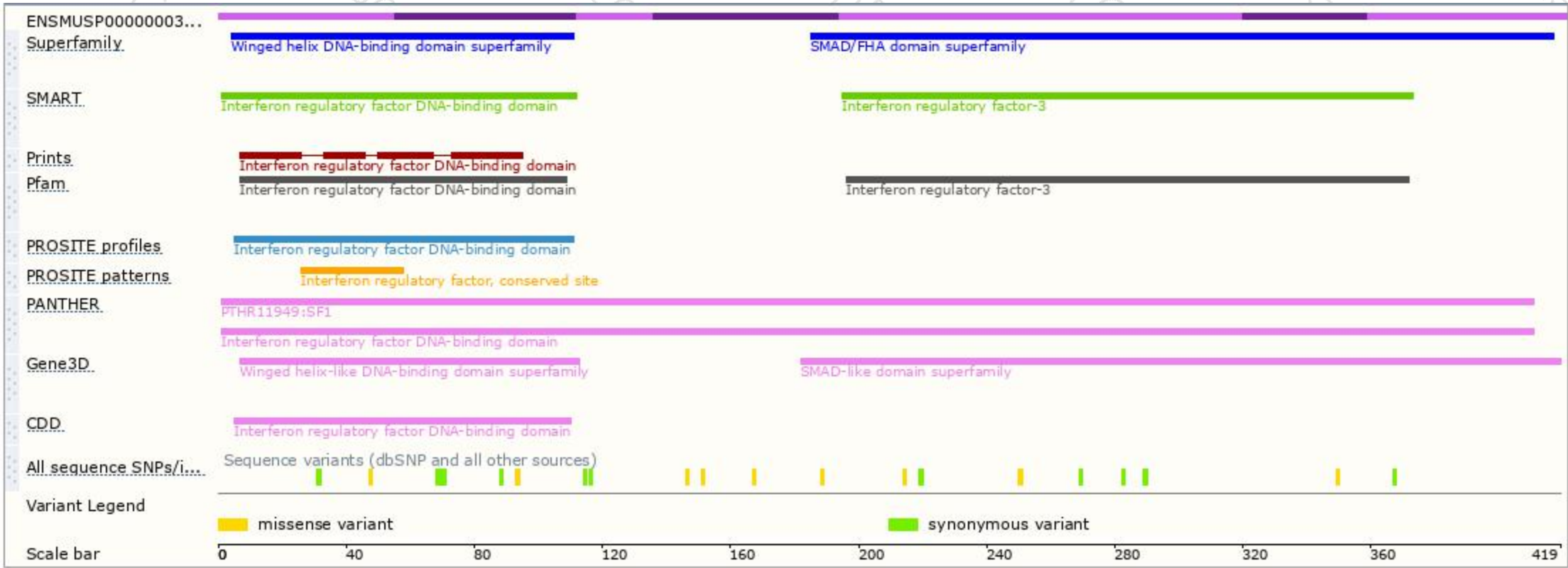
The strategy is based on the design of *Irf3-201* transcript,The transcription is shown below



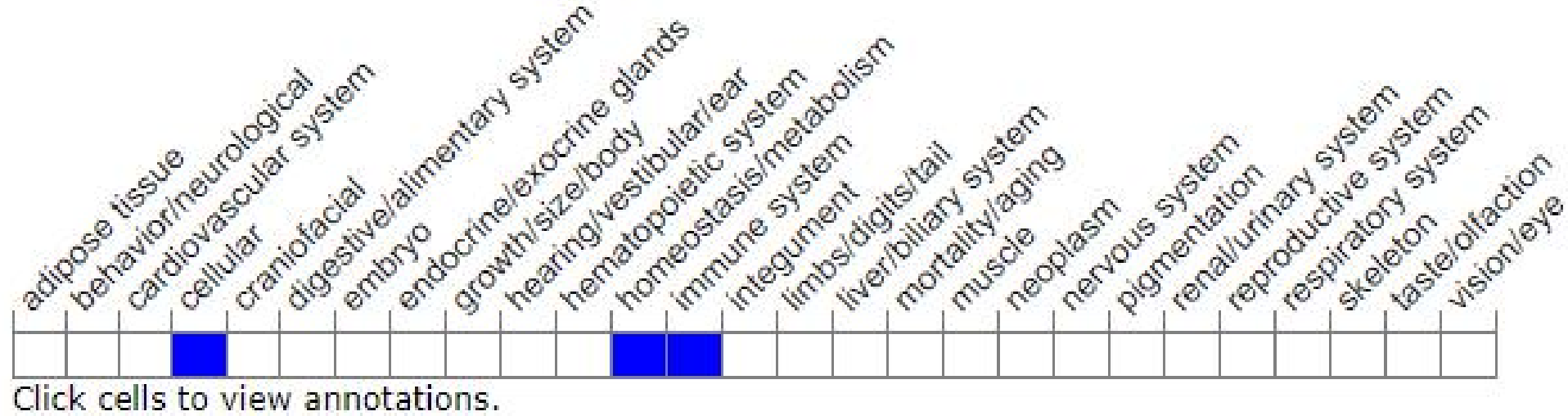
Genomic location distribution



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, homozygous null mice are more susceptible to viral infection and exhibit lower serum interferon levels in response to viral infection.

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

Tel: 400-9660890

