

Cxcr5 Cas9-CKO Strategy

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Reviewer:

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Project Overview

Project Name

Cxcr5

Project type

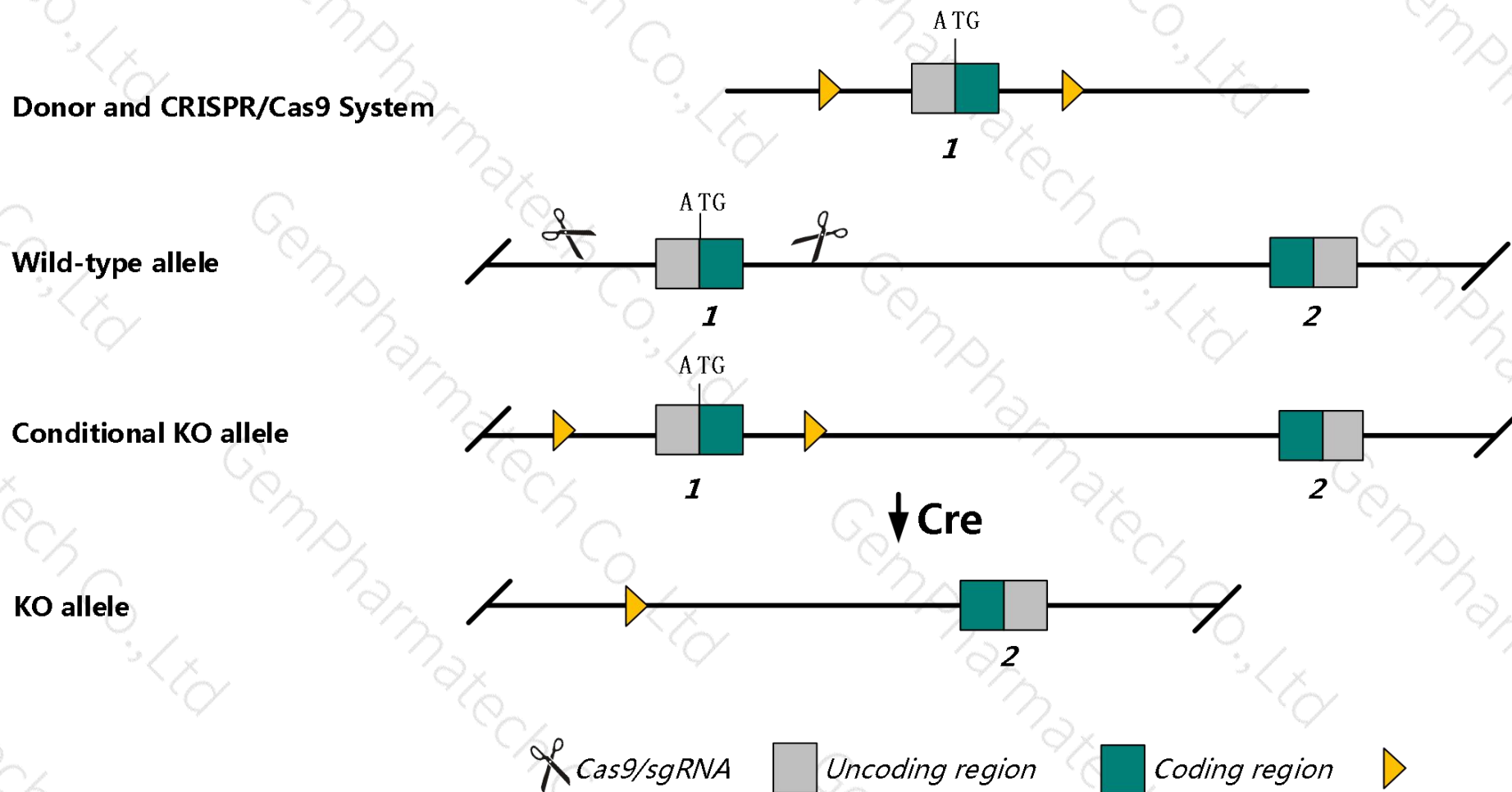
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cxcr5* gene. The schematic diagram is as follows:



- The *Cxcr5* has 5 transcripts. According to the structure of *Cxcr5* gene, exon1 of *Cxcr5*-201 (ENSMUST00000062215.7) transcript is recommended as the knockout region. The region contains 57 bp start codon ATG coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cxcr5* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA 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 or cell types.

- According to the existing MGI data , homozygous null mutants lack inguinal lymph nodes, have a few abnormal or no Peyer's patches, morphologically altered primary lymphoid follicles and no functional germinal centers in their spleen.
- The *Cxcr5* gene is located on the Chr9. 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)

Cxcr5 chemokine (C-X-C motif) receptor 5 [*Mus musculus* (house mouse)]

Gene ID: 12145, updated on 19-Nov-2019

Summary

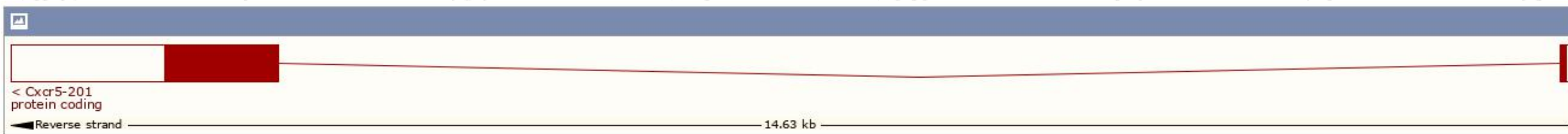
| | |
|---------------------------|---|
| Official Symbol | Cxcr5 provided by MGI |
| Official Full Name | chemokine (C-X-C motif) receptor 5 provided by MGI |
| Primary source | MGI:MGI:103567 |
| See related | Ensembl:ENSMUSG00000047880 |
| Gene type | protein coding |
| RefSeq status | VALIDATED |
| Organism | Mus musculus |
| Lineage | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| Also known as | Blr1; Gpcr6; MDR15; CXC-R5; CXCR-5 |
| Expression | Biased expression in spleen adult (RPKM 61.9), mammary gland adult (RPKM 12.5) and 1 other tissue See more |
| Orthologs | human all |

Transcript information (Ensembl)

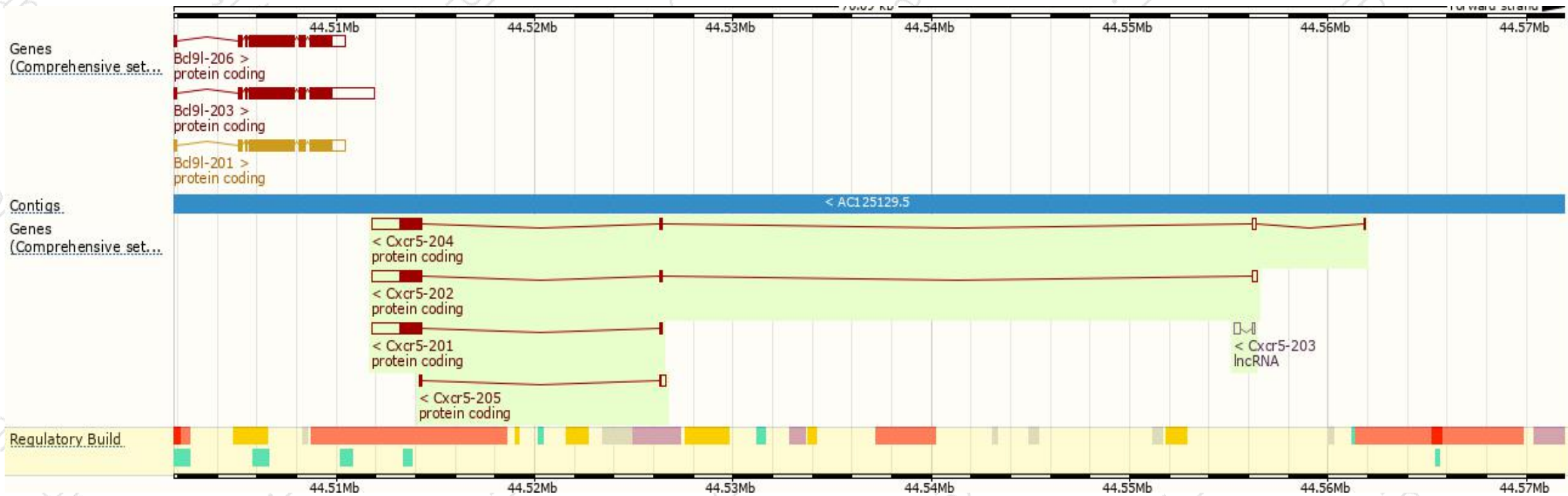
The gene has 5 transcripts, and all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|-----------|--------------------------------------|------|-----------------------|----------------|---------------------------|----------------------------|-------------------------------|
| Cxcr5-204 | ENSMUST00000215293.1 | 2846 | 374aa | Protein coding | CCDS23115 | Q04683 | TSL:1 GENCODE basic APPRIS P1 |
| Cxcr5-202 | ENSMUST00000179828.7 | 2824 | 374aa | Protein coding | CCDS23115 | Q04683 | TSL:5 GENCODE basic APPRIS P1 |
| Cxcr5-201 | ENSMUST00000062215.7 | 2614 | 374aa | Protein coding | CCDS23115 | Q04683 | TSL:1 GENCODE basic APPRIS P1 |
| Cxcr5-205 | ENSMUST00000215661.1 | 358 | 51aa | Protein coding | - | A0A1L1SRC9 | CDS 3' incomplete TSL:1 |
| Cxcr5-203 | ENSMUST00000213357.1 | 377 | No protein | lncRNA | - | - | TSL:3 |

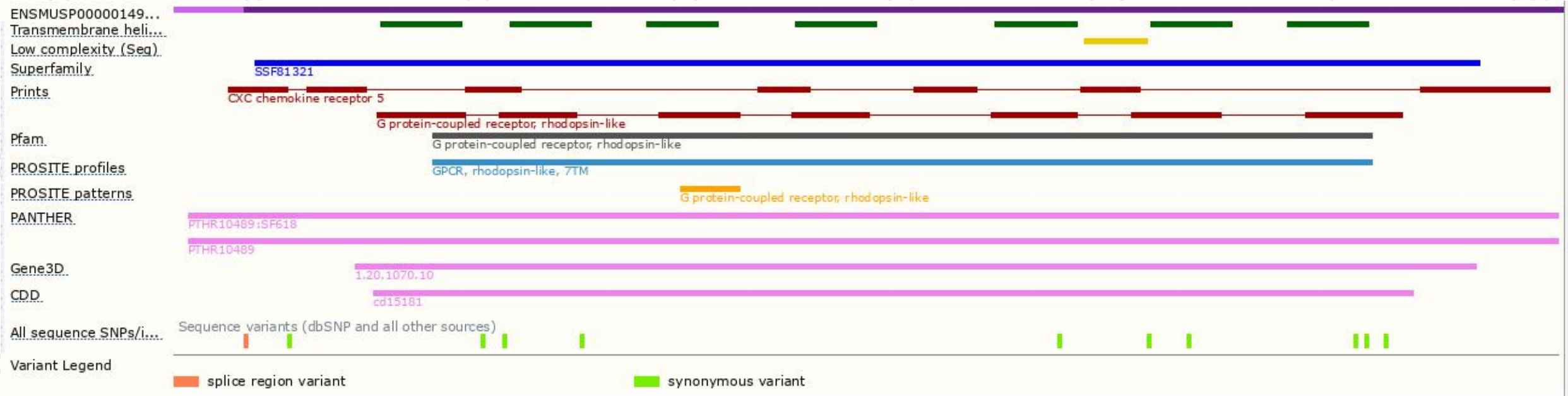
The strategy is based on the design of *Cxcr5-201* transcript, The transcription is shown below:



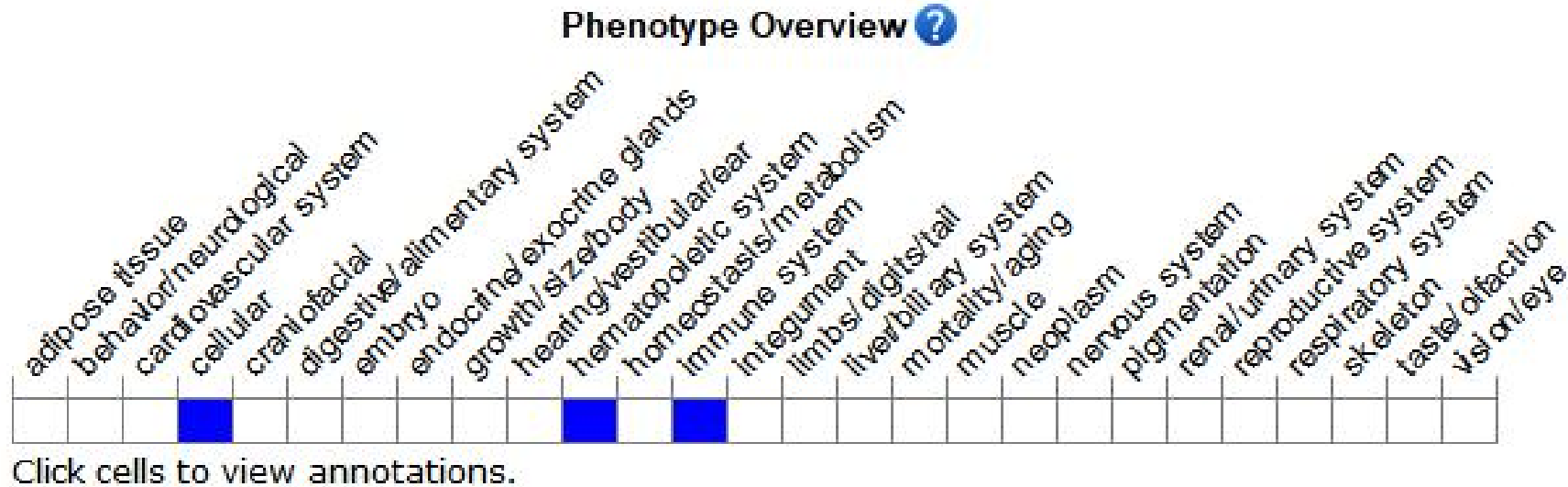
Genomic location distribution



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 mutants lack inguinal lymph nodes, have a few abnormal or no Peyer's patches, morphologically altered primary lymphoid follicles and no functional germinal centers in their spleen.