

***Cd4* Cas9-CKO Strategy**

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Project Overview

Project Name

Cd4

Project type

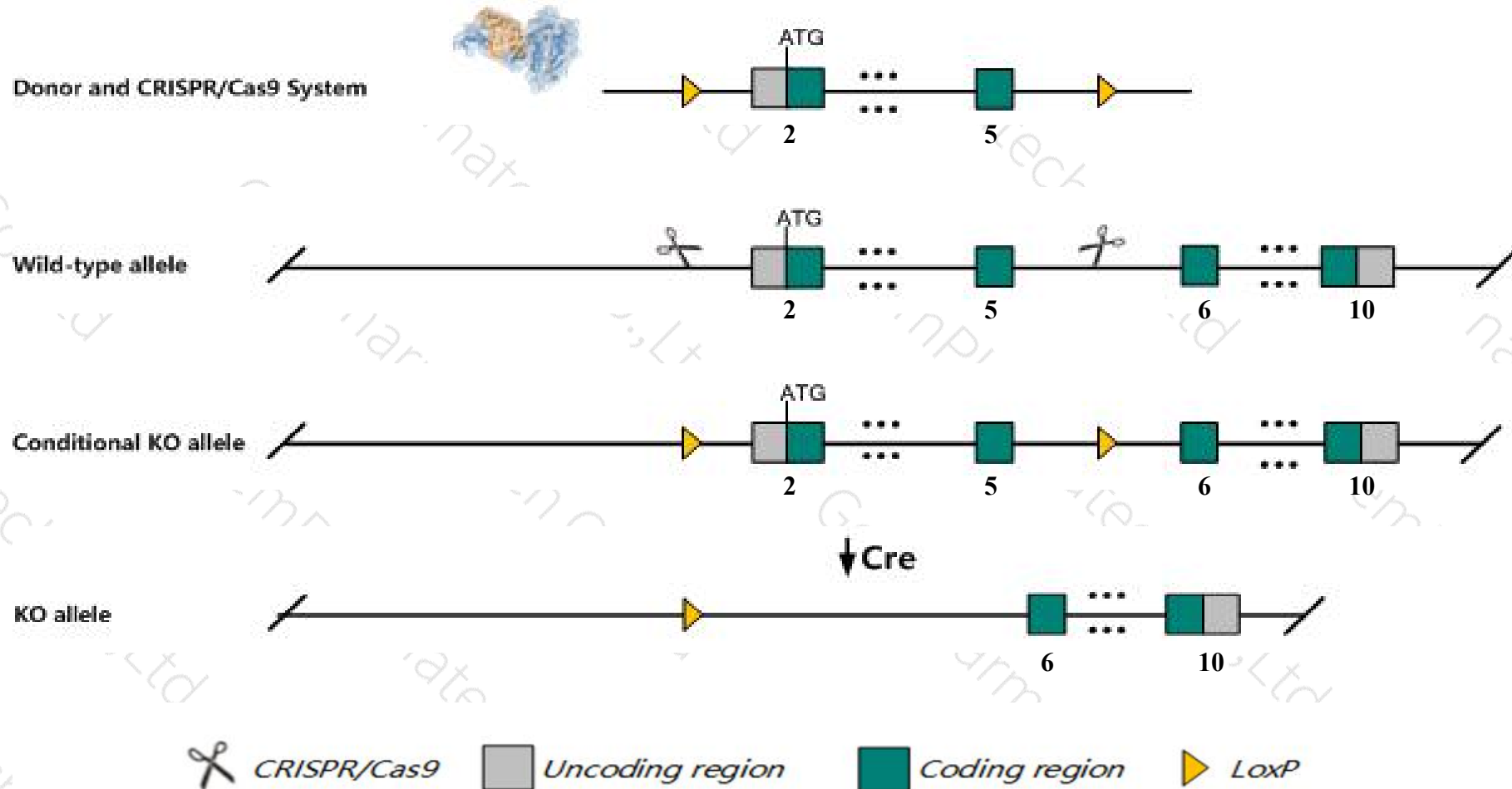
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cd4* gene. The schematic diagram is as follows:



- The *Cd4* gene has 5 transcripts. According to the structure of *Cd4* gene, exon2-exon5 of *Cd4-201* (ENSMUST00000024044.6) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cd4* 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, Mice homozygous for knock-out alleles exhibit abnormal immune system morphology and physiology.
- The *Cd4* gene is located on the Chr6. 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)

Cd4 CD4 antigen [Mus musculus (house mouse)]

Gene ID: 12504, updated on 19-Mar-2019

Summary



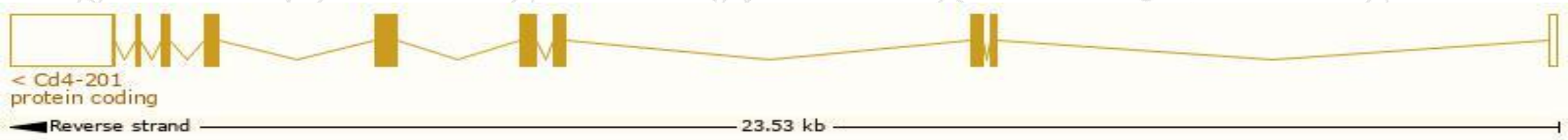
Official Symbol	Cd4 provided by MGI
Official Full Name	CD4 antigen provided by MGI
Primary source	MGI:MGI:88335
See related	Ensembl:ENSMUSG00000023274
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	L3T4, Ly-4
Expression	Biased expression in thymus adult (RPKM 167.0), spleen adult (RPKM 15.0) and 1 other tissue See more
Orthologs	human all

Transcript information (Ensembl)

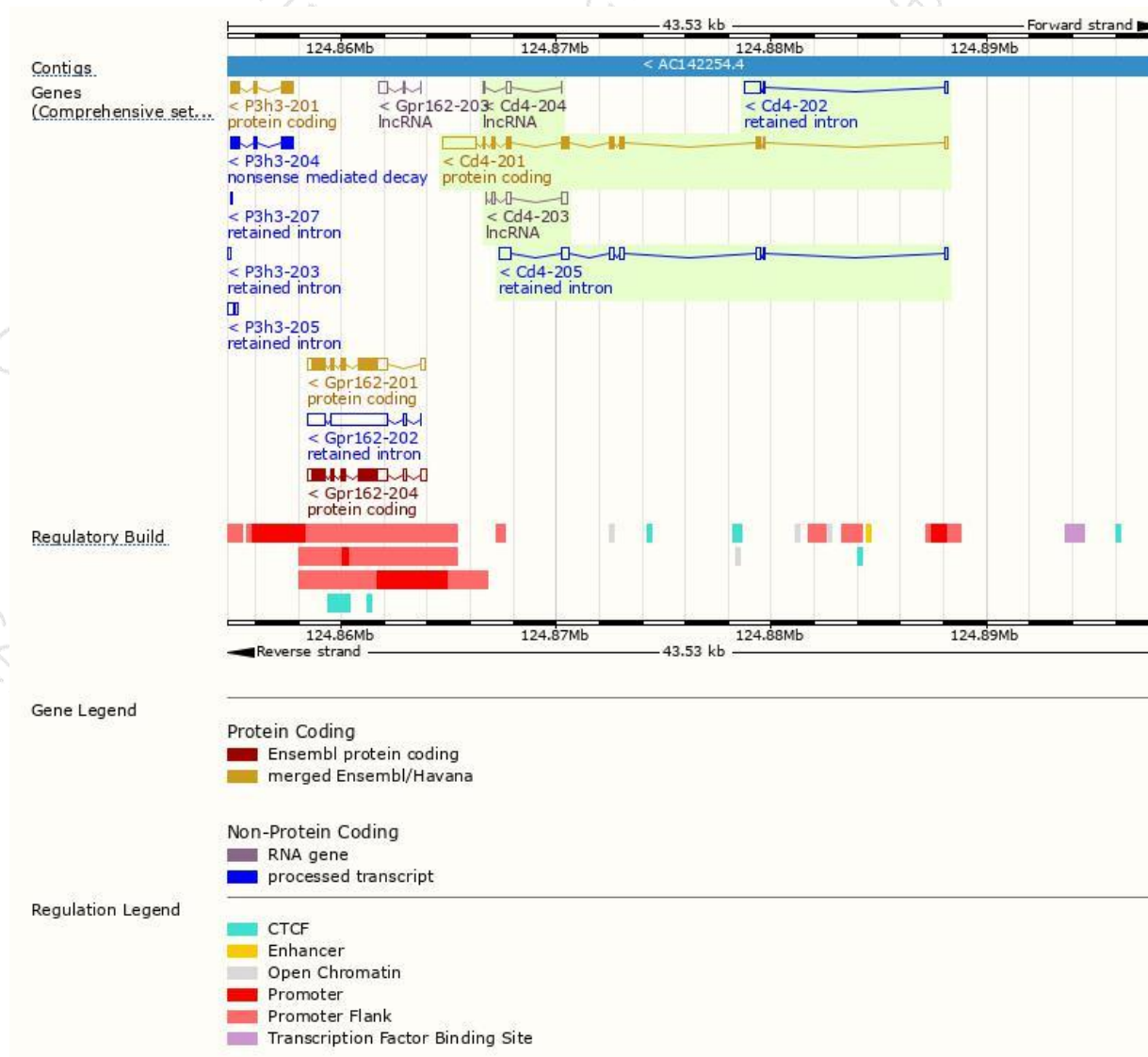
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cd4-201	ENSMUST00000024044.6	3108	457aa	Protein coding	CCDS20535	P06332 Q3TSV7	TSL:1 GENCODE basic APPRIS P1
Cd4-205	ENSMUST00000151594.7	1647	No protein	Retained intron	-	-	TSL:1
Cd4-202	ENSMUST00000130378.1	946	No protein	Retained intron	-	-	TSL:1
Cd4-203	ENSMUST00000145818.1	591	No protein	lncRNA	-	-	TSL:5
Cd4-204	ENSMUST00000145977.7	273	No protein	lncRNA	-	-	TSL:1

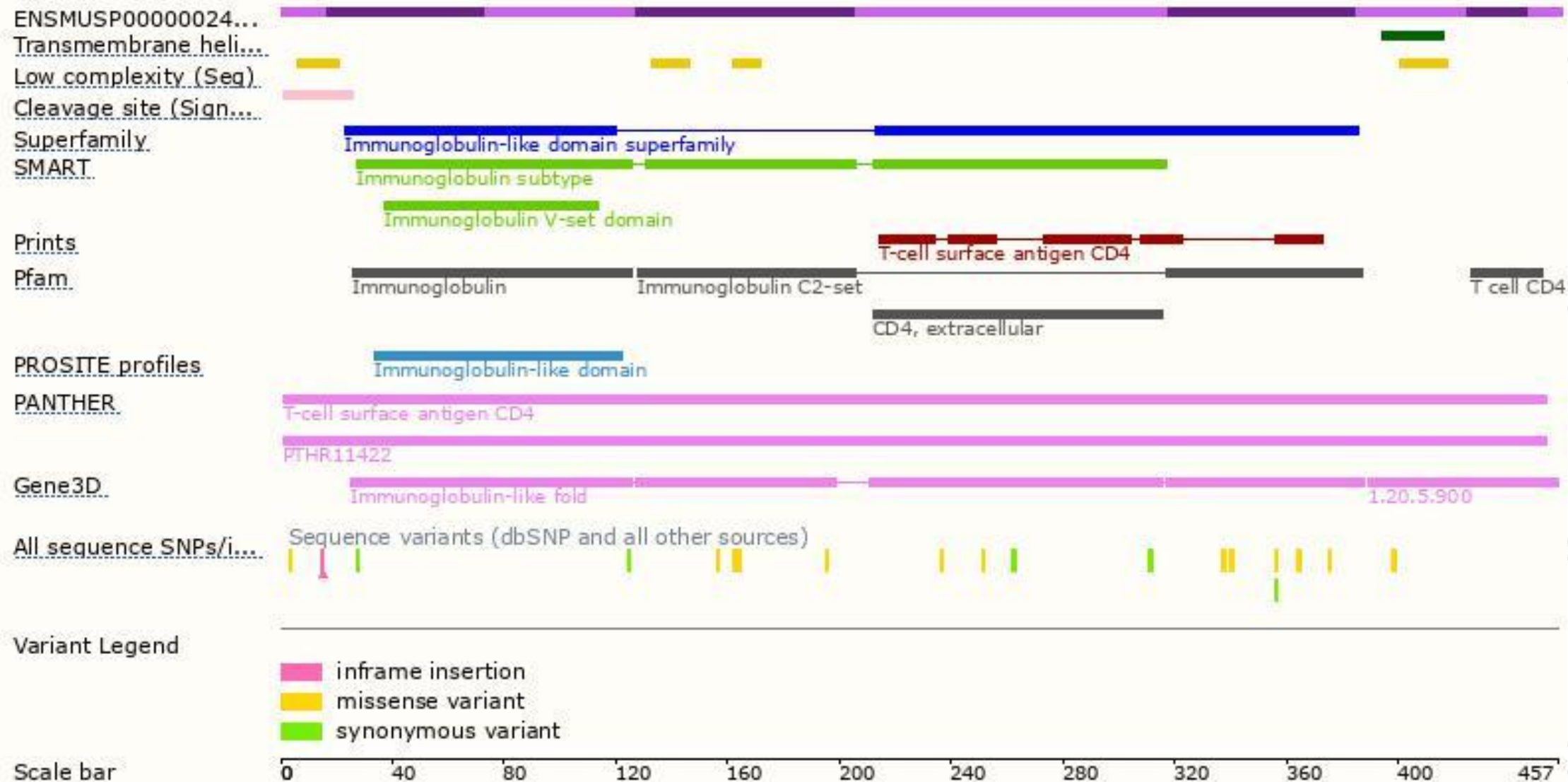
The strategy is based on the design of *Cd4-201* transcript,The transcription is shown below



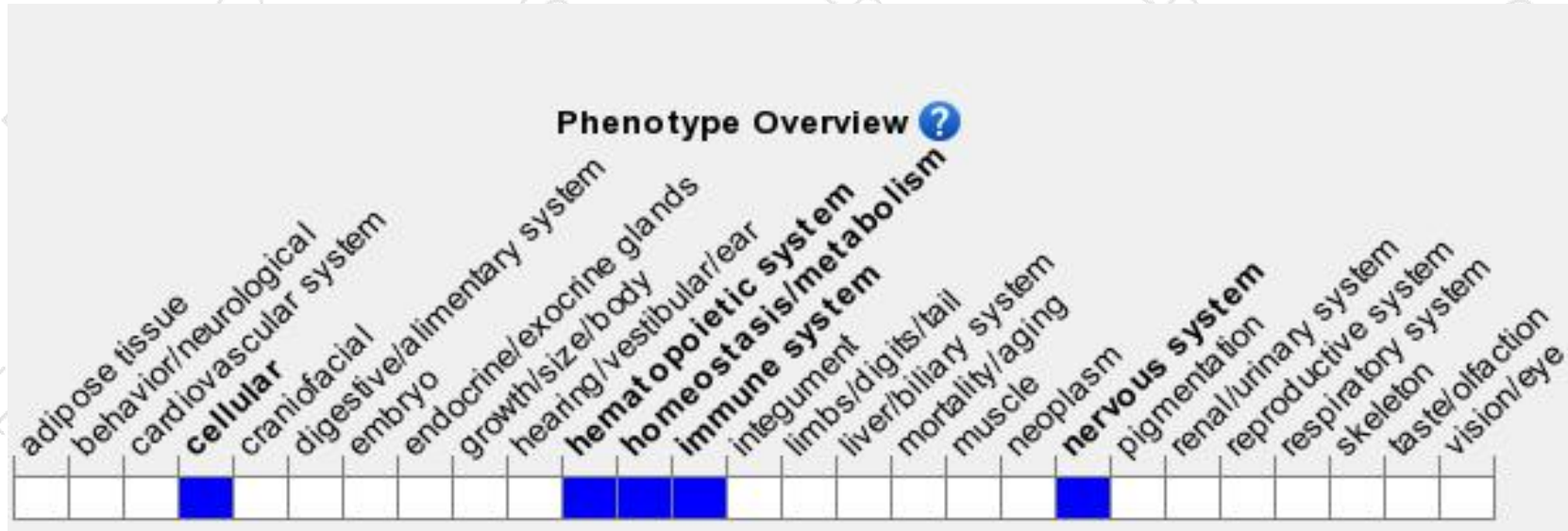
Genomic location distribution



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 knock-out alleles exhibit abnormal immune system morphology and physiology.

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

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