Zap70 Cas9-CKO Strategy

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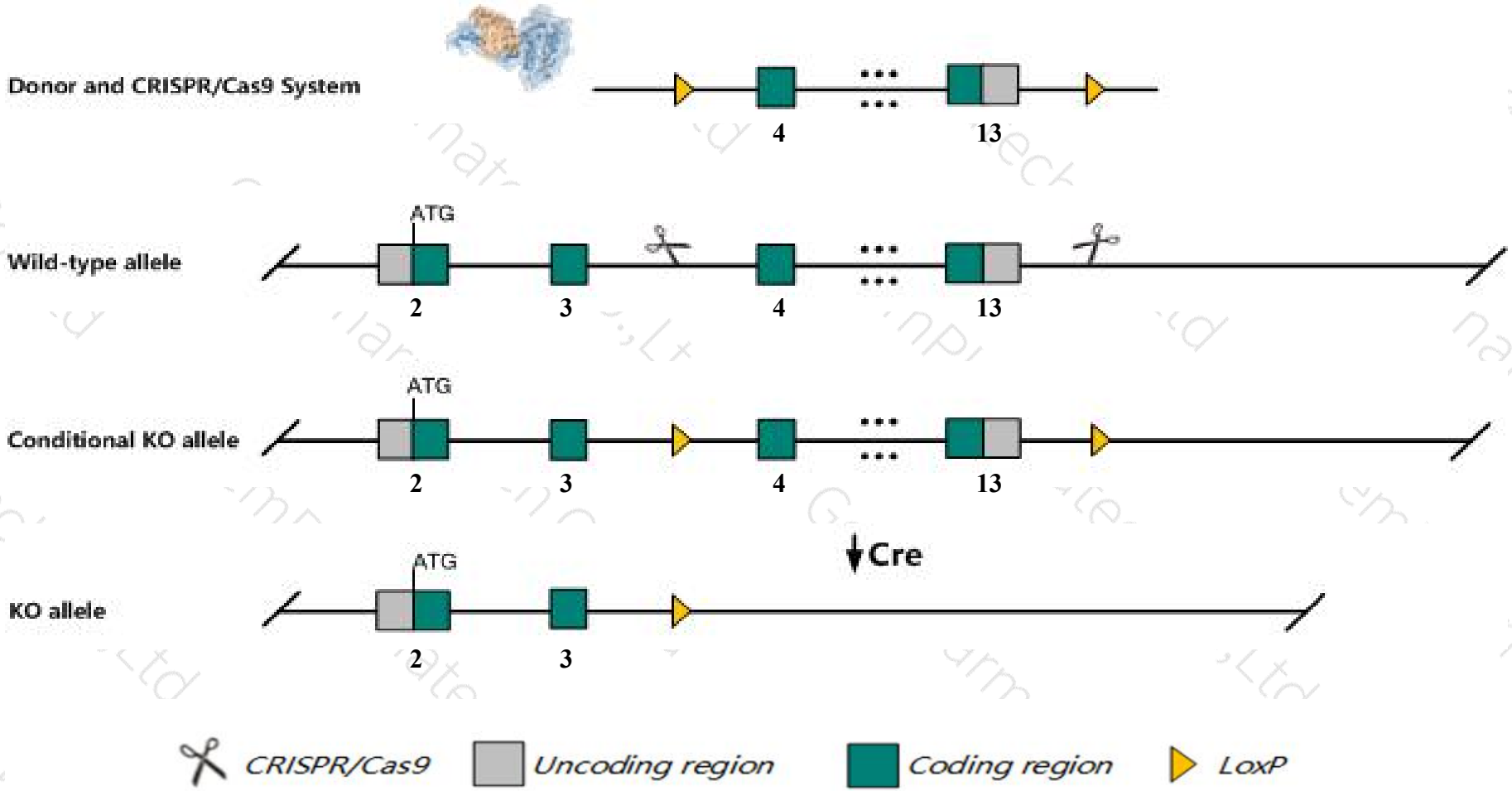
Project Overview

Project Name	***Zap70***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Zap70* gene. The schematic diagram is as follows:



Technical routes

- The *Zap70* gene has 4 transcripts. According to the structure of *Zap70* gene, exon4-exon13 of *Zap70-201* (ENSMUST00000027291.6) transcript is recommended as the knockout region.The region contains 1294bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Zap70* 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,Mutant mice show T cell defects. Null mutants lack alpha-beta T cells in the thymus and have fewer T cells in dendritic and intestinal epithelium. Spontaneous and knock-in missense mutations affect T cell receptor signaling, one of the former resulting in severe chronic arthritis.

➢ The *Zap70* gene is located on the Chr1. 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）

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Zap70 zeta-chain (TCR) associated protein kinase [Mus musculus (house mouse)]

Gene ID: 22637, updated on 5-Mar-2019

Summary

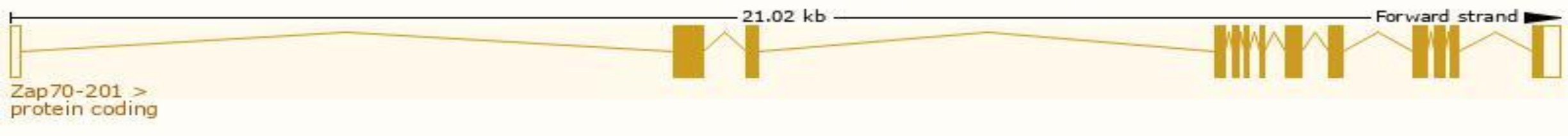
Official Symbol	Zap70 provided by MGI
Official Full Name	zeta-chain (TCR) associated protein kinase provided by MGI
Primary source	MGI:MGI:99613
See related	Ensembl:ENSMUSG00000026117
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Srk, ZAP-70, mrtle, mur
Summary	This gene encodes a member of the protein tyrosine kinase family. The encoded protein is essential for development of T lymphocytes and thymocytes, and functions in the initial step of T lymphocyte receptor-mediated signal transduction. A mutation in this gene causes chronic autoimmune arthritis, similar to rheumatoid arthritis in humans. Mice lacking this gene are deficient in alpha-beta T lymphocytes in the thymus. In humans, mutations in this gene cause selective T-cell defect, a severe combined immunodeficiency disease characterized by a selective absence of CD8-positive T lymphocytes. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Jan 2014]
Expression	Biased expression in thymus adult (RPKM 70.4), spleen adult (RPKM 17.5) and 3 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

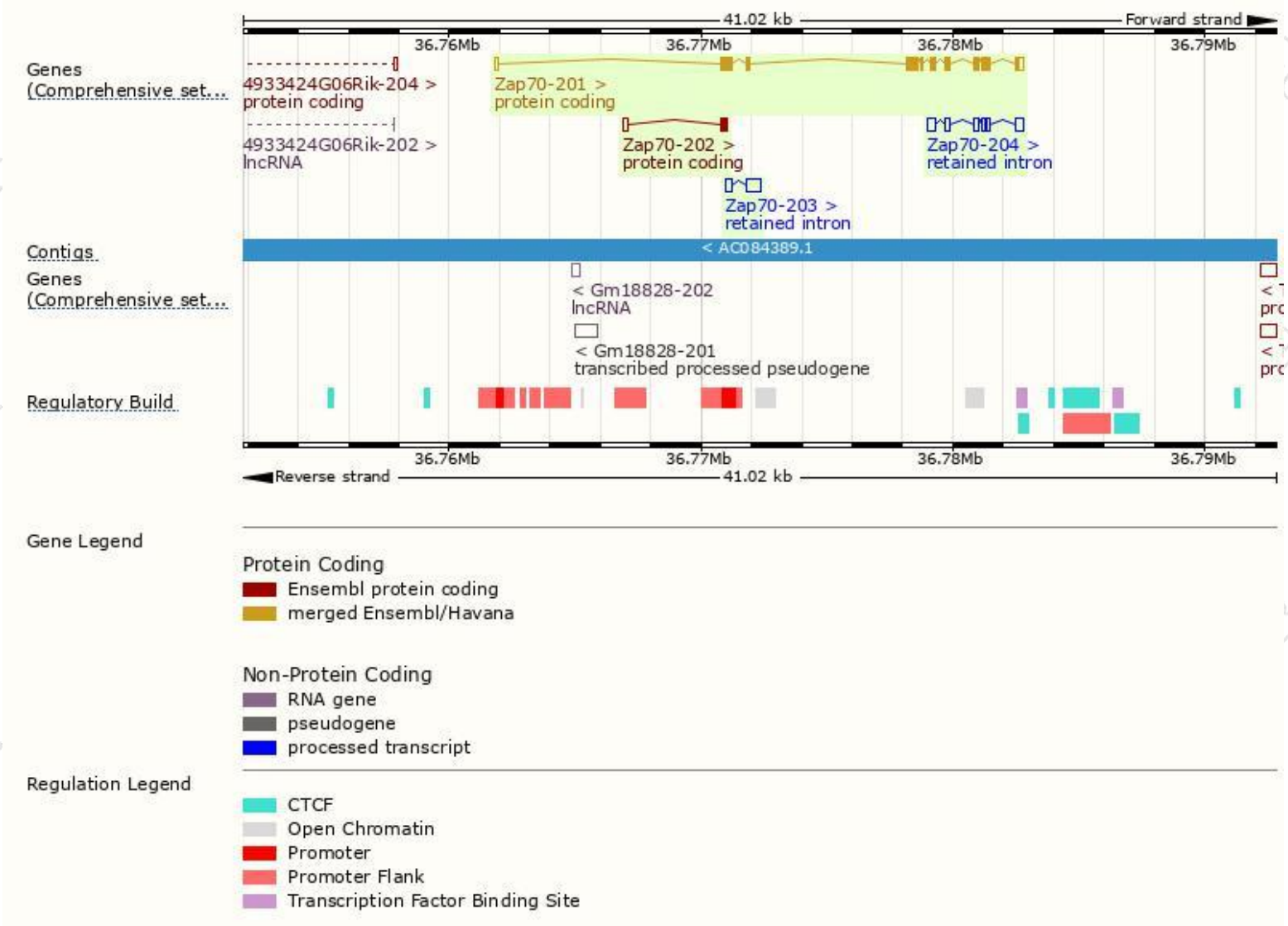
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Zap70-201	ENSMUST00000027291.6	2245	618aa	Protein coding	CCDS14888	P43404	TSL:1 GENCODE basic APPRIS P1
Zap70-202	ENSMUST00000185871.1	454	85aa	Protein coding	-	A0A087WQ05	CDS 3' incomplete TSL:2
Zap70-204	ENSMUST00000190128.1	1338	No protein	Retained intron	-	-	TSL:1
Zap70-203	ENSMUST00000186624.1	808	No protein	Retained intron	-	-	TSL:2

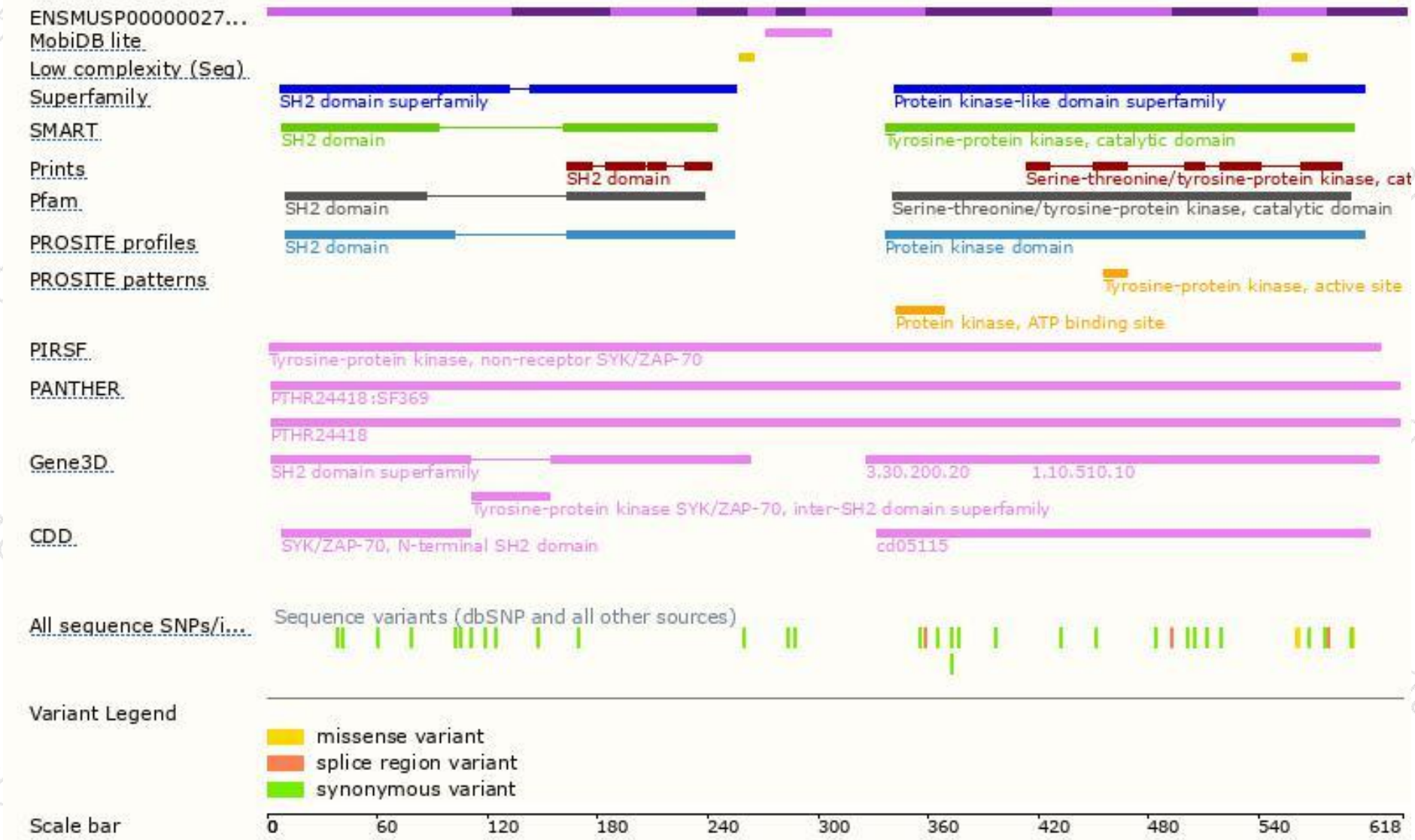
The strategy is based on the design of *Zap70-201* transcript,The transcription is shown below



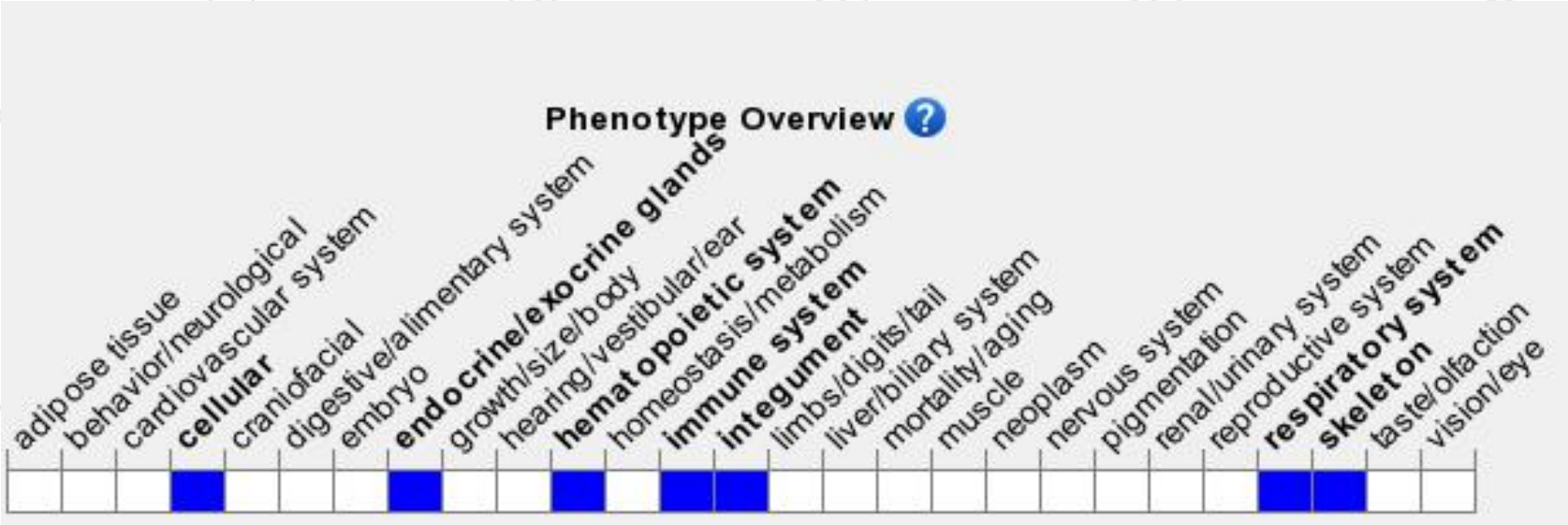
Genomic location distribution



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/).

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

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