

Ccl5 Cas9-CKO Strategy

Designer:

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Design Date:

2019-9-30

Project Overview

Project Name

Ccl5

Project type

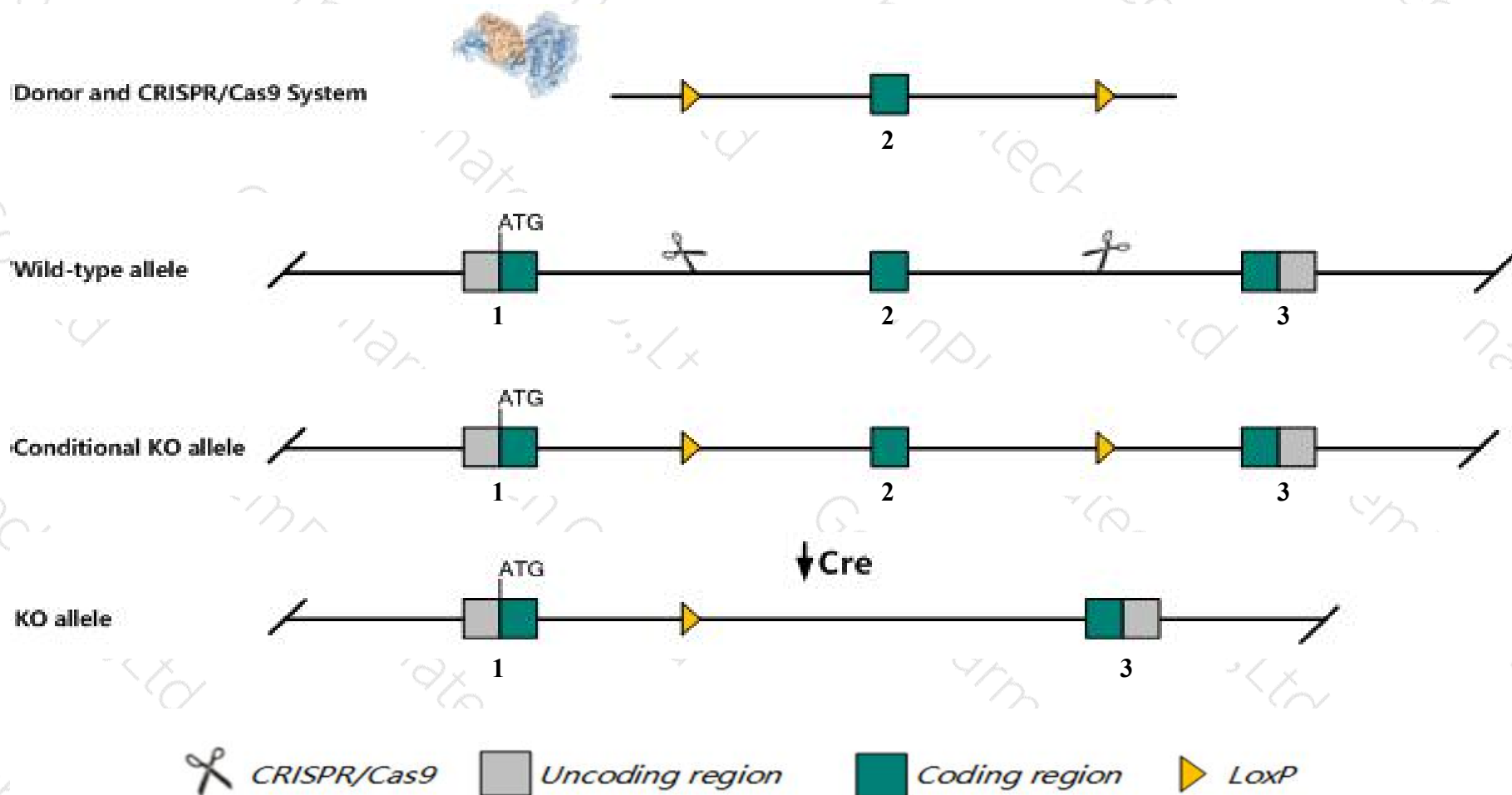
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ccl5* gene. The schematic diagram is as follows:



Technical routes

- The *Ccl5* gene has 2 transcripts. According to the structure of *Ccl5* gene, exon2 of *Ccl5-201* (ENSMUST00000035938.2) transcript is recommended as the knockout region. The region contains 112bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ccl5* 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 mutation of this gene results in decreased ear and footpad swelling following injection with DHT. T cell proliferative response is also impaired in mutant mice.
- The *Ccl5* gene is located on the Chr11. 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)

Ccl5 chemokine (C-C motif) ligand 5 [Mus musculus (house mouse)]

Gene ID: 20304, updated on 9-Apr-2019

Summary



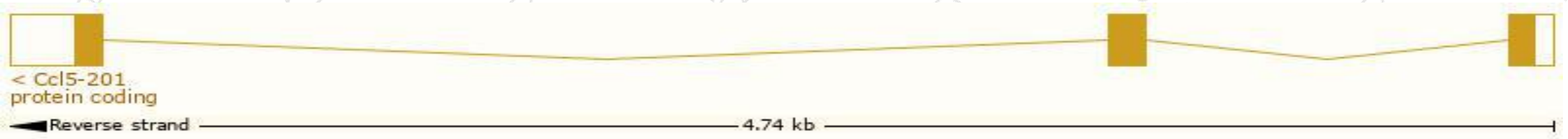
Official Symbol	Ccl5 provided by MGI
Official Full Name	chemokine (C-C motif) ligand 5 provided by MGI
Primary source	MGI:MGI:98262
See related	Ensembl:ENSMUSG00000035042
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	MuRantes, RANTES, SISd, Scya5, TCP228
Expression	Biased expression in small intestine adult (RPKM 266.1), mammary gland adult (RPKM 266.0) and 5 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

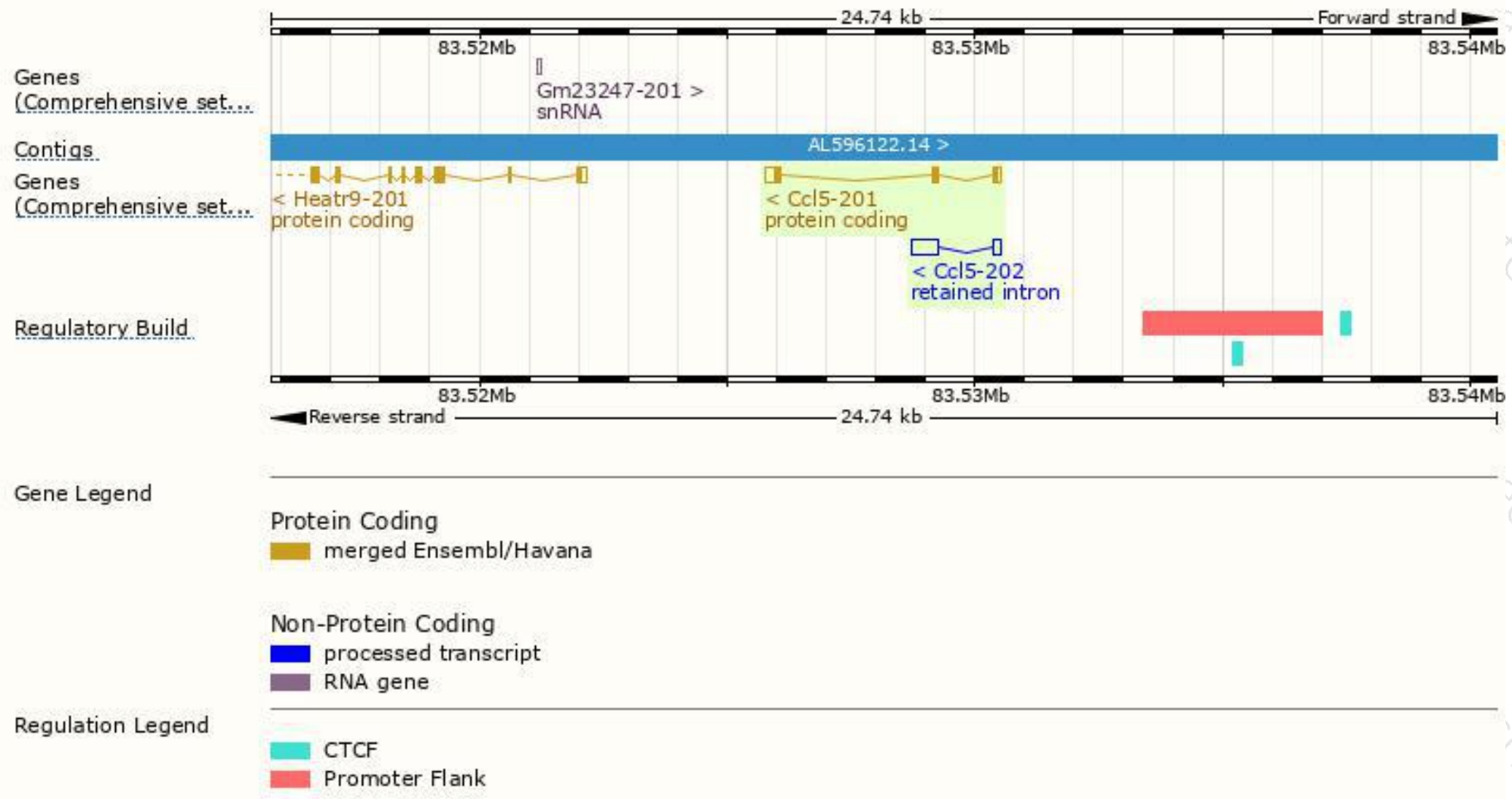
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ccl5-201	ENSMUST00000035938.2	532	91aa	Protein coding	CCDS36254	P30882 Q5XZF2	TSL:1 GENCODE basic APPRIS P1
Ccl5-202	ENSMUST00000125015.1	668	No protein	Retained intron	-	-	TSL:2

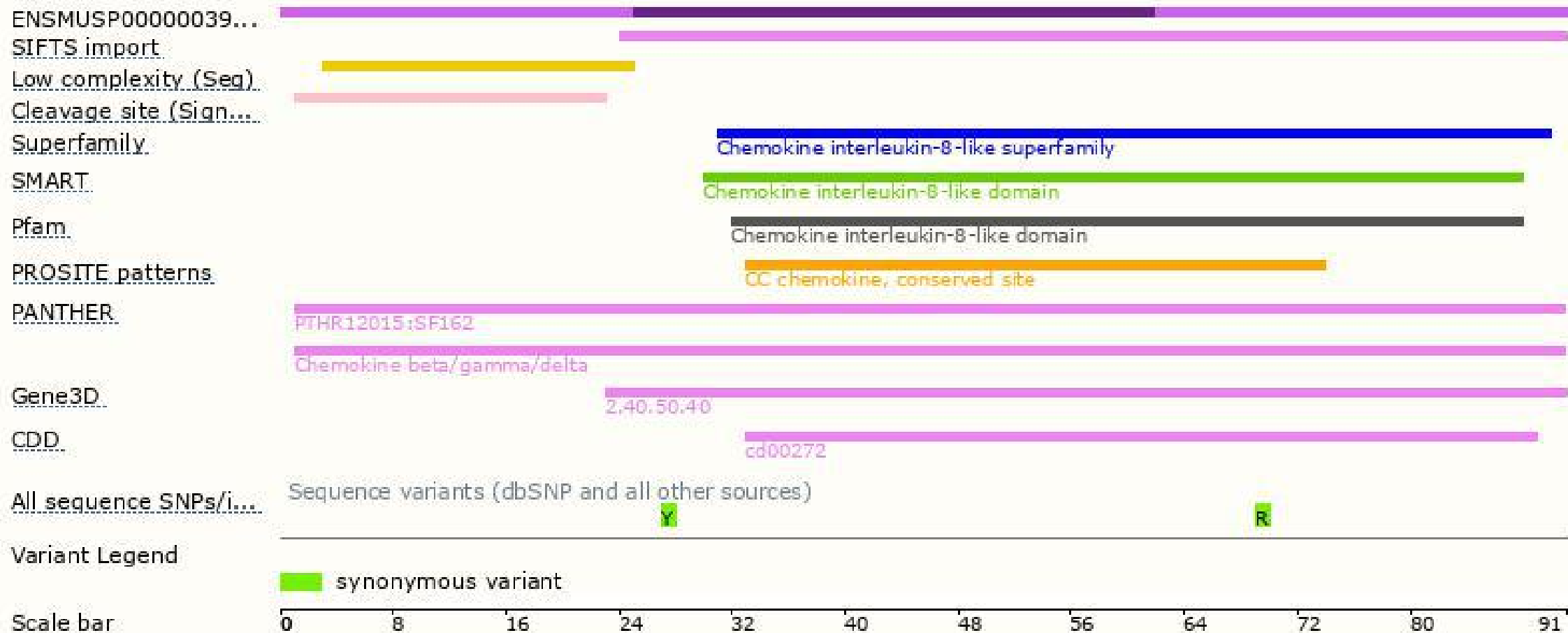
The strategy is based on the design of *Ccl5-201* transcript,The transcription is shown below



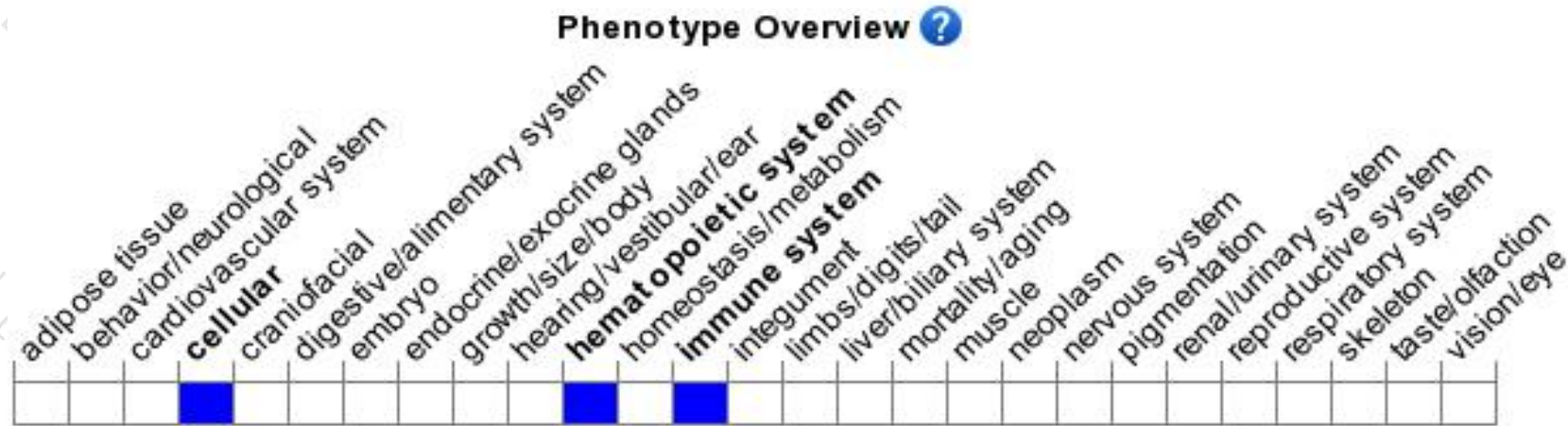
Genomic location distribution



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 mutation of this gene results in decreased ear and footpad swelling following injection with DHT. T cell proliferative response is also impaired in mutant mice.

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

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