

***Flot1* Cas9-CKO Strategy**

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Project Overview

Project Name

Flot1

Project type

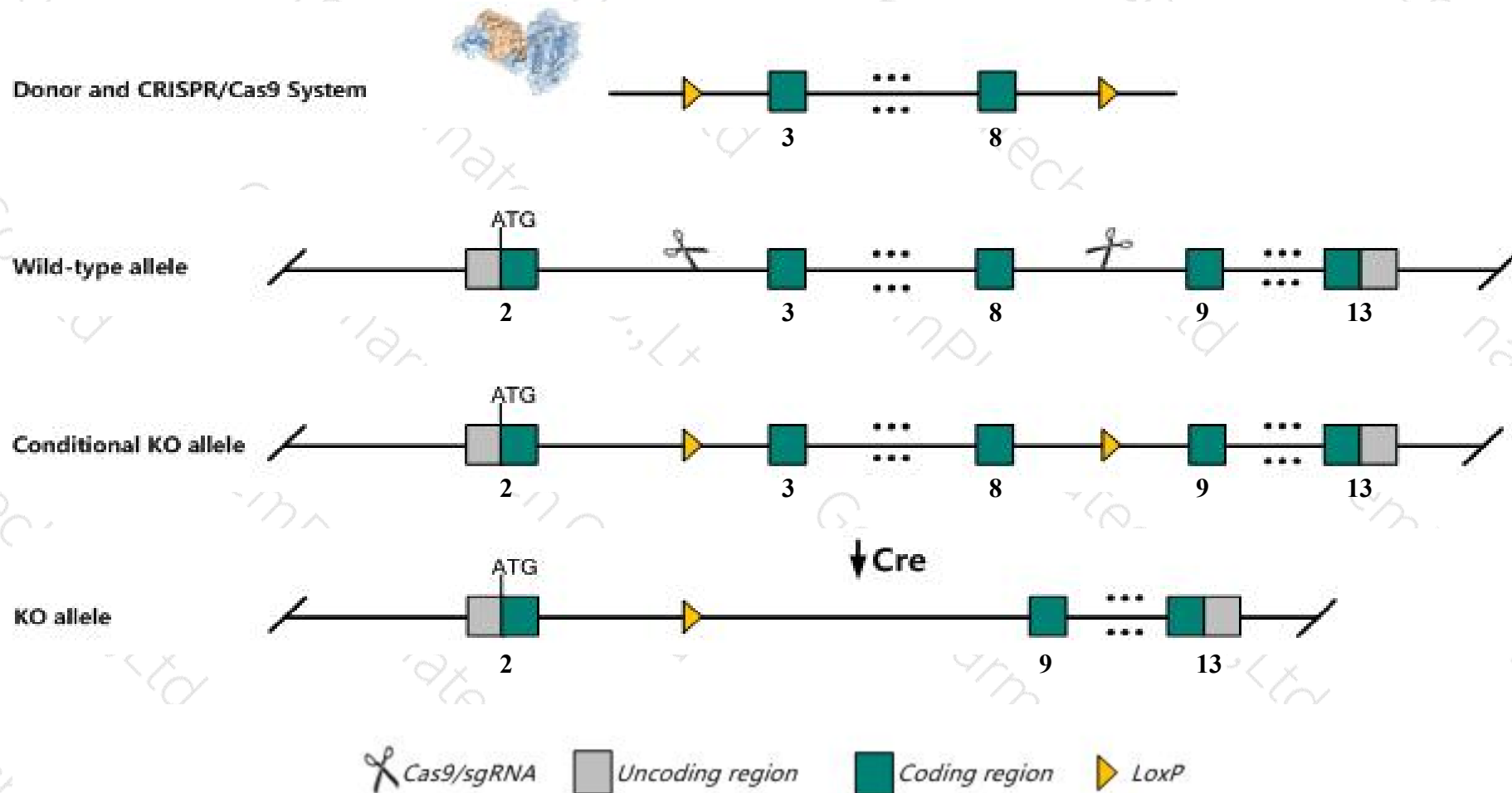
Cas9-CKO

Strain background

C57BL/6J

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Flot1* gene. The schematic diagram is as follows:



Technical routes

- The *Flot1* gene has 11 transcripts. According to the structure of *Flot1* gene, exon3-exon8 of *Flot1-201* (ENSMUST00000001569.14) transcript is recommended as the knockout region. The region contains 680bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Flot1* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed. Cas9, sgRNA and Donor were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.
- The flox mice was 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 a knock-out allele exhibit impaired neutrophil recruitment.
- Transcript *Flot1*-204&206&211 may not be affected.
- Transcript *Flot1*-205 may be directly destroyed in this strategy.
- The intron 2-3 is 263 bp, the loxp insertion may affect the regulation of *Flot1* gene.
- The floxed region is near to the C-terminal of *Ier3* gene, this strategy may influence the regulatory function of the C-terminal of *Ier3* gene.
- The *Flot1* gene is located on the Chr17. 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)

Flot1 flotillin 1 [*Mus musculus* (house mouse)]

Gene ID: 14251, updated on 8-Dec-2019

Summary

Official Symbol	Flot1 provided by MGI
Official Full Name	flotillin 1 provided by MGI
Primary source	MGI:MGI:1100500
See related	Ensembl:ENSMUSG00000059714
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	reggie-2
Expression	Ubiquitous expression in bladder adult (RPKM 47.3), heart adult (RPKM 42.6) and 28 other tissues See more
Orthologs	human all

Genomic context

Location: 17; 17 B1

See Flot1 in [Genome Data Viewer](#)

Exon count: 14

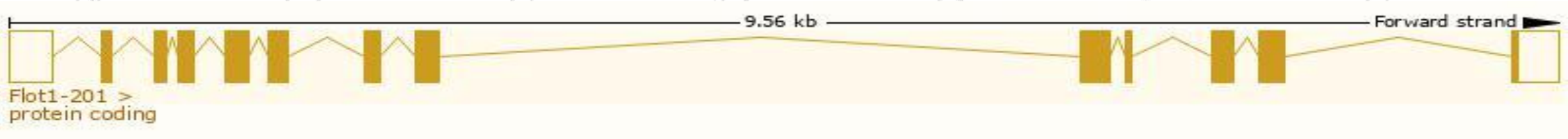
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	17	NC_000083.6 (35823320..35832789)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	17	NC_000083.5 (35960302..35969732)

Transcript information (Ensembl)

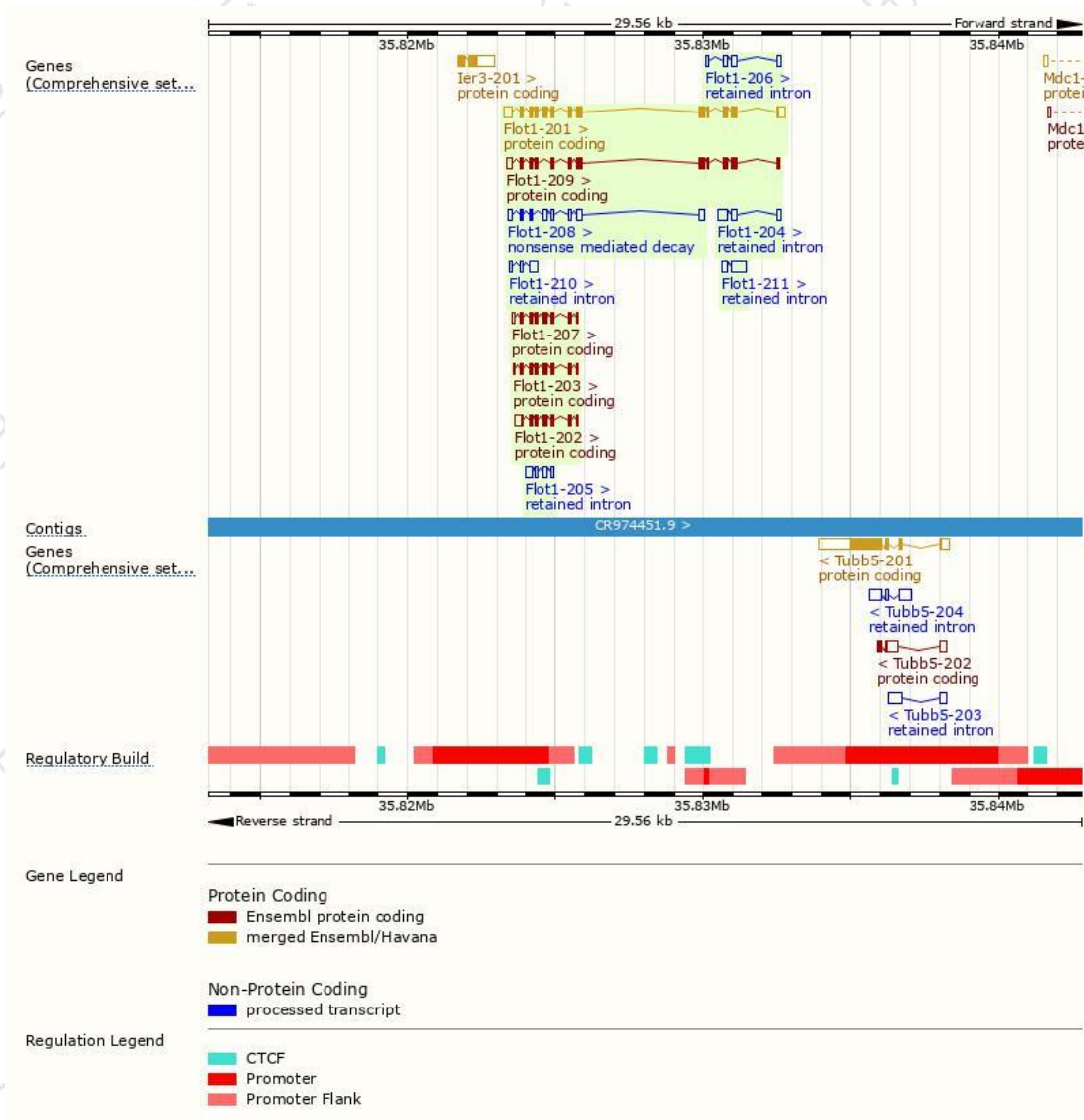
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Flot1-201	ENSMUST00000001569.14	1825	428aa	Protein coding	CCDS28705	O08917 Q54014	TSL:1 GENCODE basic APPRIS P1
Flot1-209	ENSMUST00000174080.7	1383	380aa	Protein coding	-	G3UYU4	TSL:5 GENCODE basic
Flot1-202	ENSMUST00000172846.1	805	192aa	Protein coding	-	G3UZZ5	CDS 3' incomplete TSL:2
Flot1-207	ENSMUST00000173493.7	697	192aa	Protein coding	-	G3UZZ5	CDS 3' incomplete TSL:5
Flot1-203	ENSMUST00000173147.7	651	196aa	Protein coding	-	G3UWW8	CDS 3' incomplete TSL:5
Flot1-208	ENSMUST00000173628.7	948	40aa	Nonsense mediated decay	-	G3XA73	TSL:5
Flot1-211	ENSMUST00000174297.1	682	No protein	Retained intron	-	-	TSL:1
Flot1-204	ENSMUST00000173273.1	592	No protein	Retained intron	-	-	TSL:2
Flot1-205	ENSMUST00000173337.1	538	No protein	Retained intron	-	-	TSL:3
Flot1-206	ENSMUST00000173381.7	502	No protein	Retained intron	-	-	TSL:2
Flot1-210	ENSMUST00000174227.1	405	No protein	Retained intron	-	-	TSL:3

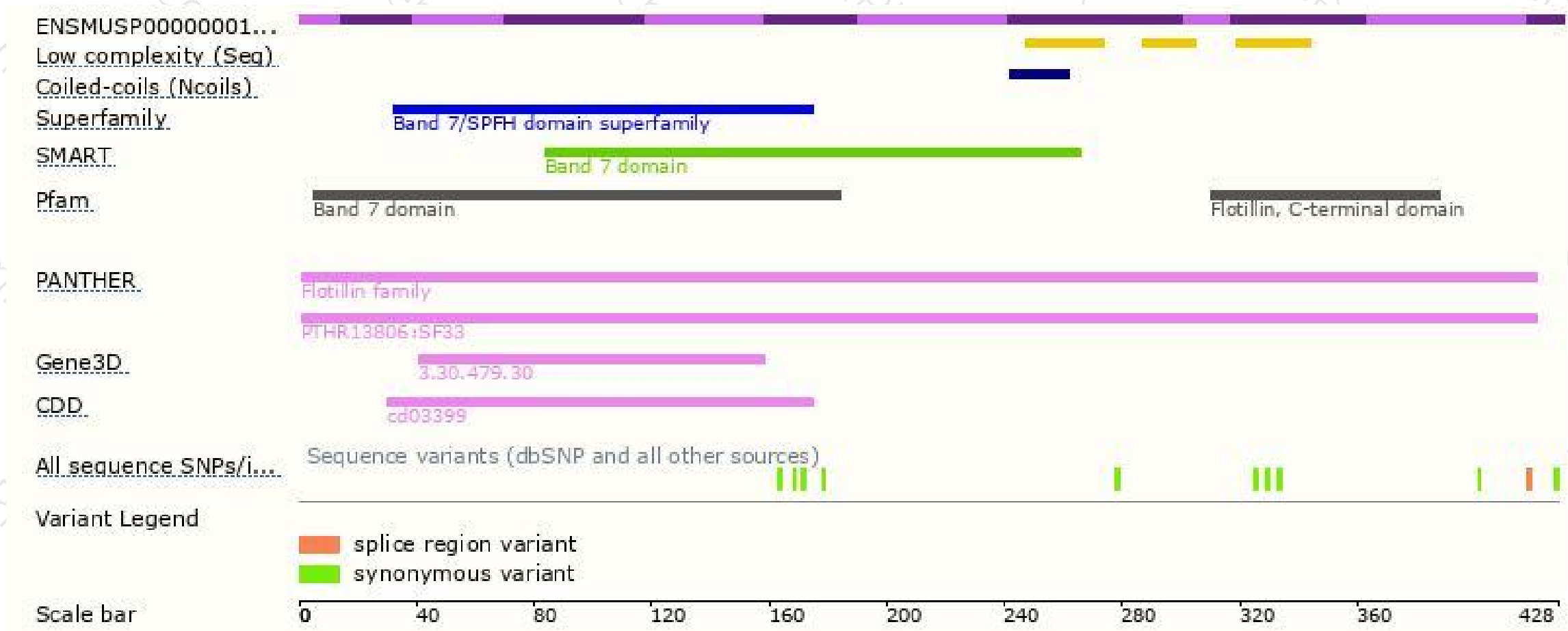
The strategy is based on the design of *Flot1-201* transcript,The transcription is shown below



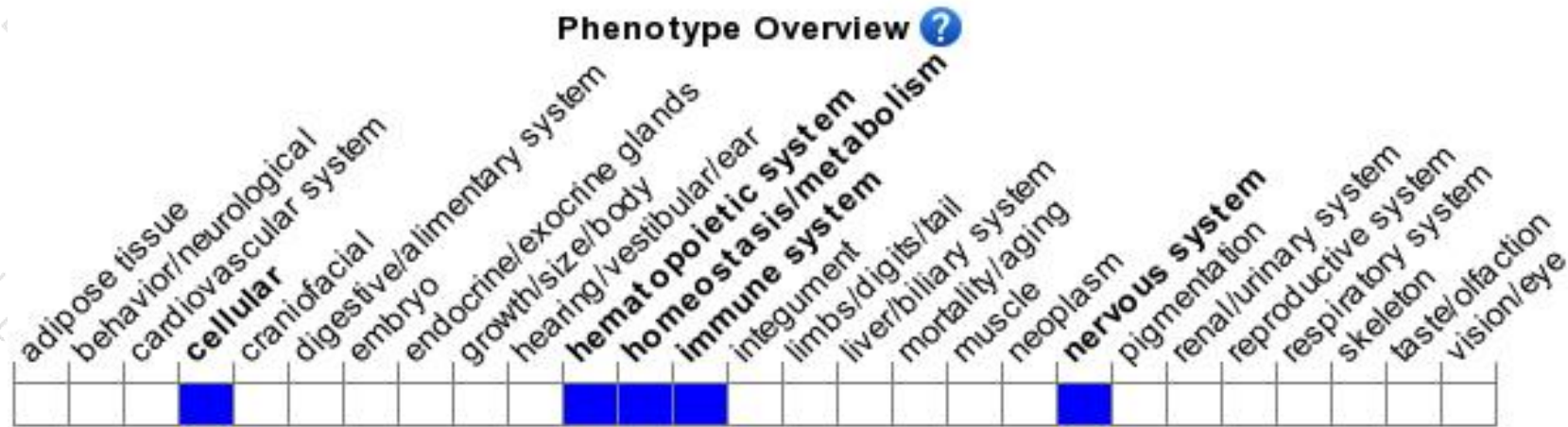
Genomic location distribution



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 a knock-out allele exhibit impaired neutrophil recruitment.

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

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