

Cfd Cas9-CKO Strategy

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Project Overview

Project Name

Cfd

Project type

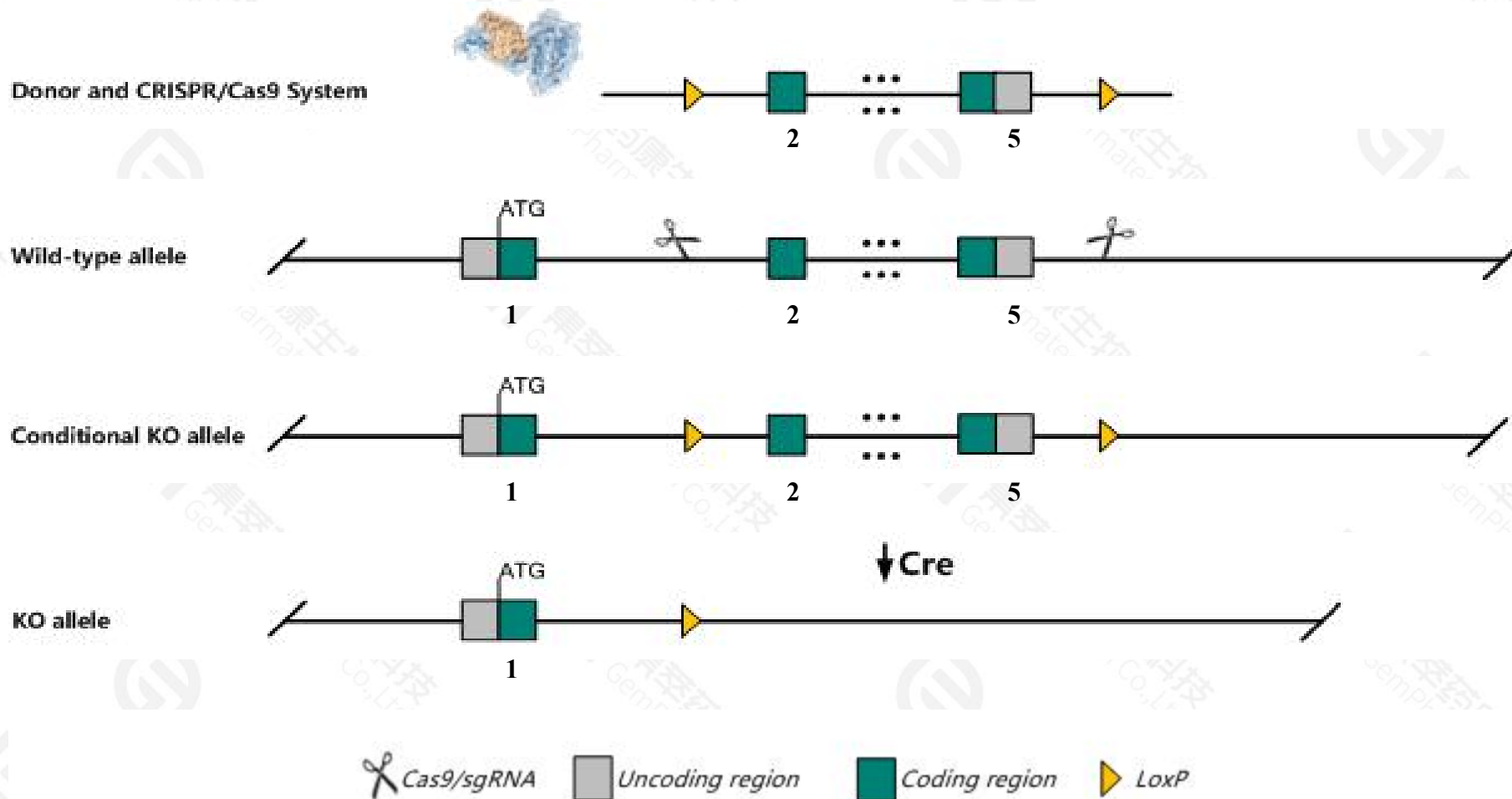
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- The *Cfd* gene has 2 transcripts. According to the structure of *Cfd* gene, exon2-exon5 of *Cfd-201*(ENSMUST00000061653.9) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cfd* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor was constructed. Cas9, sgRNA 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 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 show impaired complement activation by alternative pathway activators, and increased susceptibility to pneumococcal infection.
- The Intron1-2 is only 475bp, loxp insertion may affect mRNA splicing.
- The *Cfd* gene is located on the Chr10. 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)

Cfd complement factor D (adipsin) [Mus musculus (house mouse)]

Gene ID: 11537, updated on 13-Mar-2020

Summary

Official Symbol Cfd provided by [MGI](#)

Official Full Name complement factor D (adipsin) provided by [MGI](#)

Primary source [MGI:MGI:87931](#)

See related [Ensembl:ENSMUSG00000061780](#)

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 Adn, DF

Summary This gene encodes a serine protease that plays an important role in the alternative pathway of complement activation for pathogen recognition and elimination. The encoded preproprotein undergoes proteolytic processing to generate a mature, functional enzyme that in turn cleaves factor B in the complement pathway. This gene is expressed in adipocytes and the mature enzyme is secreted into the bloodstream. Mice lacking the encoded product cannot initiate alternative pathway of complement activation. [provided by RefSeq, Jul 2016]

Expression Biased expression in mammary gland adult (RPKM 11182.1), subcutaneous fat pad adult (RPKM 7523.9) and 4 other tissues [See more](#)

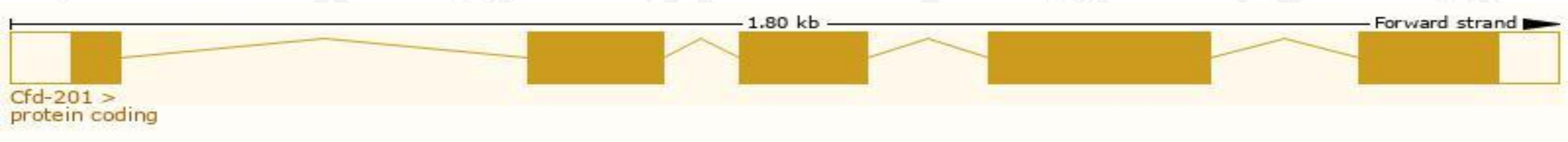
Orthologs [human](#) [all](#)

Transcript information (Ensembl)

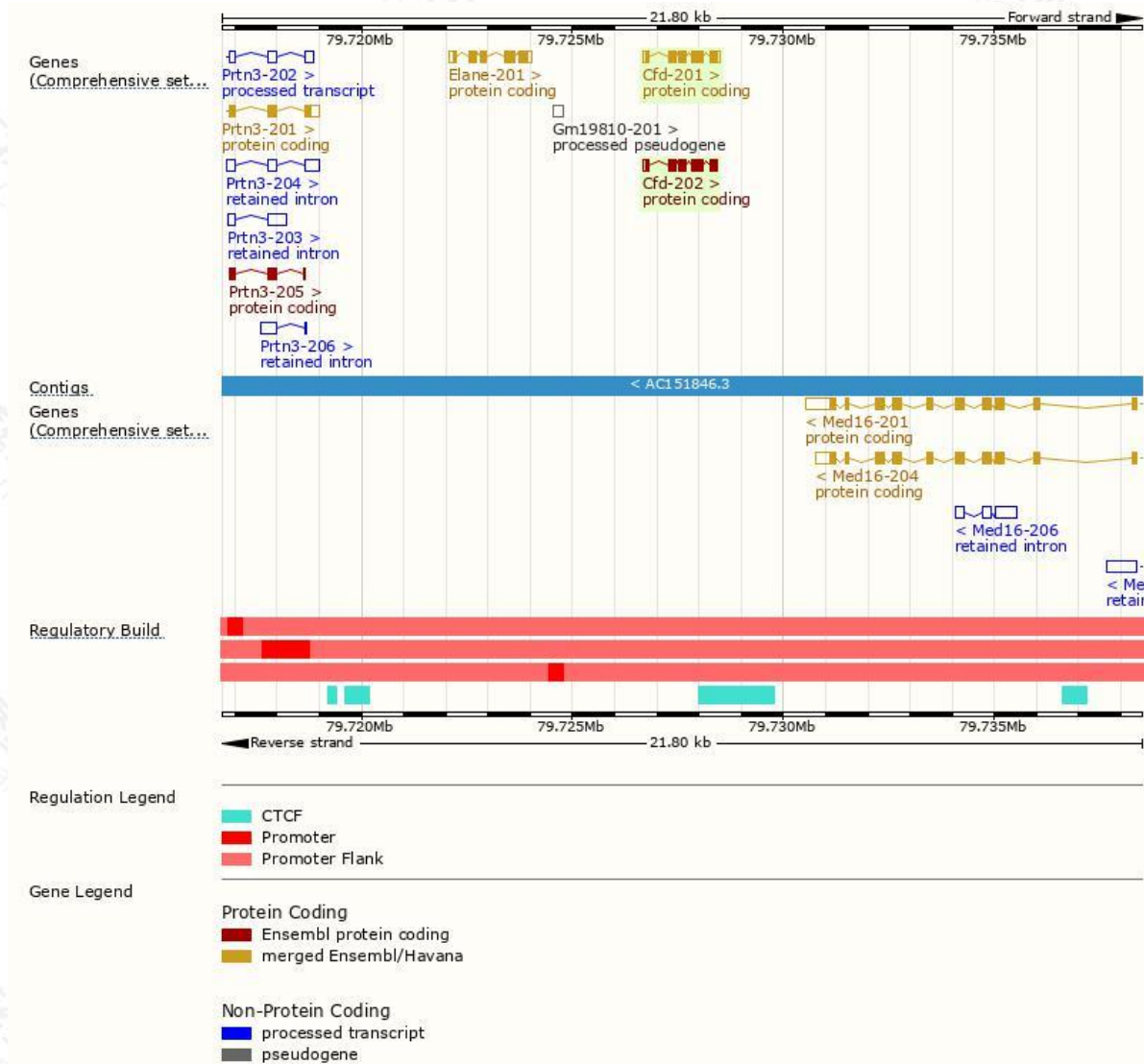
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cfd-201	ENSMUST00000061653.8	922	259aa	Protein coding	CCDS23995	P03953 Q3UP47	TSL:1 GENCODE basic APPRIS P2
Cfd-202	ENSMUST00000217837.1	871	258aa	Protein coding	-	B7ZNS9 P03953	TSL:1 GENCODE basic APPRIS ALT2

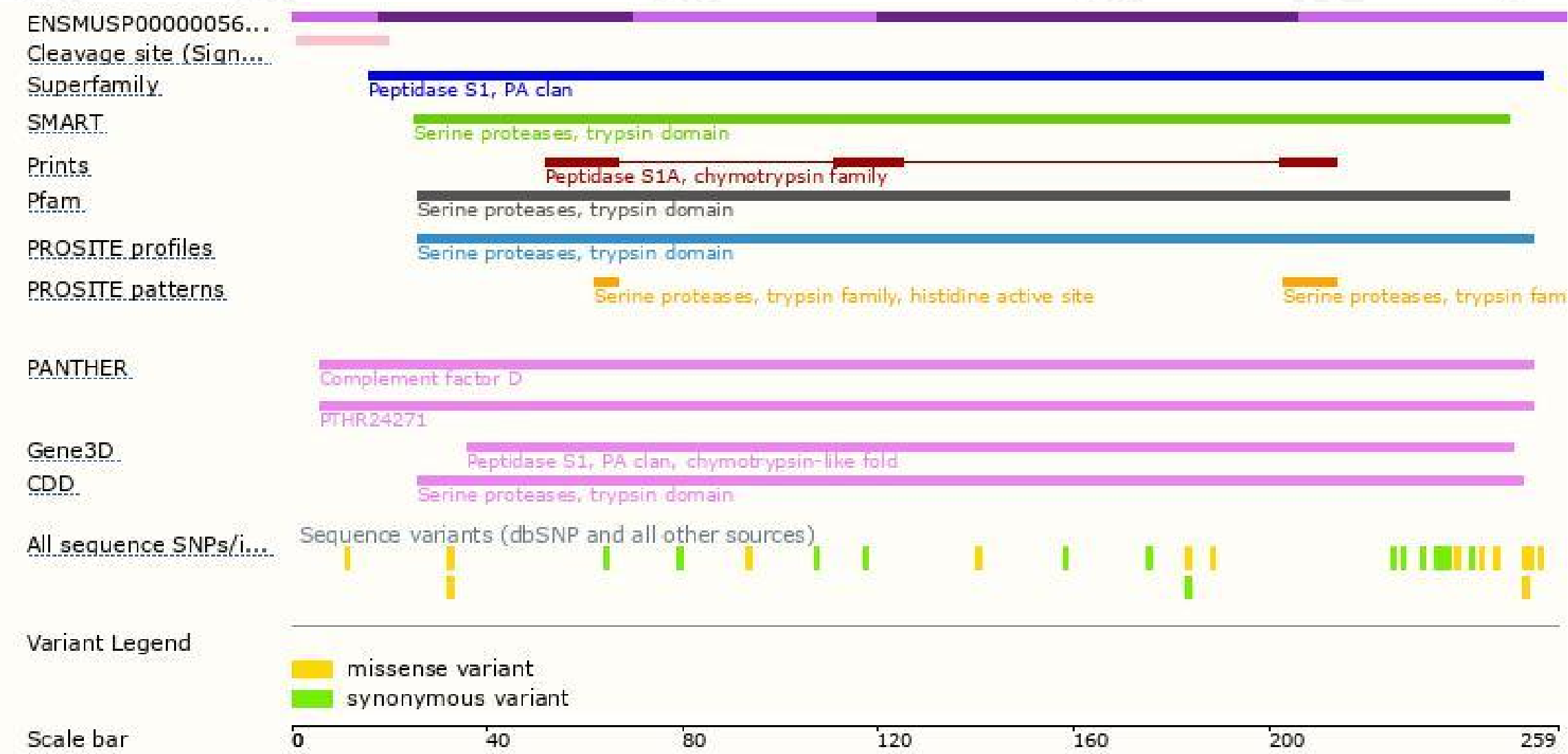
The strategy is based on the design of *Cfd-201* transcript,the transcription is shown below:



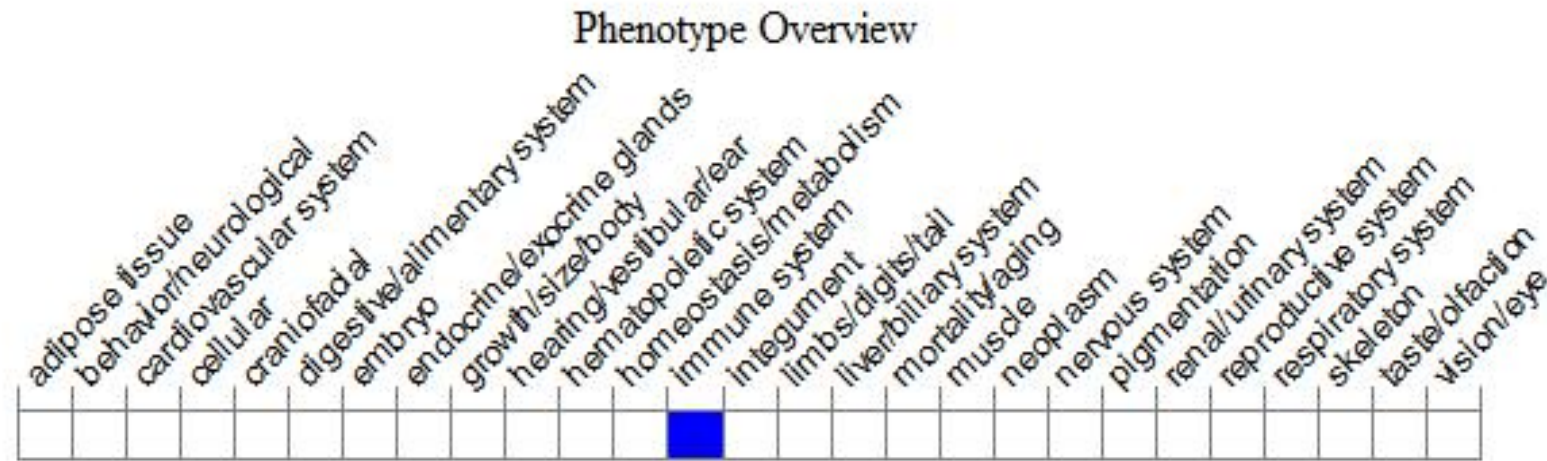
Genomic location distribution



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 show impaired complement activation by alternative pathway activators, and increased susceptibility to pneumococcal infection.

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

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