Tnf Cas9-CKO Strategy

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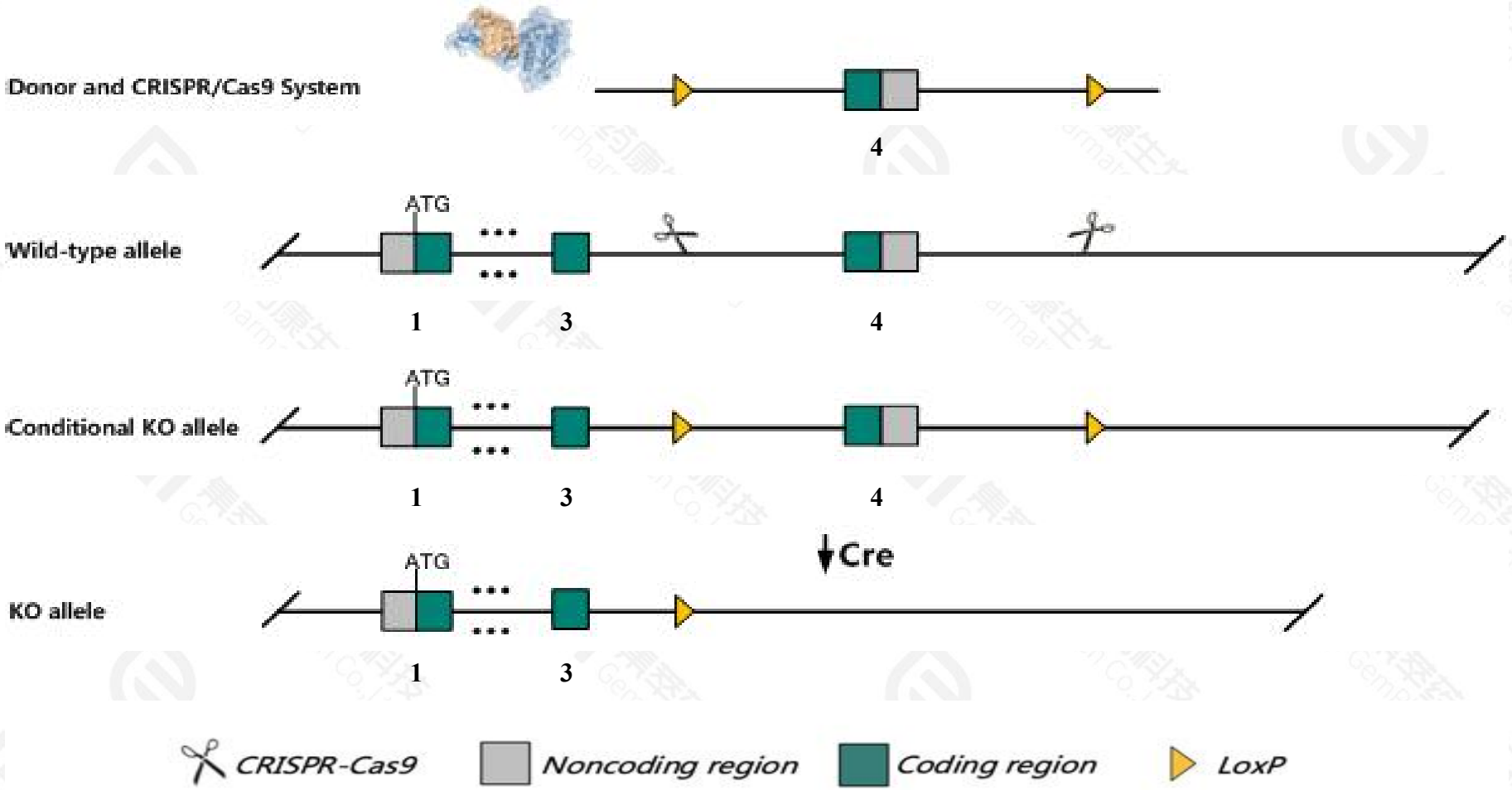
Project Overview

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

Conditional Knockout strategy[1]

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



Technical routes

➢ The *Tnf* gene has 2 transcripts. According to the structure of *Tnf* gene, exon4 of *Tnf-201*(ENSMUST00000025263.15) transcript is recommended as the knockout region.The region contains 419bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR-Cas9 technology to modify *Tnf* 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,mutations at this locus primarily affect the immune system, causing increased susceptibility to infection, failure to form splenic B-cell follicles, increased inflammation and impaired contact hypersensitivity. Homozygotes also may show metabolic defects.

➢ The Intron3 is only 294bp,loxp insertion may affect mRNA splicing.

➢ The *Tnf* 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）

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Tnf tumor necrosis factor [Mus musculus (house mouse)]

Gene ID: 21926, updated on 22-Mar-2020

Summary

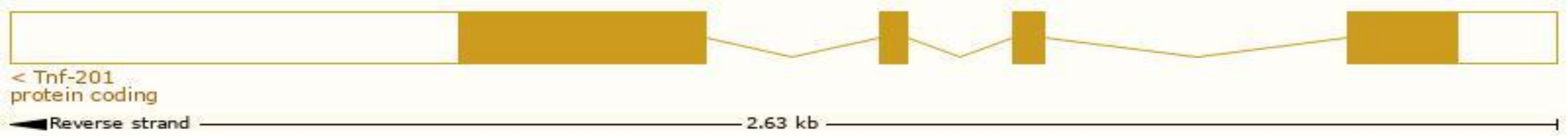
Official Symbol	Tnf provided by MGI
Official Full Name	tumor necrosis factor provided by MGI
Primary source	MGI:MGI:104798
See related	Ensembl:ENSMUSG00000024401
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	DIF, TNF-a, TNF-alpha, TNFSF2, TNFalpha, Tnfa, Tnfsf1a, Tnlg1f
Summary	This gene encodes a multifunctional proinflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily. Members of this family are classified based on primary sequence, function, and structure. This protein is synthesized as a type-II transmembrane protein and is reported to be cleaved into products that exert distinct biological functions. It plays an important role in the innate immune response as well as regulating homeostasis but is also implicated in diseases of chronic inflammation. In mouse deficiency of this gene is associated with defects in response to bacterial infection, with defects in forming organized follicular dendritic cell networks and germinal centers, and with a lack of primary B cell follicles. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Jun 2013]
Expression	Broad expression in spleen adult (RPKM 4.1), thymus adult (RPKM 3.0) and 16 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

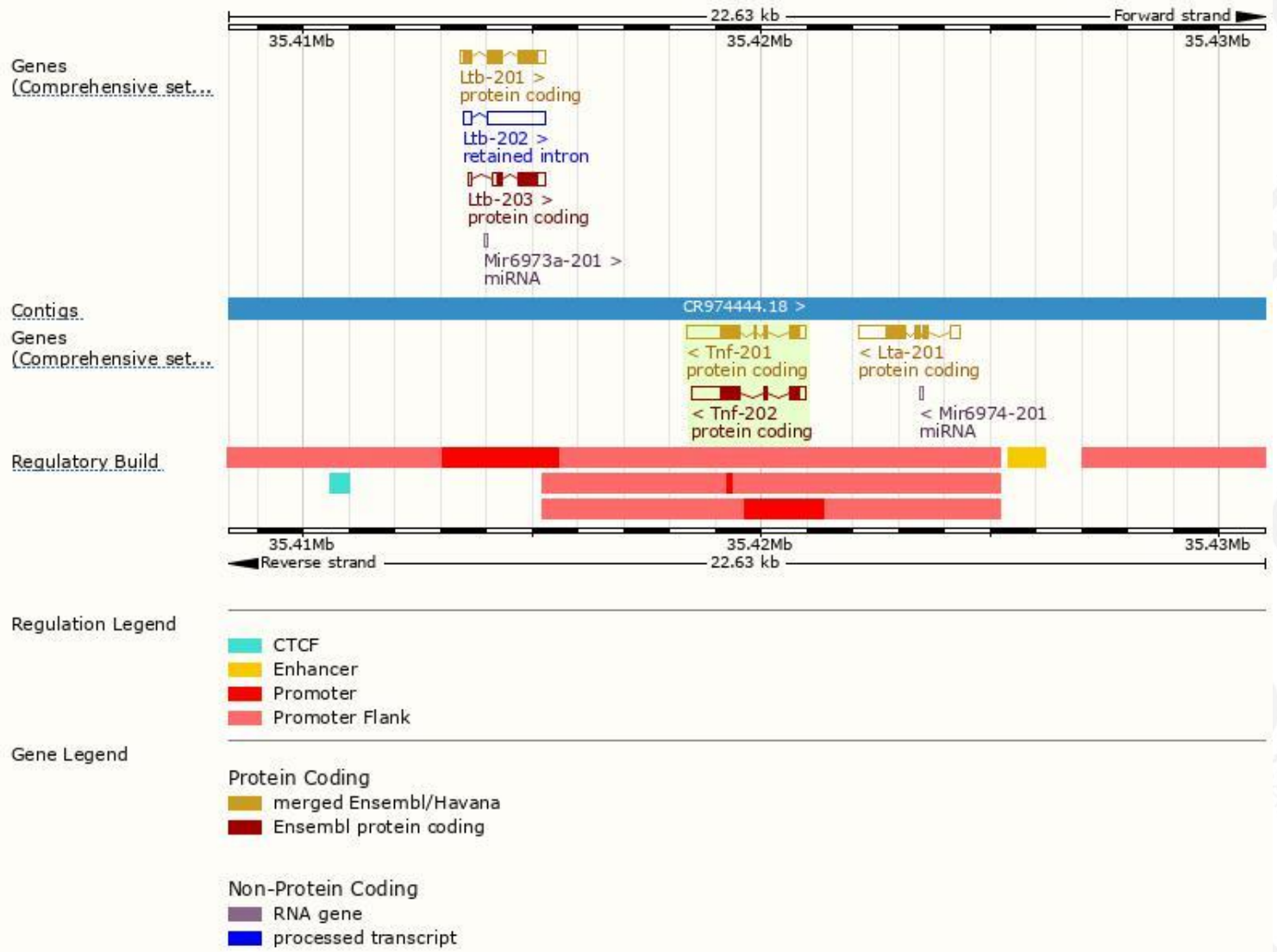
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tnf-201	ENSMUST00000025263.14	1639	235aa	Protein coding	CCDS28691	P06804 Q3U593	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Tnf-202	ENSMUST00000167924.1	1462	219aa	Protein coding	CCDS70801	A0A0R4J210	TSL:1 GENCODE basic

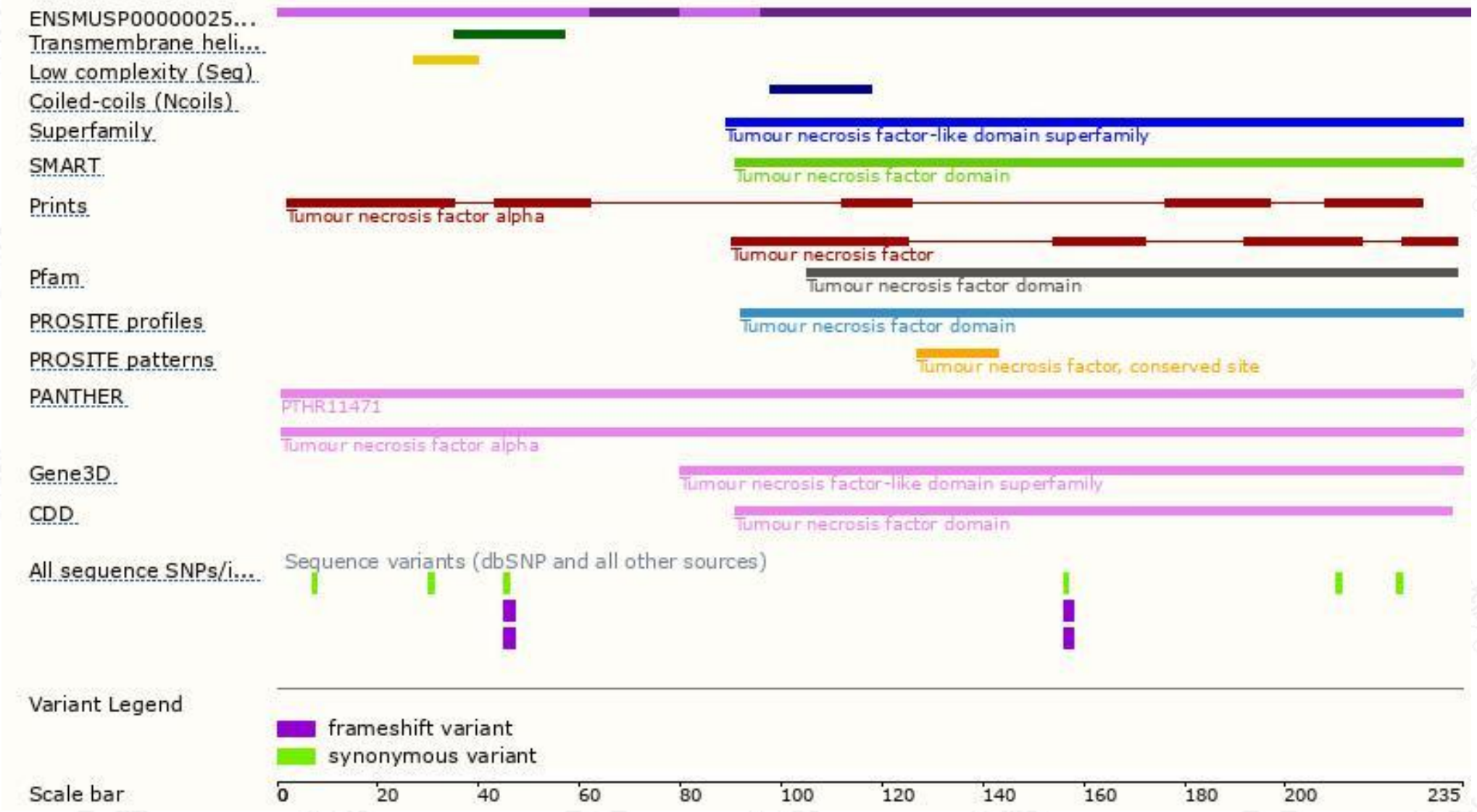
The strategy is based on the design of *Tnf-201* transcript,the transcription is shown below:



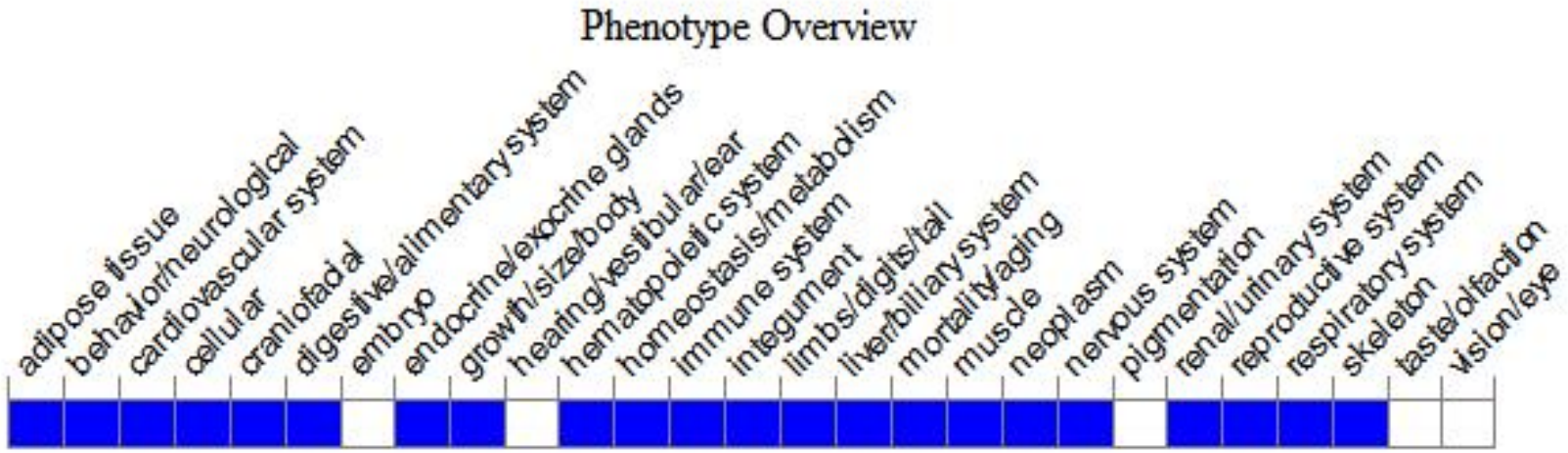
Genomic location distribution



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,mutations at this locus primarily affect the immune system, causing increased susceptibility to infection, failure to form splenic B-cell follicles, increased inflammation and impaired contact hypersensitivity. Homozygotes also may show metabolic defects.

Reference

[1] Grivennikov S I , Tumanov A V , Liepinsh D J , et al. Distinct and Nonredundant In Vivo Functions of TNF Produced by T Cells and Macrophages/Neutrophils[J]. IMMUNITY -CAMBRIDGE MA-, 2005.

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