Alox12b Cas9-CKO Strategy

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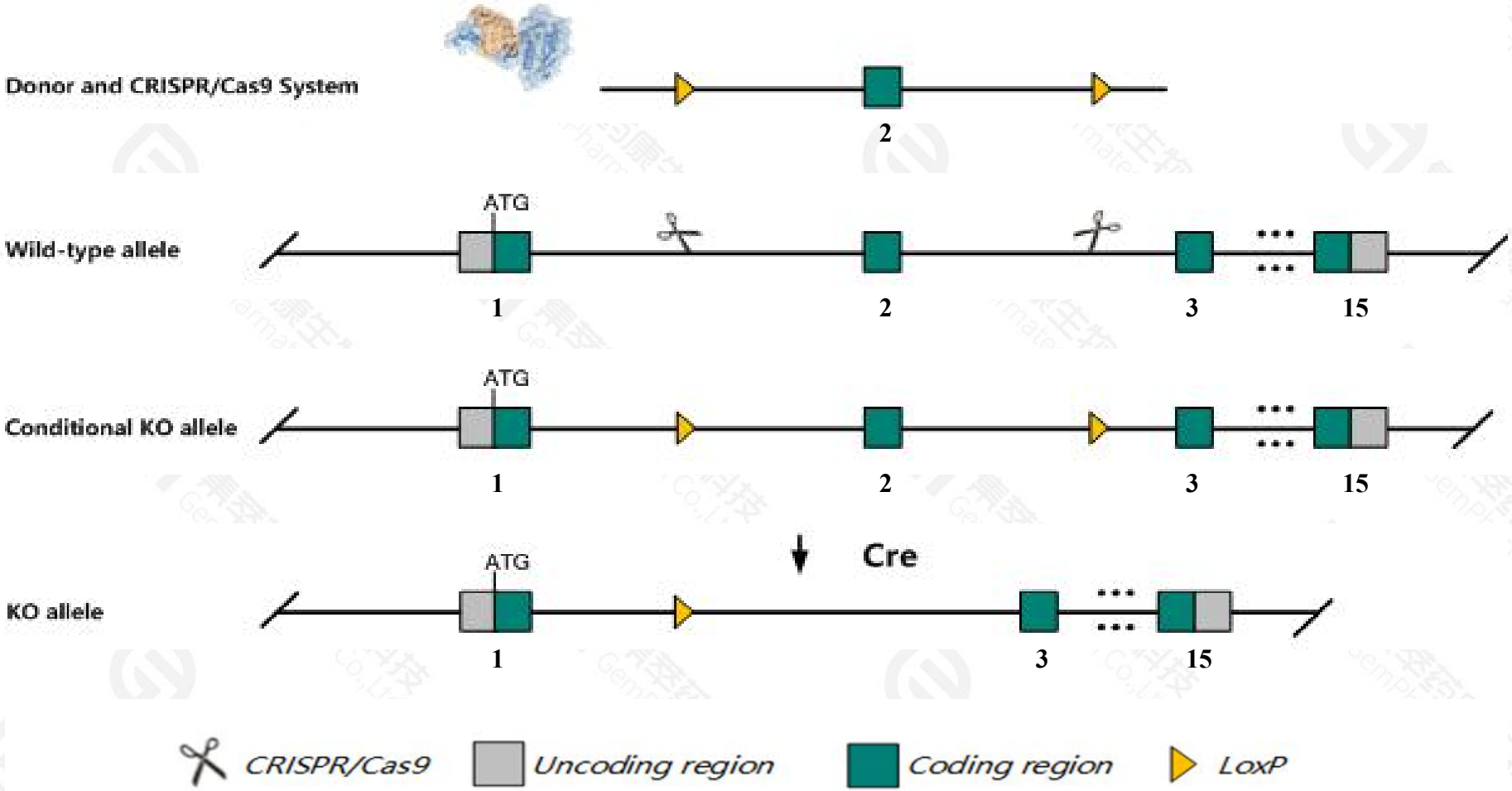
Project Overview

Project Name	***Alox12b***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Alox12b* gene. The schematic diagram is as follows:



Technical routes

➢ The *Alox12b* gene has 1 transcript. According to the structure of *Alox12b* gene, exon2 of *Alox12b-201*(ENSMUST00000036424.2) transcript is recommended as the knockout region.The region contains 205bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Alox12b* 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,neonatal homozygous mutant mice exhibit reddened skin that quickly dehydrates and appears scaly. The epidermis is hyperkeratotic, and its permeability barrier function is compromised. Homozygotes die within 24 hours of birth.

➢ The *Alox12b* 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）

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Alox12b arachidonate 12-lipoxygenase, 12R type [Mus musculus (house mouse)]

Gene ID: 11686, updated on 13-Mar-2020

Summary

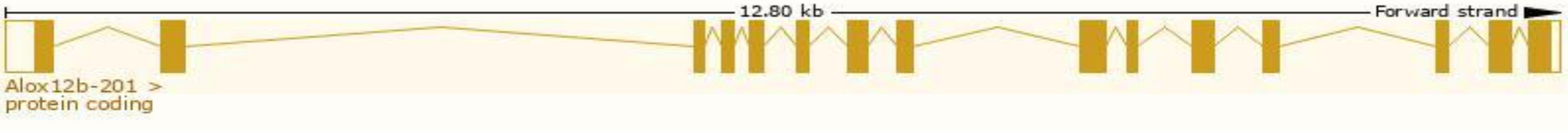
Official Symbol	Alox12b provided by MGI
Official Full Name	arachidonate 12-lipoxygenase, 12R type provided by MGI
Primary source	MGI:MGI:1274782
See related	Ensembl:ENSMUSG00000032807
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	12R-LOX, Aloxe2, e-LOX2
Summary	This gene encodes an enzyme involved in the conversion of arachidonic acid to 12R-hydroxyeicosatetraenoic acid. Mutations in this gene can prevent the formation of the epidermal permeability barrier and cause an ichthyosiform phenotype. [provided by RefSeq, Sep 2015]
Expression	Biased expression in stomach adult (RPKM 18.4), lung adult (RPKM 9.8) and 1 other tissue See more
Orthologs	human all

Transcript information（Ensembl）

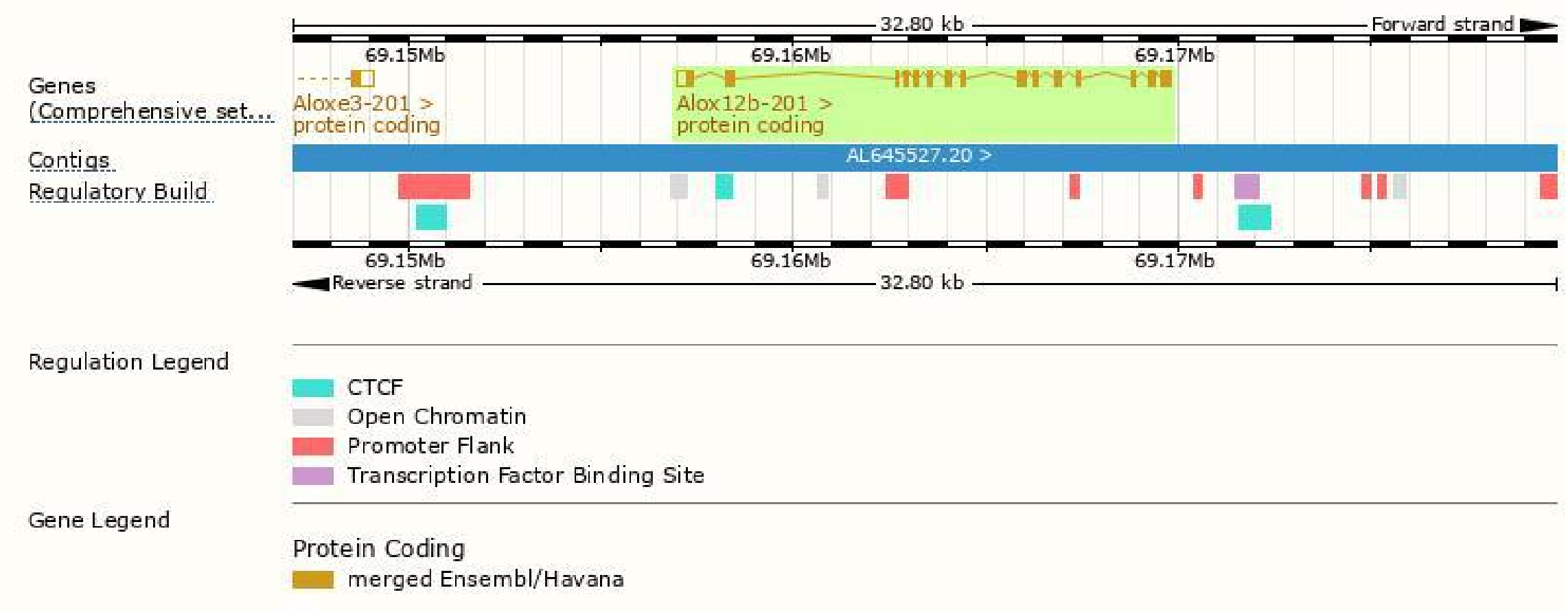
The gene has 1 transcript,and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Alox12b-201	ENSMUST00000036424.2	2431	701aa	Protein coding	CCDS24885	O70582 Q2KHL0	TSL:1 GENCODE basic APPRIS P1

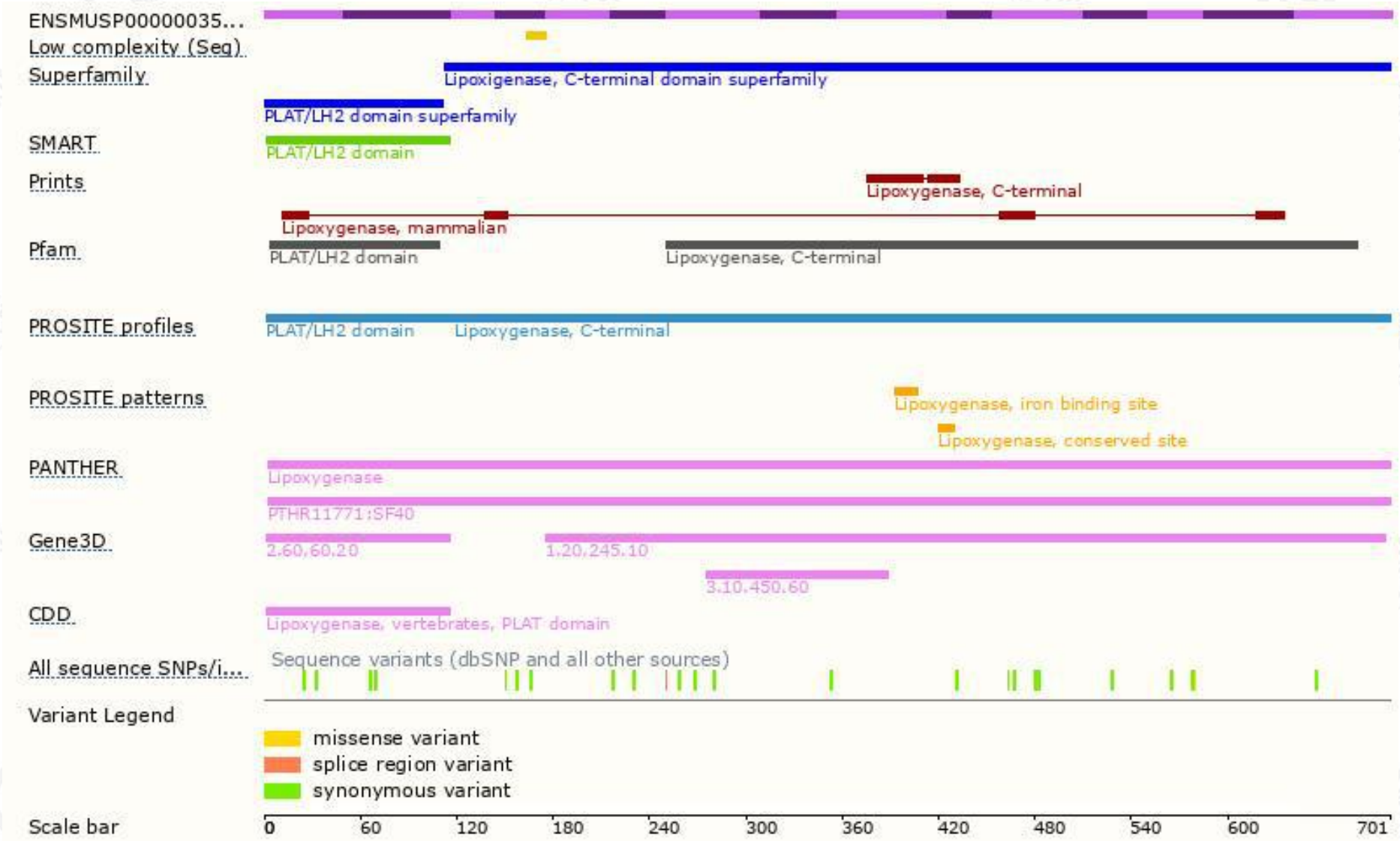
The strategy is based on the design of *Alox12b-201* transcript,the transcription is shown below:



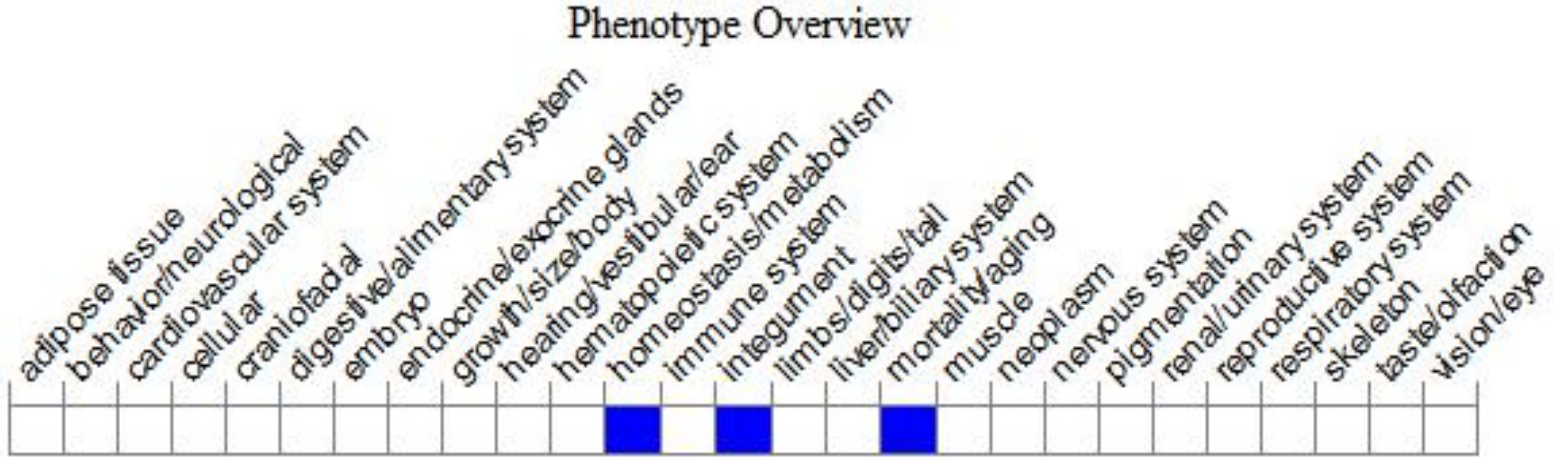
Genomic location distribution



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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