Creb3l3 Cas9-CKO Strategy

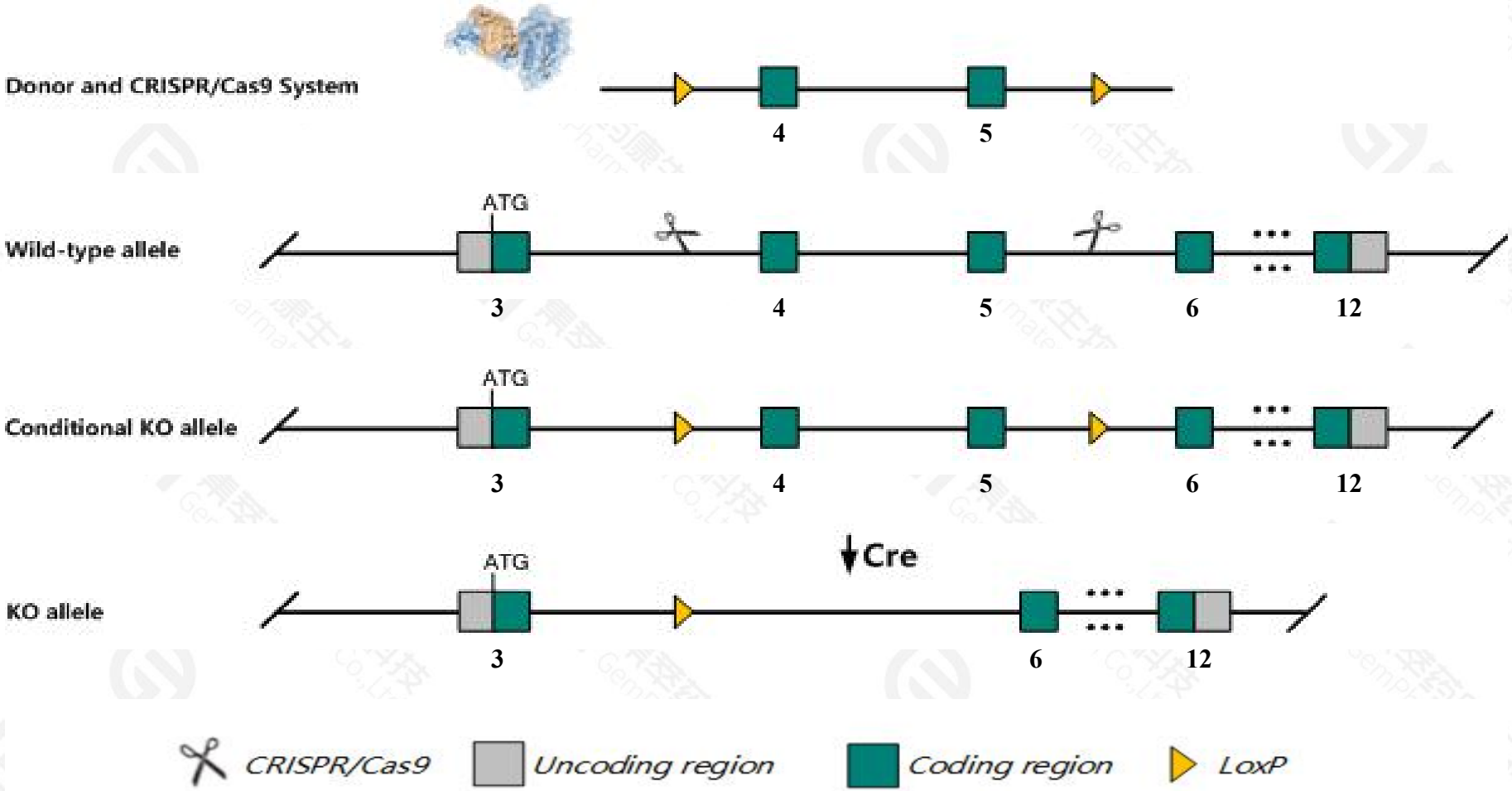
Designer: Qiong Zhou

Project Overview

Project Name	***Creb3l3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Creb3l3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Creb3l3* gene has 4 transcripts. According to the structure of *Creb3l3* gene, exon4-exon5 of *Creb3l3-201*(ENSMUST00000117422.2) transcript is recommended as the knockout region.The region contains 430bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Creb3l3* 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,mice homozygous for a knock-out allele are viable and healthy but exhibit reduced expression of acute phase response proteins following treatment with tunicamycin that induces ER stress. Mice homozygous for a different knock-out allele exhibit resistanceto sulpyrine-induced shock.

➢ The Intron3 is only 573bp,loxp insertion may affect mRNA splicing.

➢ Transcript *Creb3l3-202* may not be affected.

➢ The *Creb3l3* 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）

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Creb3l3 cAMP responsive element binding protein 3-like 3 [Mus musculus (house mouse)]

Gene ID: 208677, updated on 1-Mar-2021

Summary

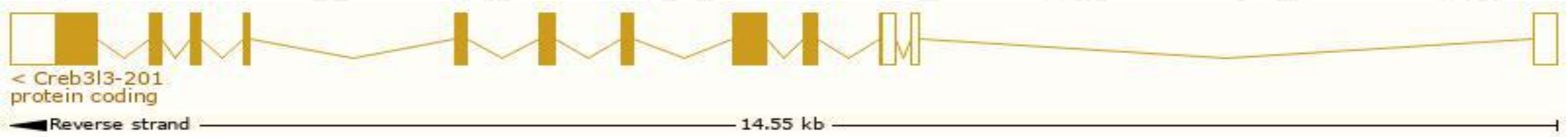
Official Symbol	Creb3l3 provided by MGI
Official Full Name	cAMP responsive element binding protein 3-like 3 provided by MGI
Primary source	MGI:MGI:2384786
See related	Ensembl:ENSMUSG00000035041
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	BC010786, CREB-, CREB-H, D10Bur1, D10Bur1e
Expression	Biased expression in duodenum adult (RPKM 141.6), small intestine adult (RPKM 102.5) and 6 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

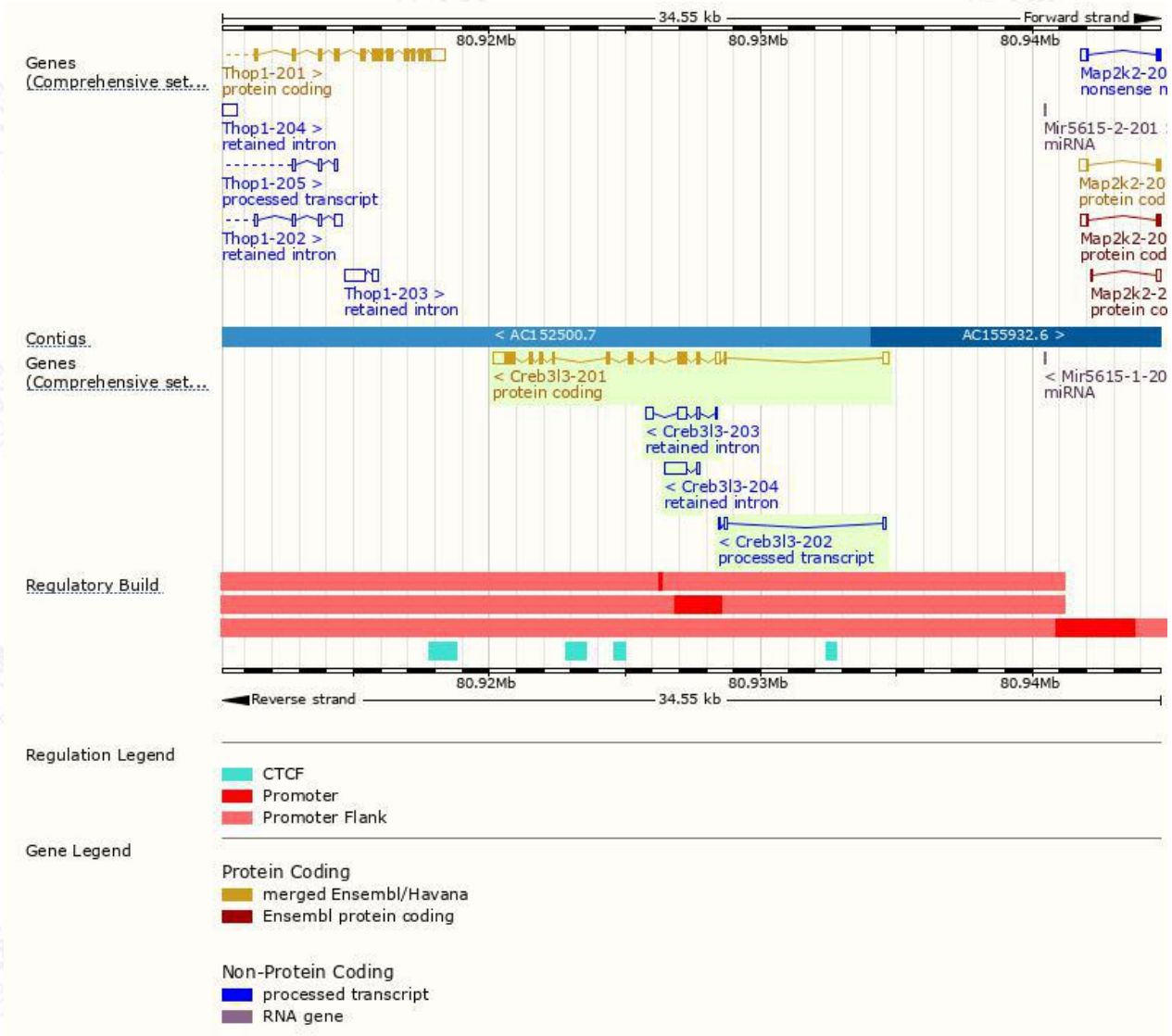
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Creb3l3-201	ENSMUST00000117422.2	2294	479aa	Protein coding	CCDS24043		TSL:1 , GENCODE basic , APPRIS P1 ,
Creb3l3-202	ENSMUST00000125907.2	275	No protein	Processed transcript	-		TSL:5 ,
Creb3l3-204	ENSMUST00000143969.2	922	No protein	Retained intron	-		TSL:3 ,
Creb3l3-203	ENSMUST00000127374.2	744	No protein	Retained intron	-		TSL:2 ,

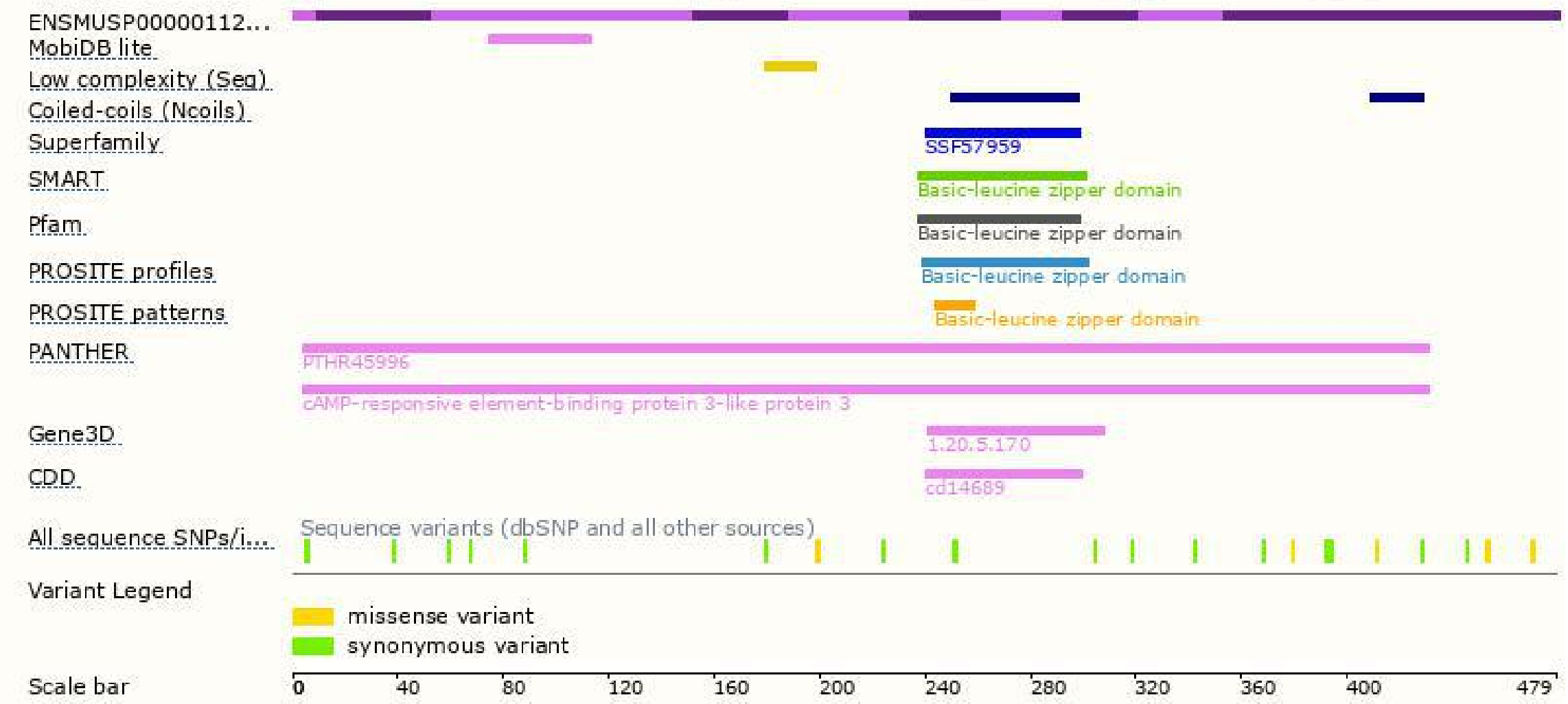
The strategy is based on the design of *Creb3l3-201* transcript,the transcription is shown below:



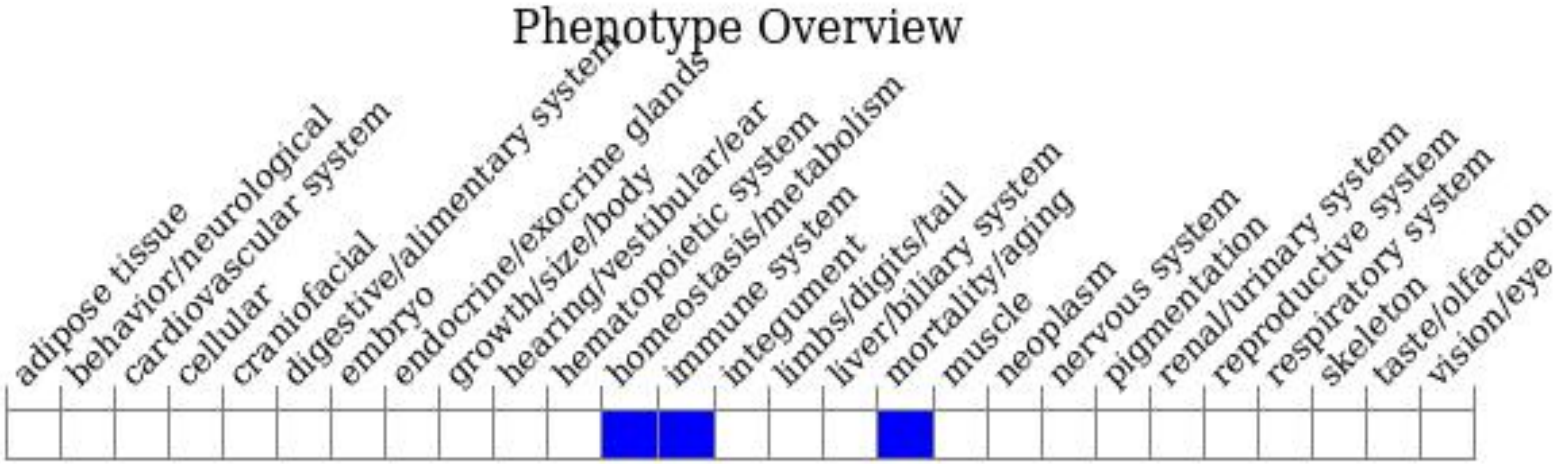
Genomic location distribution



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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