Lamp2 Cas9-KO Strategy

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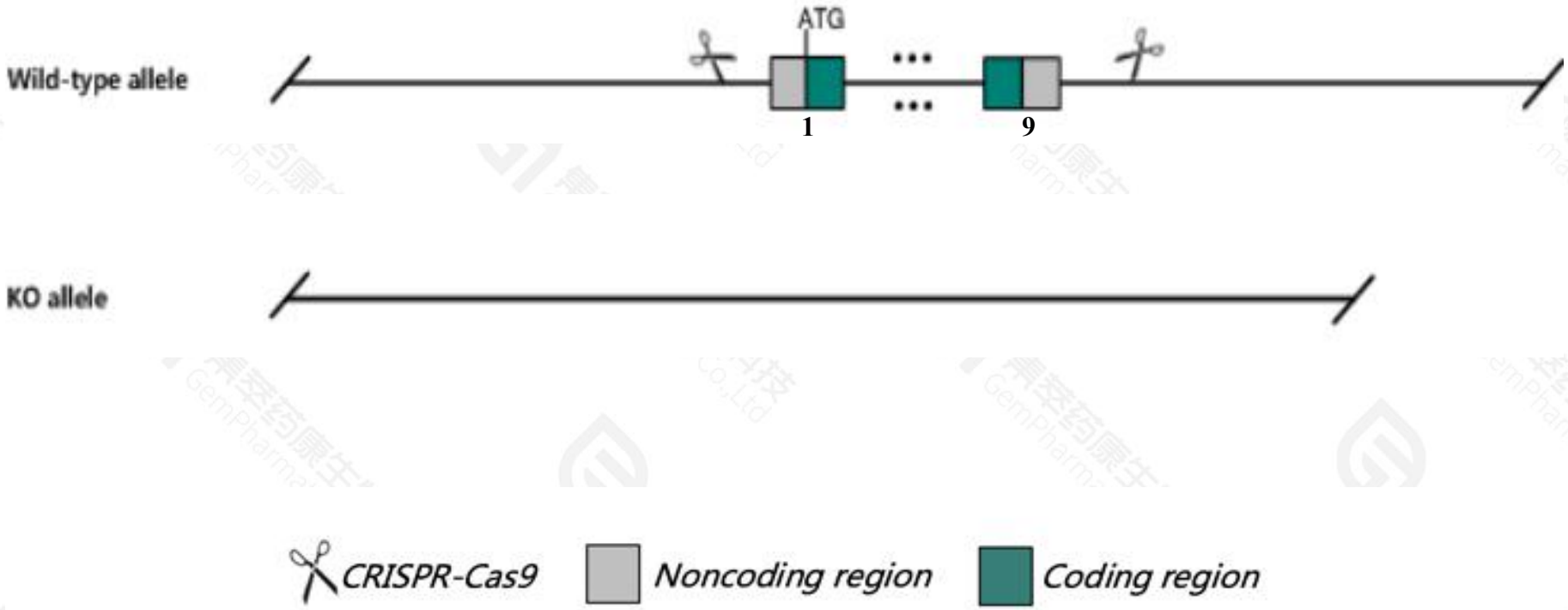
Design Date: 2022-8-5

Project Overview

Project Name	***Lamp2***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR-Cas9 technology to edit the *Lamp2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Lamp2* gene has 5 transcripts. According to the structure of *Lamp2* gene, exon1-exon9 of *Lamp2-203*(ENSMUST00000074913.12) transcript is recommended as the knockout region.The region contains all 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 *Lamp2* gene. The brief process is as follows: CRISPR-Cas9 system 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.

Notice

➢ According to the existing MGI data,the majority of hemizygous or homozygous mutant mice die prematurely displaying cardiomyopathy and accumulation of autophagic vacuoles in several tissues including liver, pancreas, spleen, kidney and skeletal and cardiac muscle.

➢ The *Lamp2* gene is located on the ChrX. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

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Lamp2 lysosomal-associated membrane protein 2 [Mus musculus (house mouse)]

Gene ID: 16784, updated on 12-Jul-2022

Summary

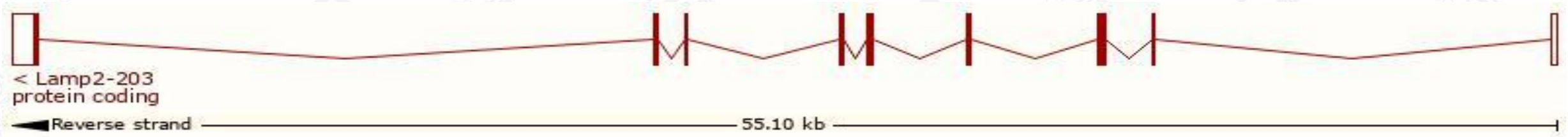
Official Symbol	Lamp2 provided by MGI
Official Full Name	lysosomal-associated membrane protein 2 provided byMGI
Primary source	MGI:MGI:96748
See related	Ensembl:ENSMUSG00000016534
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	CD107b, LGP-B, Lamp II, Lamp-2, Lamp-2a, Lamp-2b, Lamp-2c, Mac3
Expression	Ubiquitous expression in kidney adult (RPKM 53.2), placenta adult (RPKM 47.8) and 25 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

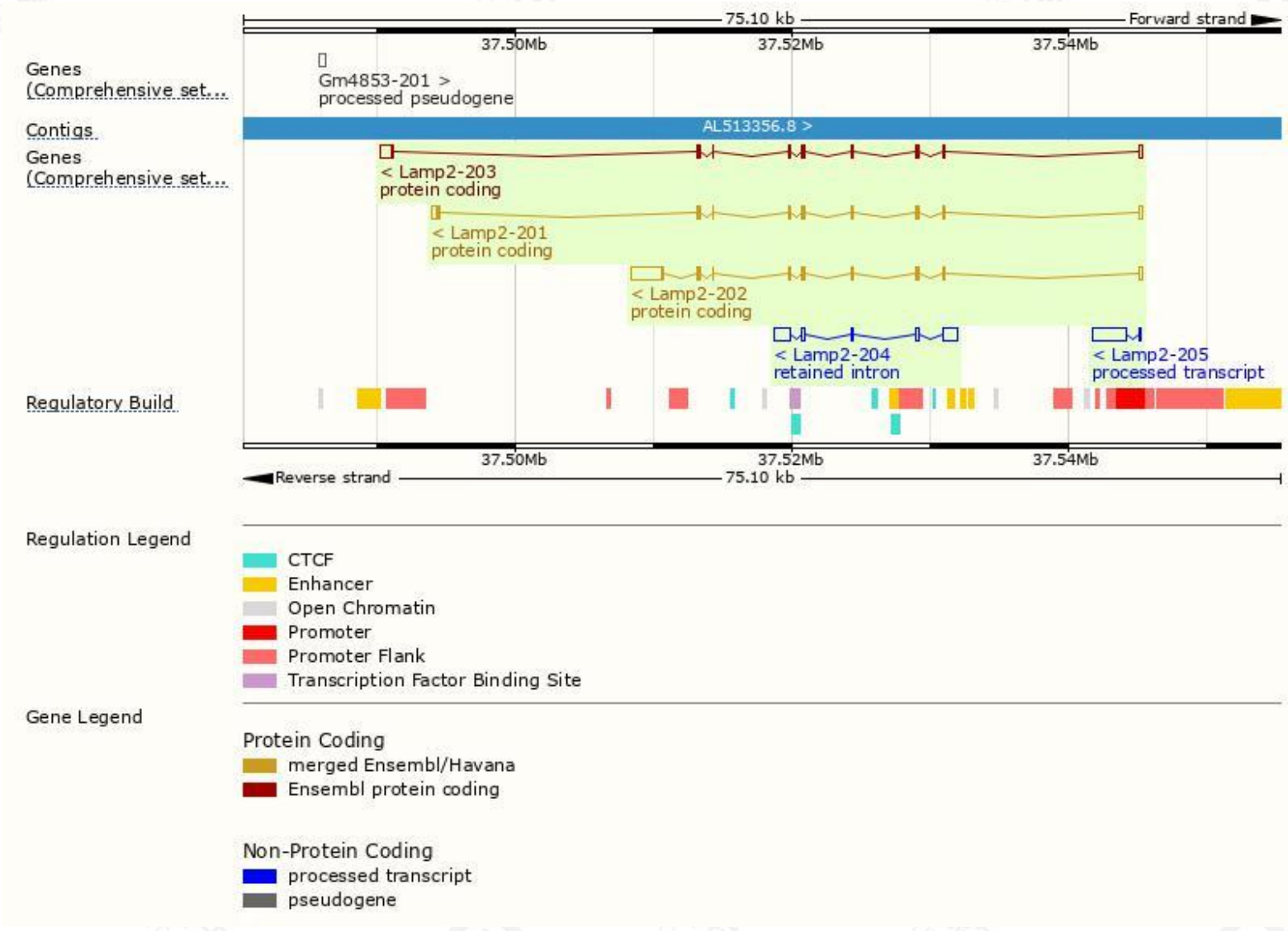
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lamp2-202	ENSMUST00000061755.9	3604	415aa	Protein coding	CCDS30093		TSL:1 , GENCODE basic , APPRIS ALT2 ,
Lamp2-203	ENSMUST00000074913.12	2184	416aa	Protein coding	CCDS72369		TSL:1 , GENCODE basic , APPRIS ALT2 ,
Lamp2-201	ENSMUST00000016678.14	1769	415aa	Protein coding	CCDS30092		TSL:1 , GENCODE basic , APPRIS P5 ,
Lamp2-205	ENSMUST00000144663.2	2633	No protein	Processed transcript	-		TSL:1 ,
Lamp2-204	ENSMUST00000136817.2	2870	No protein	Retained intron	-		TSL:2 ,

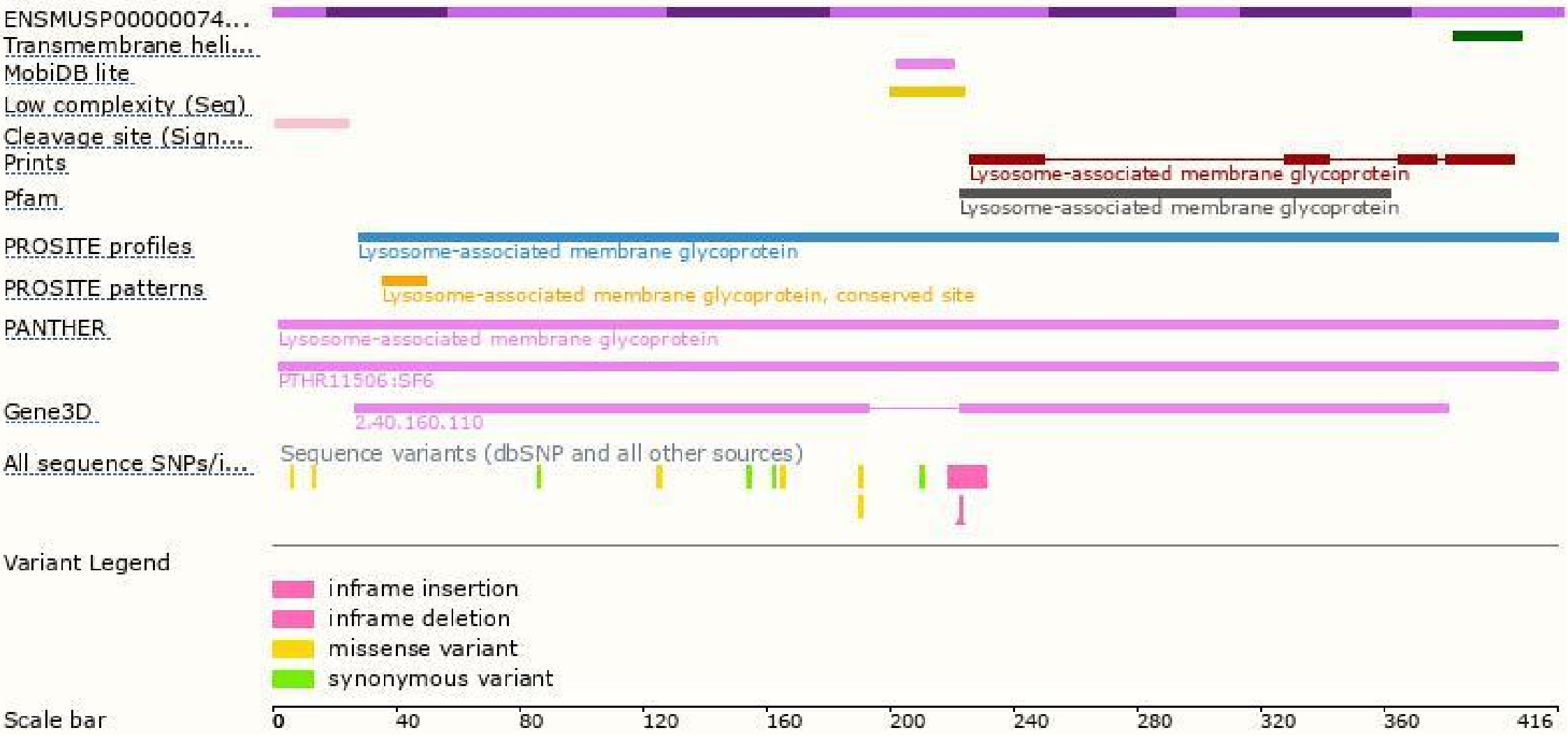
The strategy is based on the design of *Lamp2-203* transcript,the transcription is shown below:



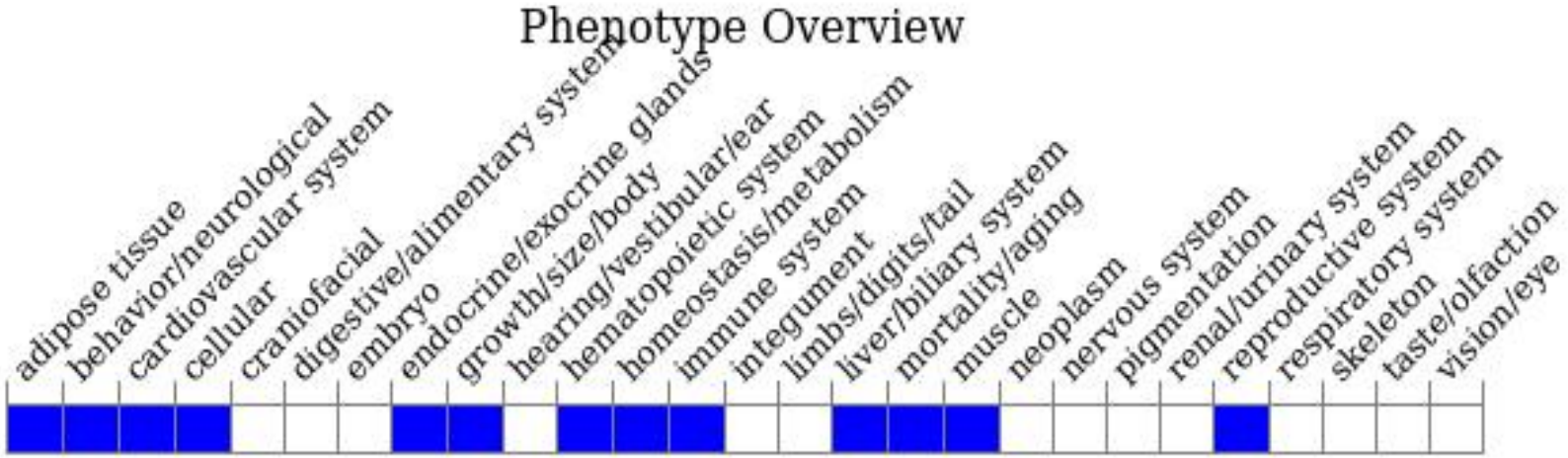
Genomic location distribution



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,the majority of hemizygous or homozygous mutant mice die prematurely displaying cardiomyopathy and accumulation of autophagic vacuoles in several tissues including liver, pancreas, spleen, kidney and skeletal and cardiac muscle.

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