Pfkm Cas9-CKO Strategy

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Project Overview

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

Conditional Knockout strategy

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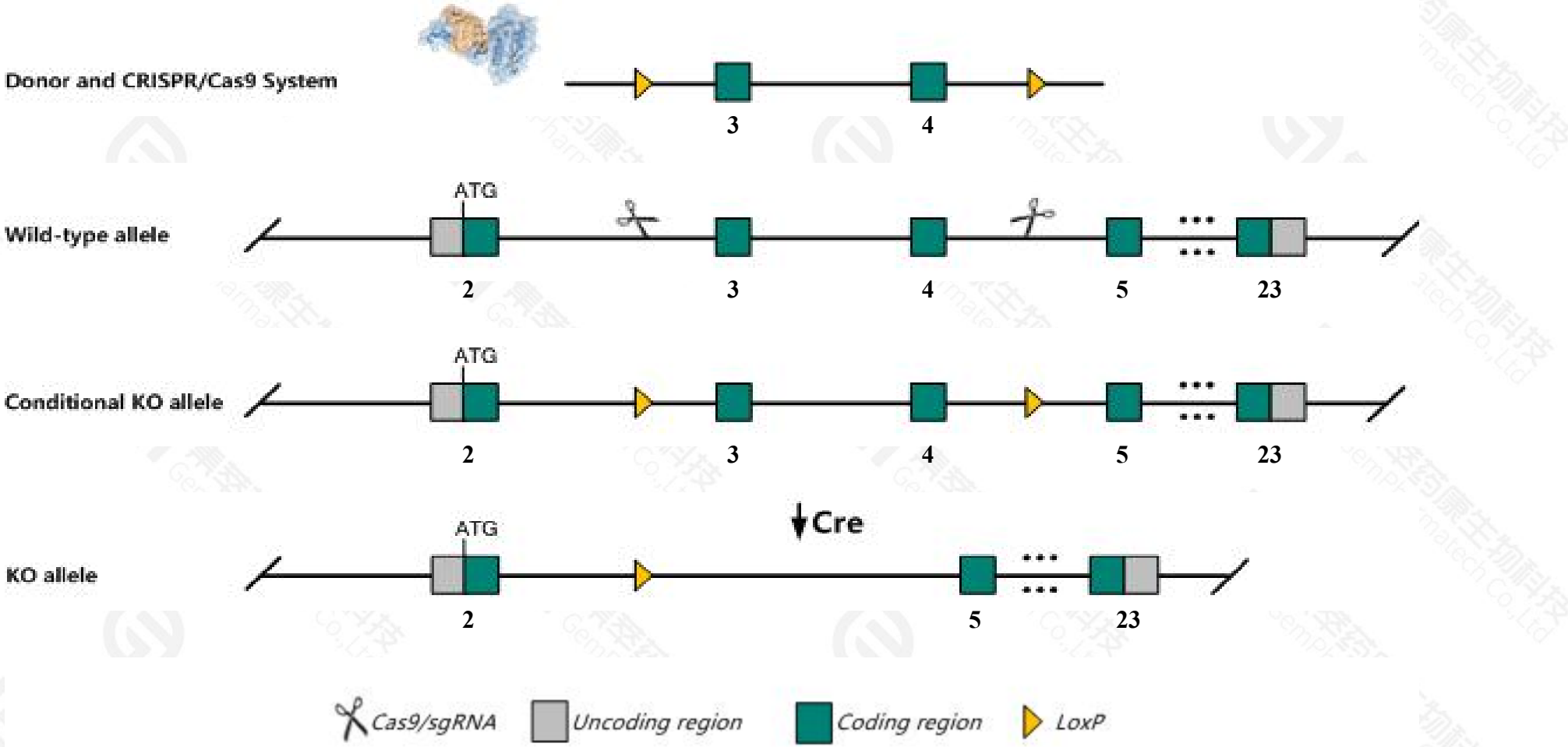
This model will use CRISPR/Cas9 technology to edit the *Pfkm* gene. The schematic diagram is as follows:



Technical routes

➢ The *Pfkm* gene has 5 transcripts. According to the structure of *Pfkm* gene, exon3-exon4 of *Pfkm-201*(ENSMUST00000051226.8) transcript is recommended as the knockout region.The region contains 152bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Pfkm* 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.

Notice

➢ According to the existing MGI data,mice homozygous for a gene trapped allele exhibit abnormal glucose homeostasis. Mice homozygous for a knock-out allele exhibit premature death, exercise intolerance, abnormal glucose homeostasis, cardiomegaly, splenomegaly, and abnormal muscle morphology and physiology.

➢ The *Pfkm* gene is located on the Chr15. 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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Pfkm phosphofructokinase, muscle [Mus musculus (house mouse)]

Gene ID: 18642, updated on 10-Jan-2021

Summary

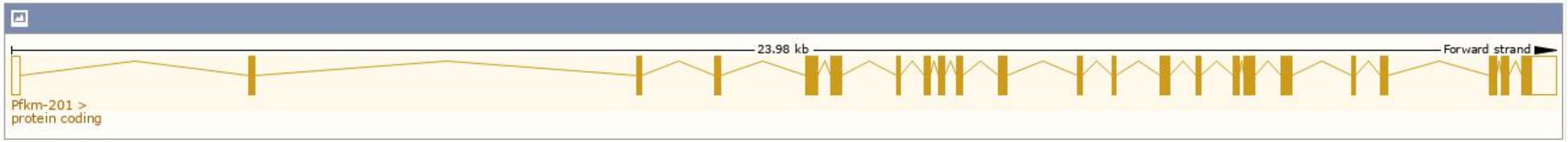
Official Symbol	Pfkm provided by MGI
Official Full Name	phosphofructokinase, muscle provided byMGI
Primary source	MGI:MGI:97548
See related	Ensembl:ENSMUSG00000033065
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI131669, ATP-PFK, PFK-A, PFK-M, Pfk, Pfk-, Pfk-4, Pfk4, Pfka, Pfkx
Expression	Broad expression in heart adult (RPKM 178.4), cerebellum adult (RPKM 100.9) and 18 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

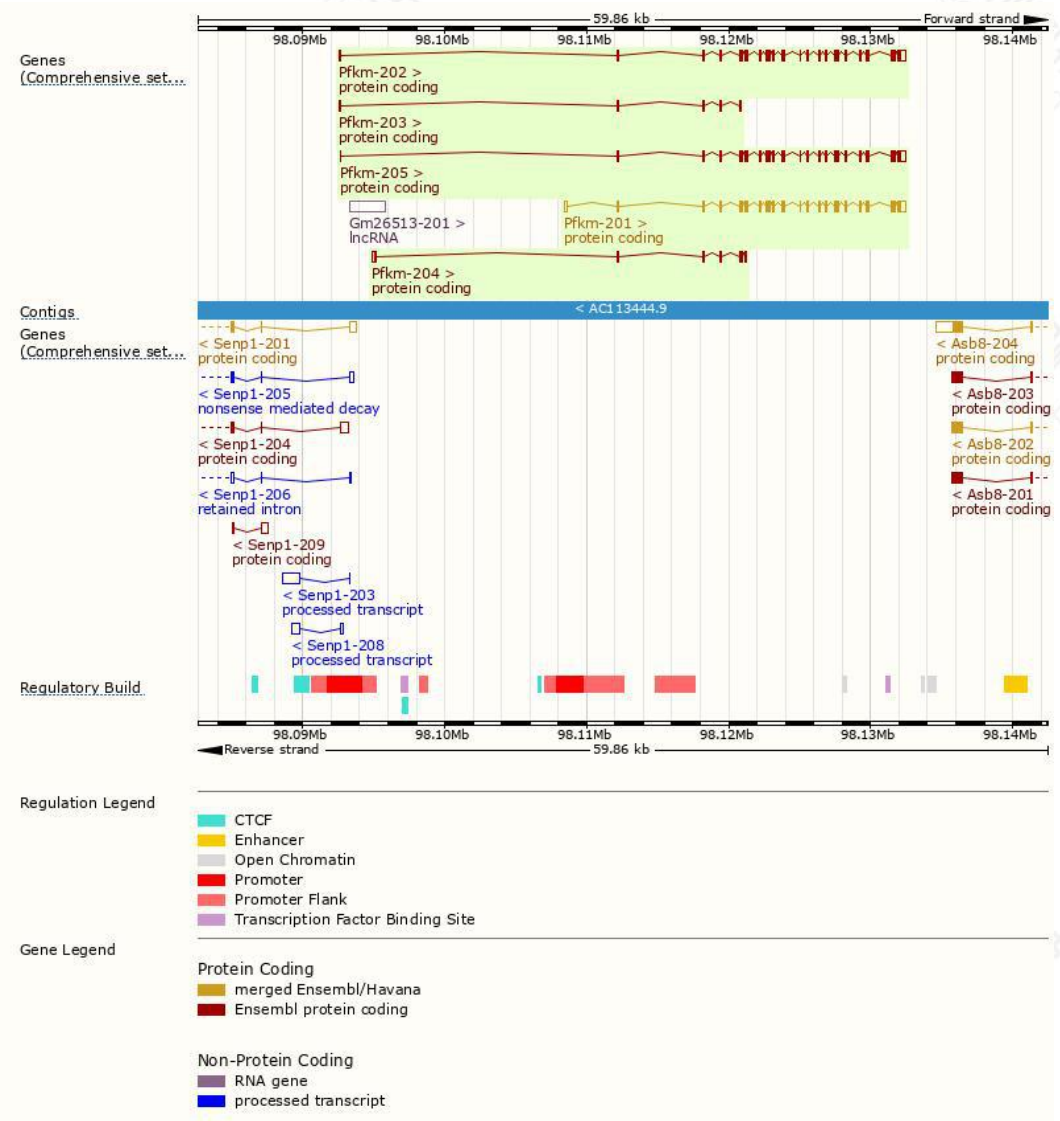
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pfkm-201	ENSMUST00000051226.8	2879	780aa	Protein coding	CCDS27786		TSL:1 , GENCODE basic , APPRIS P1 ,
Pfkm-202	ENSMUST00000163507.8	2816	780aa	Protein coding	CCDS27786		TSL:5 , GENCODE basic , APPRIS P1 ,
Pfkm-205	ENSMUST00000230445.2	2798	780aa	Protein coding	CCDS27786		GENCODE basic , APPRIS P1 ,
Pfkm-204	ENSMUST00000229344.2	785	197aa	Protein coding	-		CDS 3' incomplete ,
Pfkm-203	ENSMUST00000229280.2	438	117aa	Protein coding	-		CDS 3' incomplete ,

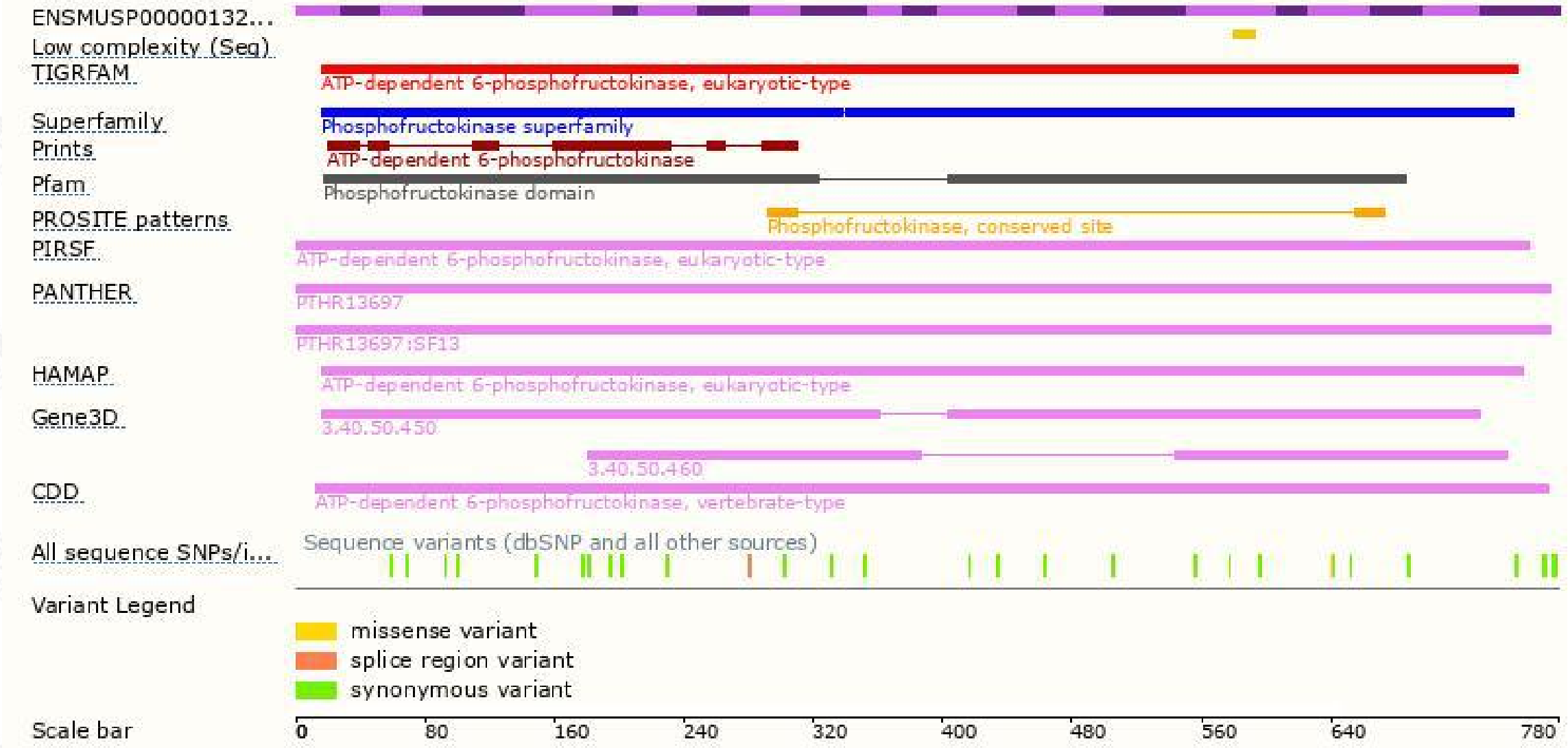
The strategy is based on the design of *Pfkm-201* transcript,the transcription is shown below:



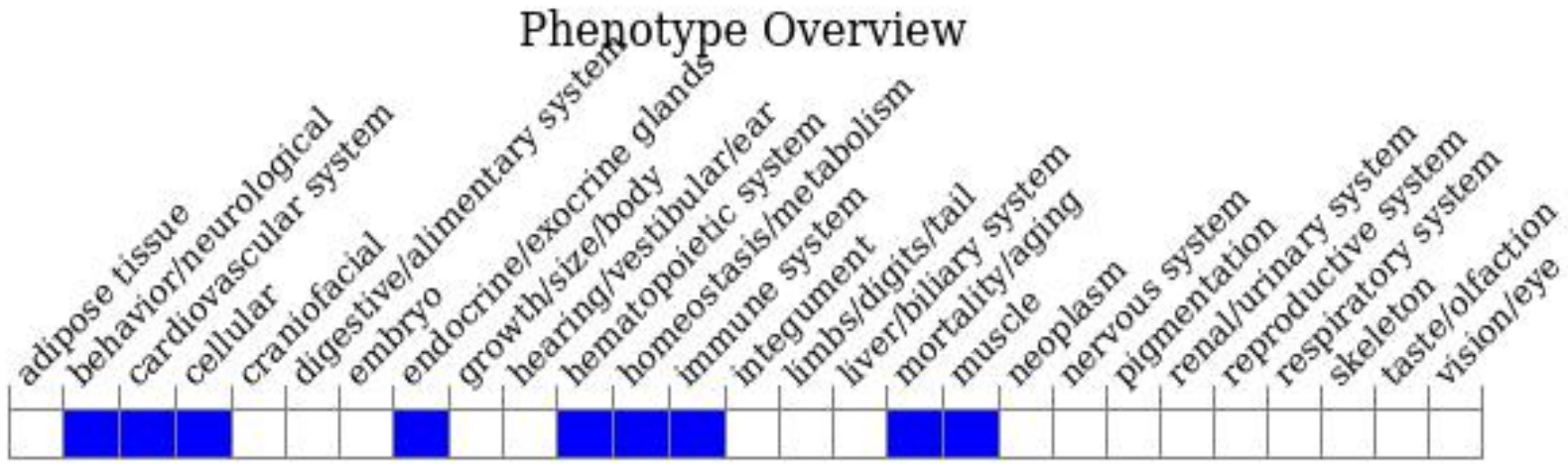
Genomic location distribution



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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