Acadvl Cas9-CKO Strategy

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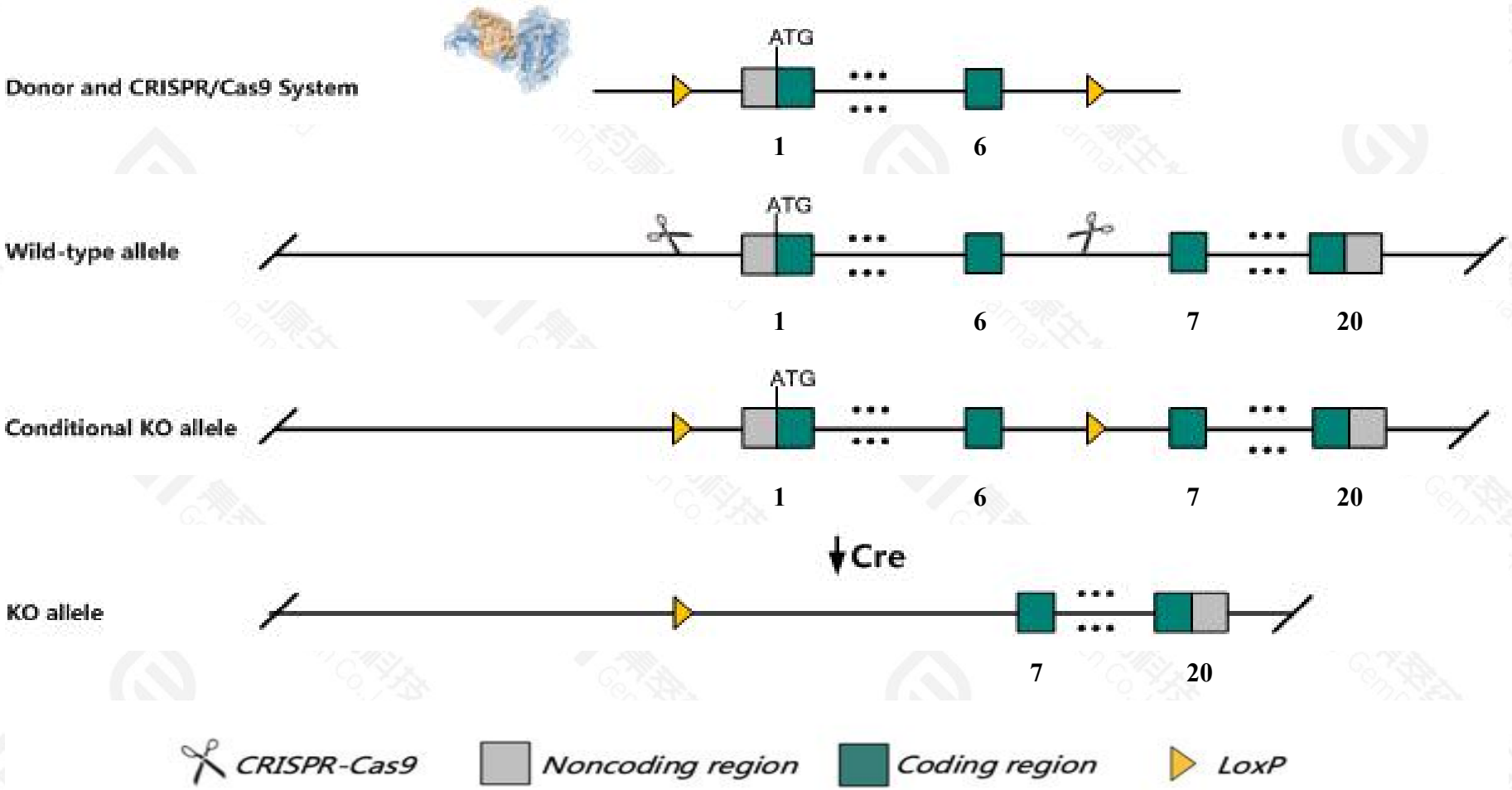
Project Overview

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

Conditional Knockout strategy

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



Technical routes

➢ The *Acadvl* gene has 7 transcripts. According to the structure of *Acadvl* gene, exon1-exon6 of *Acadvl-202*(ENSMUST00000102574.10) transcript is recommended as the knockout region.The region contains start codon ATG.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR-Cas9 technology to modify *Acadvl* 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,homozygous mutant animals exhibit mild steatosis, lipid accumulation in myocytes, increased fatigue, impaired temperature regulation, increased susceptibility to arrhythmia, accumulation of long-chain acylcarnitines, and lower free carnitine levels.

➢ The insertion of loxp may affect the 5' regulation function of *Dlg4*.

➢ The knockout region is about 1kb away from the 5- terminal of *Dlg4*, which may affect its 5-terminal regulation function.

➢ The *Acadvl* 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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Acadvl acyl-Coenzyme A dehydrogenase, very long chain [Mus musculus (house mouse)]

Gene ID: 11370, updated on 13-Mar-2020

Summary

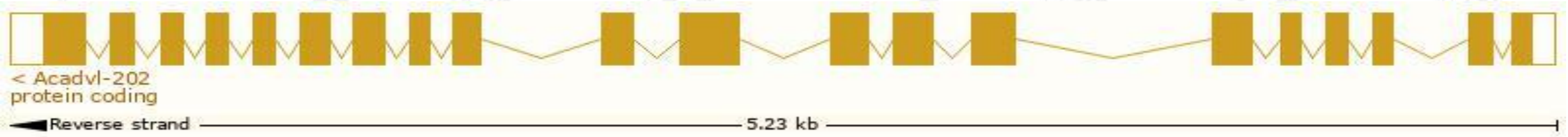
Official Symbol	Acadvl provided by MGI
Official Full Name	acyl-Coenzyme A dehydrogenase, very long chain provided byMGI
Primary source	MGI:MGI:895149
See related	Ensembl:ENSMUSG00000018574
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	vlcad
Summary	This gene encodes a homodimeric mitochondrial flavoprotein and is a member of the acyl-CoA dehydrogenase family. Members of this family catalyze the first step of fatty acid beta-oxidation, forming a C2-C3 trans-double bond in a FAD-dependent reaction. As beta-oxidation cycles through its four steps, each member of the acyl-CoA dehydrogenase family works at an optimum fatty acid chain-length. This enzyme has its optimum length between C16- and C20-acylCoA and localizes to the inner mitochondrial membrane (unlike related acyl-CoA dehydrogenases). In mice, deficiency of this gene can cause ventricular arrhythmias as well as fasting and cold intolerance. [provided by RefSeq, Nov 2012]
Expression	Broad expression in heart adult (RPKM 185.6), liver E18 (RPKM 80.6) and 25 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

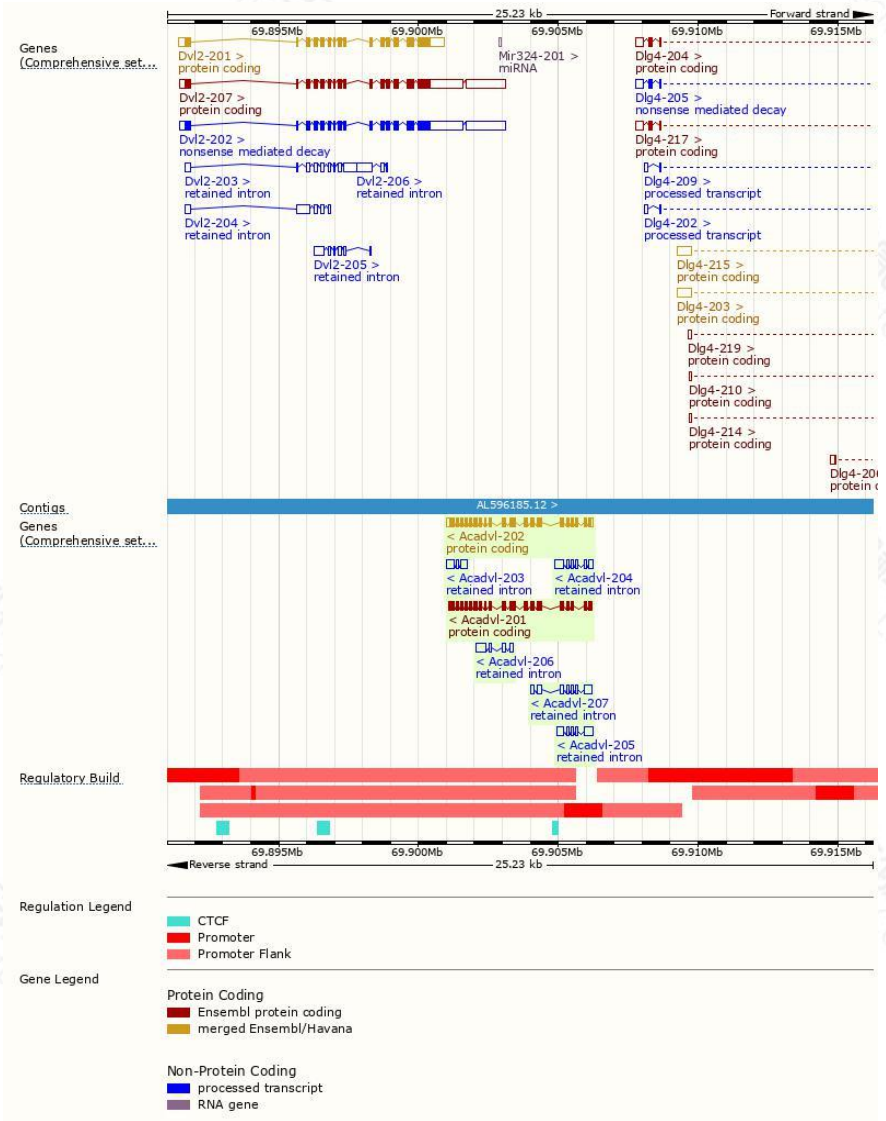
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Acadvl-202	ENSMUST00000102574.9	2168	656aa	Protein coding	CCDS24931	P50544	TSL:1 GENCODE basic APPRIS P2
Acadvl-201	ENSMUST00000018718.7	2020	634aa	Protein coding	-	B1AR28	TSL:5 GENCODE basic APPRIS ALT2
Acadvl-207	ENSMUST00000156733.7	858	No protein	Retained intron	-	-	TSL:5
Acadvl-204	ENSMUST00000137187.7	769	No protein	Retained intron	-	-	TSL:2
Acadvl-205	ENSMUST00000145478.1	749	No protein	Retained intron	-	-	TSL:3
Acadvl-206	ENSMUST00000146129.1	696	No protein	Retained intron	-	-	TSL:2
Acadvl-203	ENSMUST00000134516.1	538	No protein	Retained intron	-	-	TSL:2

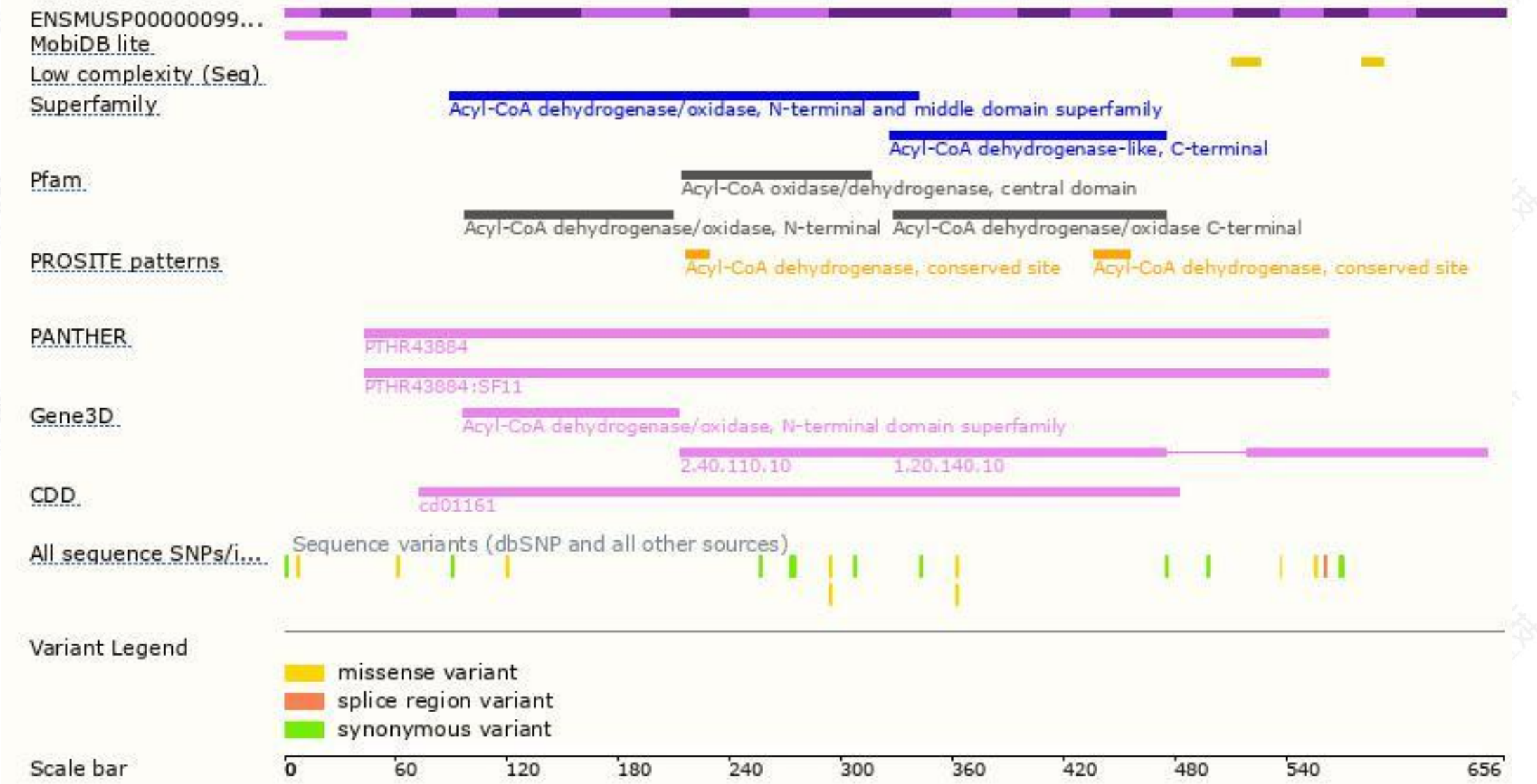
The strategy is based on the design of *Acadvl-202* transcript,the transcription is shown below:



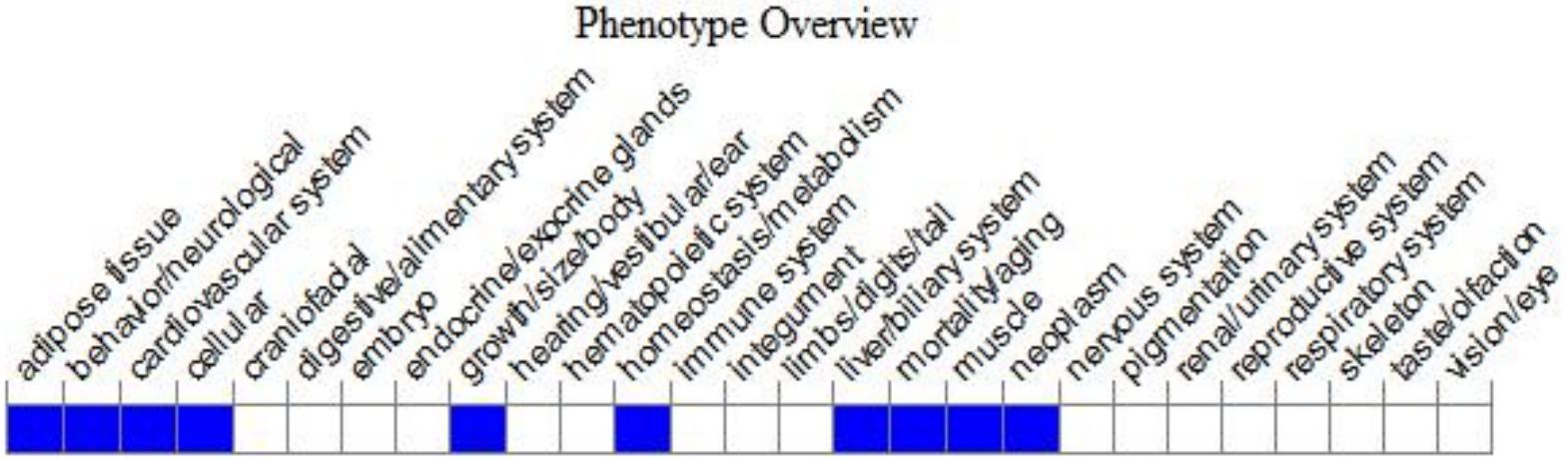
Genomic location distribution



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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