Dlat Cas9-CKO Strategy

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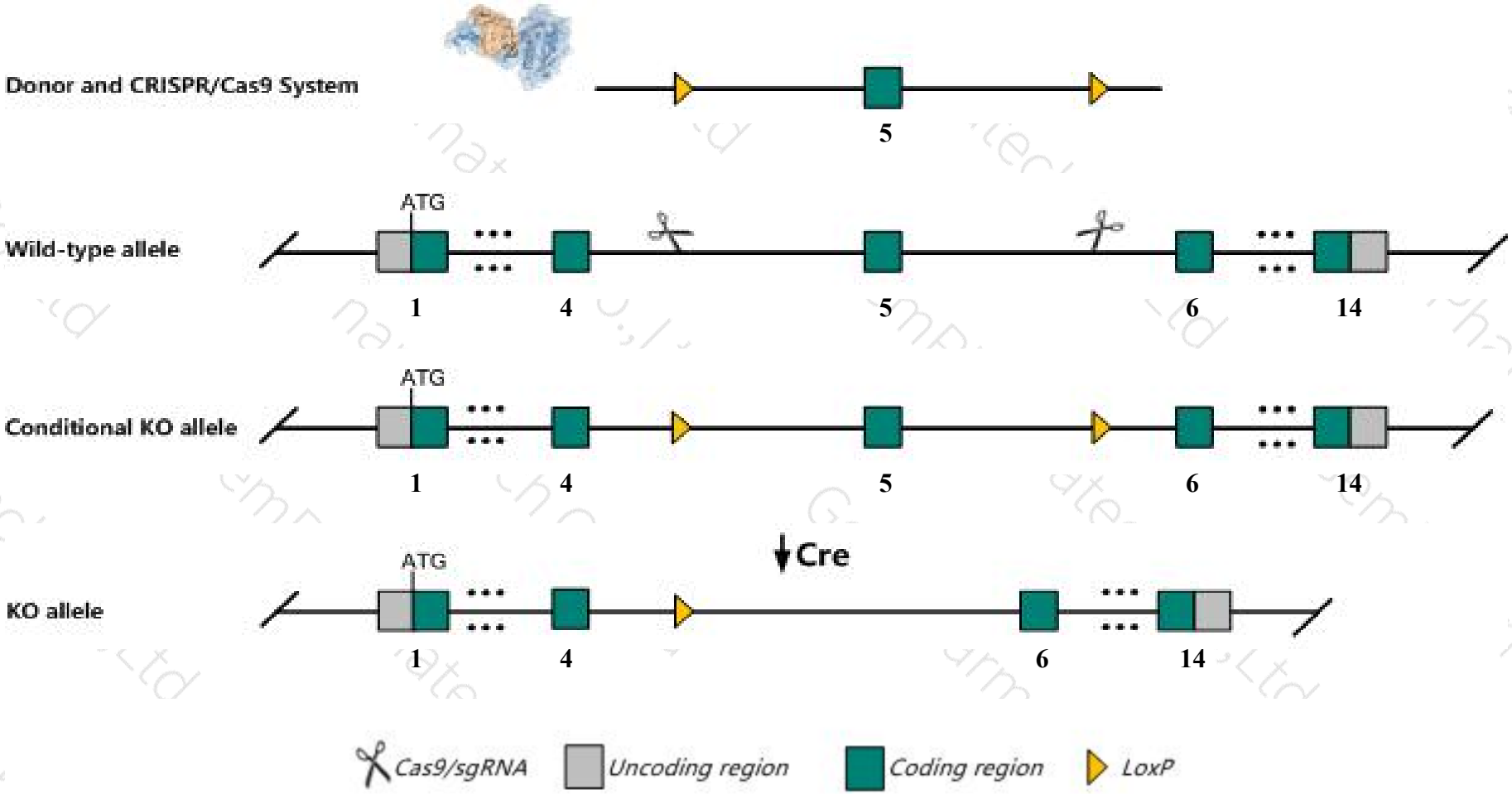
Project Overview

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

Conditional Knockout strategy

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



Technical routes

➢ The *Dlat* gene has 6 transcripts. According to the structure of *Dlat* gene, exon5 of *Dlat-201*(ENSMUST00000034567.3) transcript is recommended as the knockout region.The region contains 127bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Dlat* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector 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

➢ The *Dlat* gene is located on the Chr9. 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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Dlat dihydrolipoamide S-acetyltransferase (E2 component of pyruvate dehydrogenase complex) [Mus musculus (house mouse)]

Gene ID: 235339, updated on 13-Mar-2020

Summary

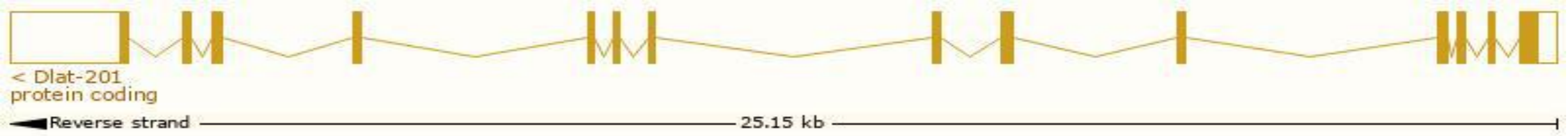
Official Symbol	Dlat provided by MGI
Official Full Name	dihydrolipoamide S-acetyltransferase (E2 component of pyruvate dehydrogenase complex) provided by MGI
Primary source	MGI:MGI:2385311
See related	Ensembl:ENSMUSG00000000168
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	6332404G05Rik, DLTA, PDC-E2
Expression	Ubiquitous expression in heart adult (RPKM 33.1), mammary gland adult (RPKM 17.1) and 27 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

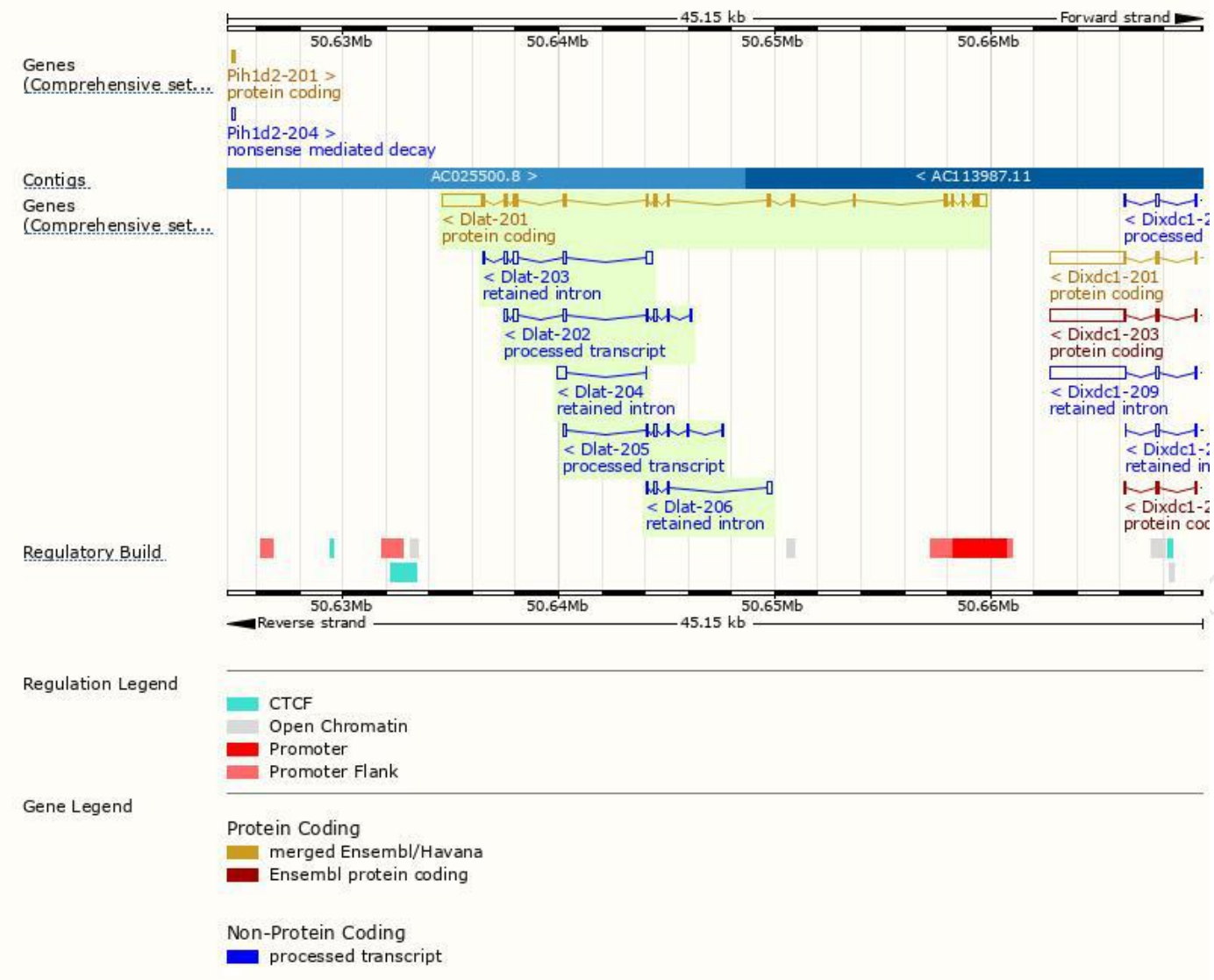
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dlat-201	ENSMUST00000034567.3	4035	642aa	Protein coding	CCDS23168	Q8BMF4	TSL:1 GENCODE basic APPRIS P1
Dlat-202	ENSMUST00000125919.7	770	No protein	Processed transcript	-	-	TSL:5
Dlat-205	ENSMUST00000142275.7	484	No protein	Processed transcript	-	-	TSL:3
Dlat-203	ENSMUST00000126933.7	779	No protein	Retained intron	-	-	TSL:2
Dlat-206	ENSMUST00000155417.1	526	No protein	Retained intron	-	-	TSL:2
Dlat-204	ENSMUST00000132455.1	459	No protein	Retained intron	-	-	TSL:2

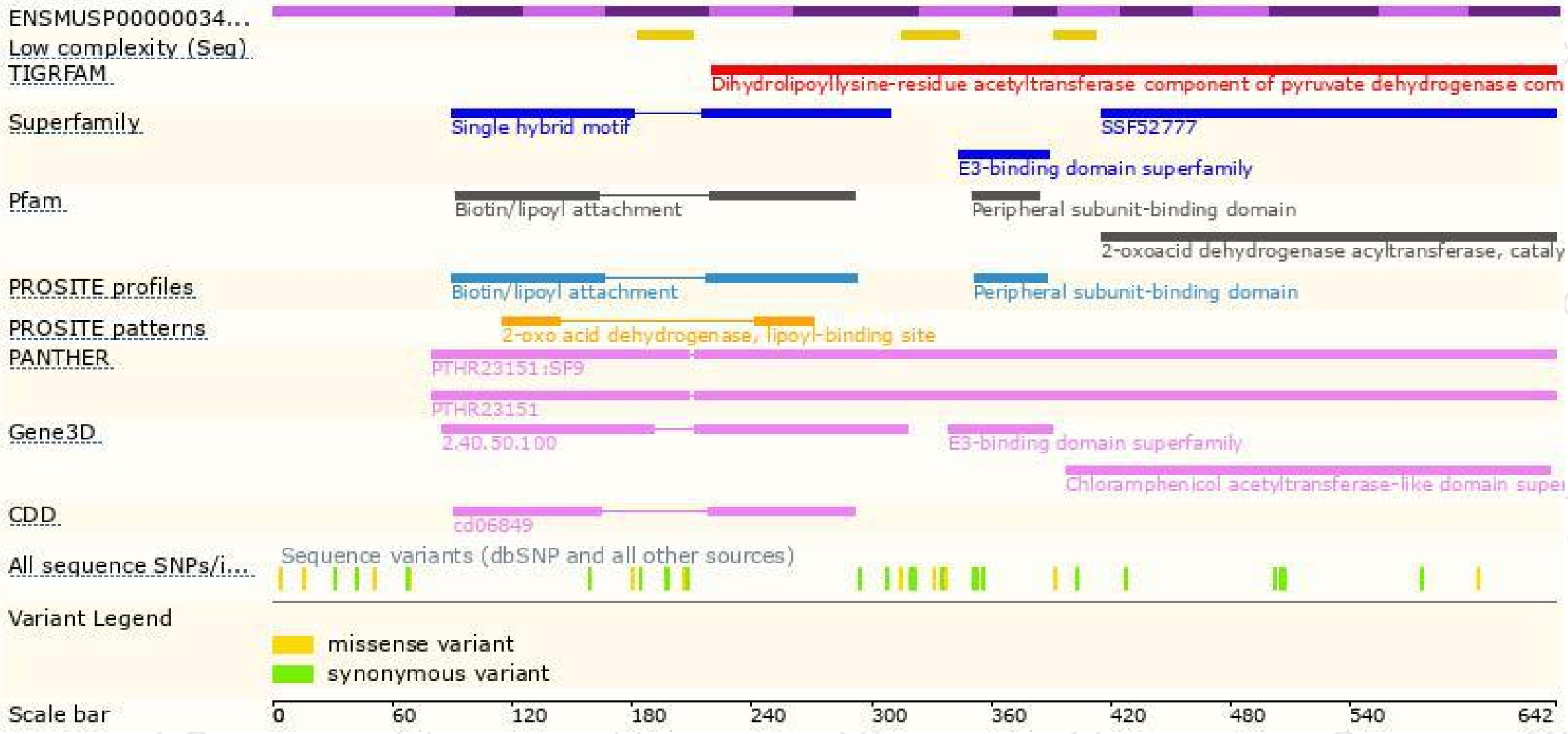
The strategy is based on the design of *Dlat-201* transcript,the transcription is shown below:



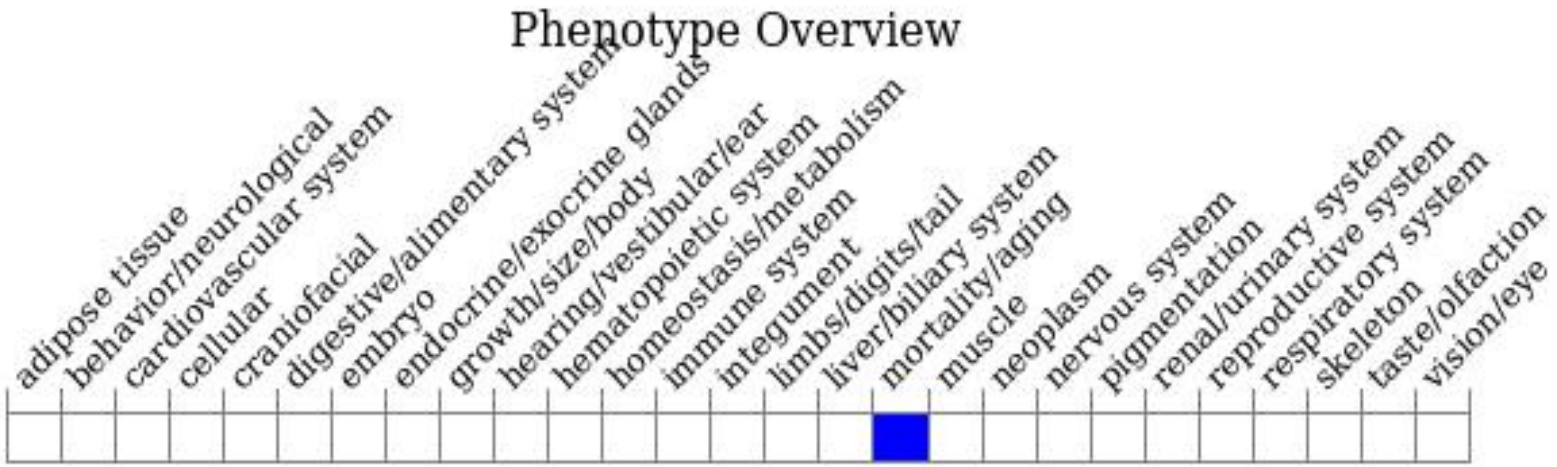
Genomic location distribution



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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