Cpt1a Cas9-CKO Strategy

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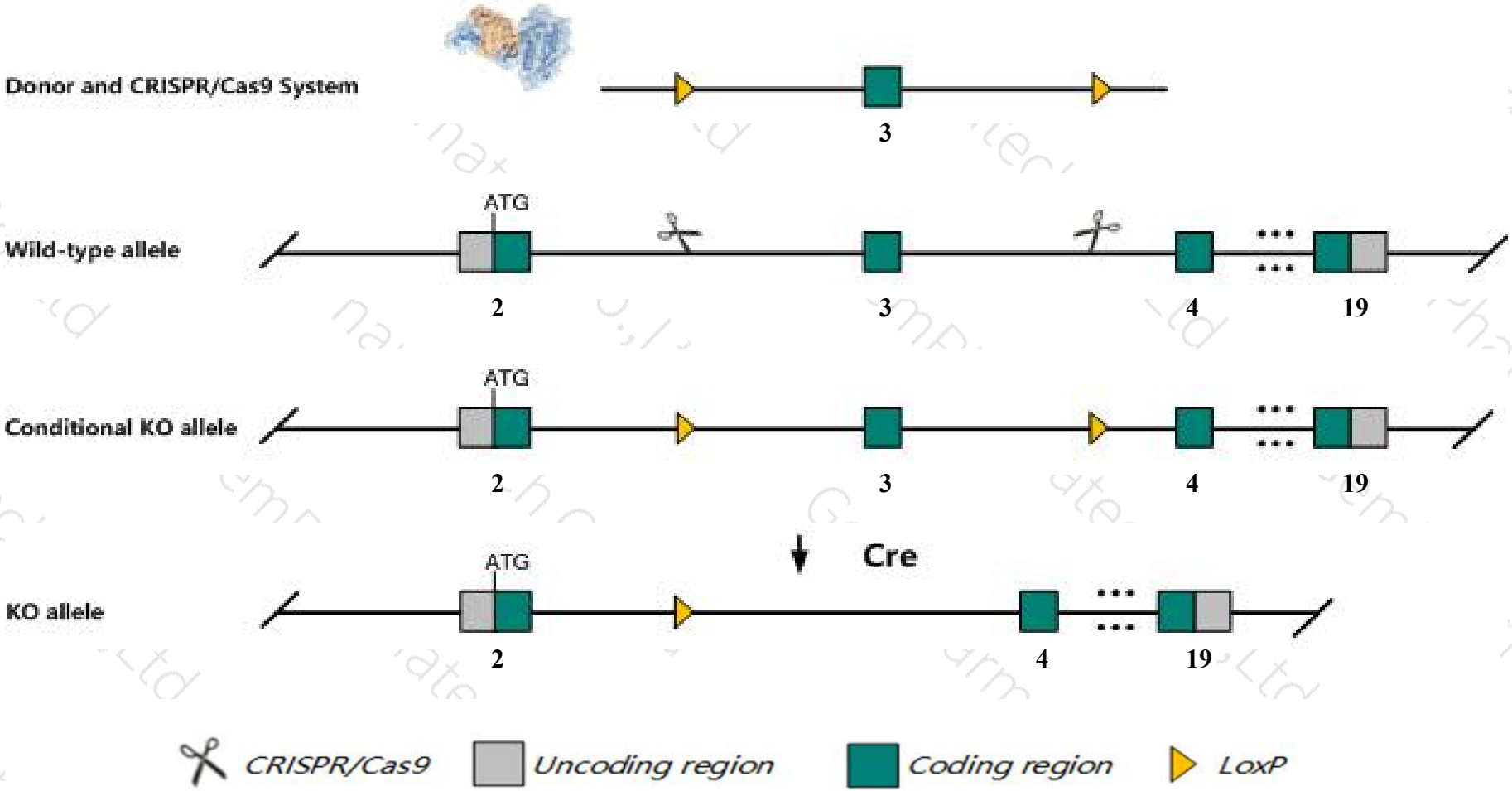
Project Overview

Project Name	***Cpt1a***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cpt1a* gene. The schematic diagram is as follows:



Technical routes

➢ The *Cpt1a* gene has 8 transcripts. According to the structure of *Cpt1a* gene, exon3 of *Cpt1a-201*(ENSMUST00000025835.5) transcript is recommended as the knockout region.The region contains 140bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Cpt1a* 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 null mice display embryonic lethality. Heterozygous null mice display decreased serum glucose and increased serum free fatty acid levels after fasting.

➢ The *Cpt1a* gene is located on the Chr19. 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）

集萃药康 GemPharmatech

Cpt1a carnitine palmitoyltransferase 1a, liver [Mus musculus (house mouse)]

Gene ID: 12894, updated on 13-Mar-2020

Summary

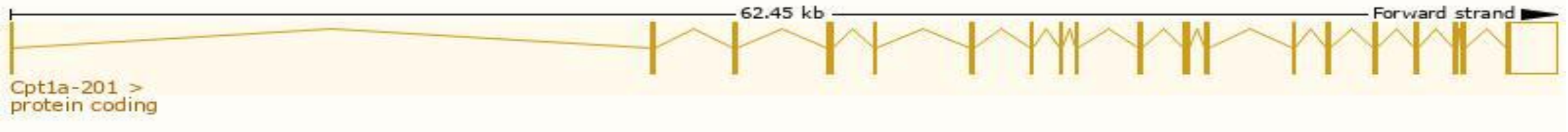
Official Symbol	Cpt1a provided by MGI
Official Full Name	carnitine palmitoyltransferase 1a, liver provided byMGI
Primary source	MGI:MGI:1098296
See related	Ensembl:ENSMUSG00000024900
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	C730027G07, CPTI, Cpt1
Expression	Broad expression in kidney adult (RPKM 56.8), adrenal adult (RPKM 53.2) and 26 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

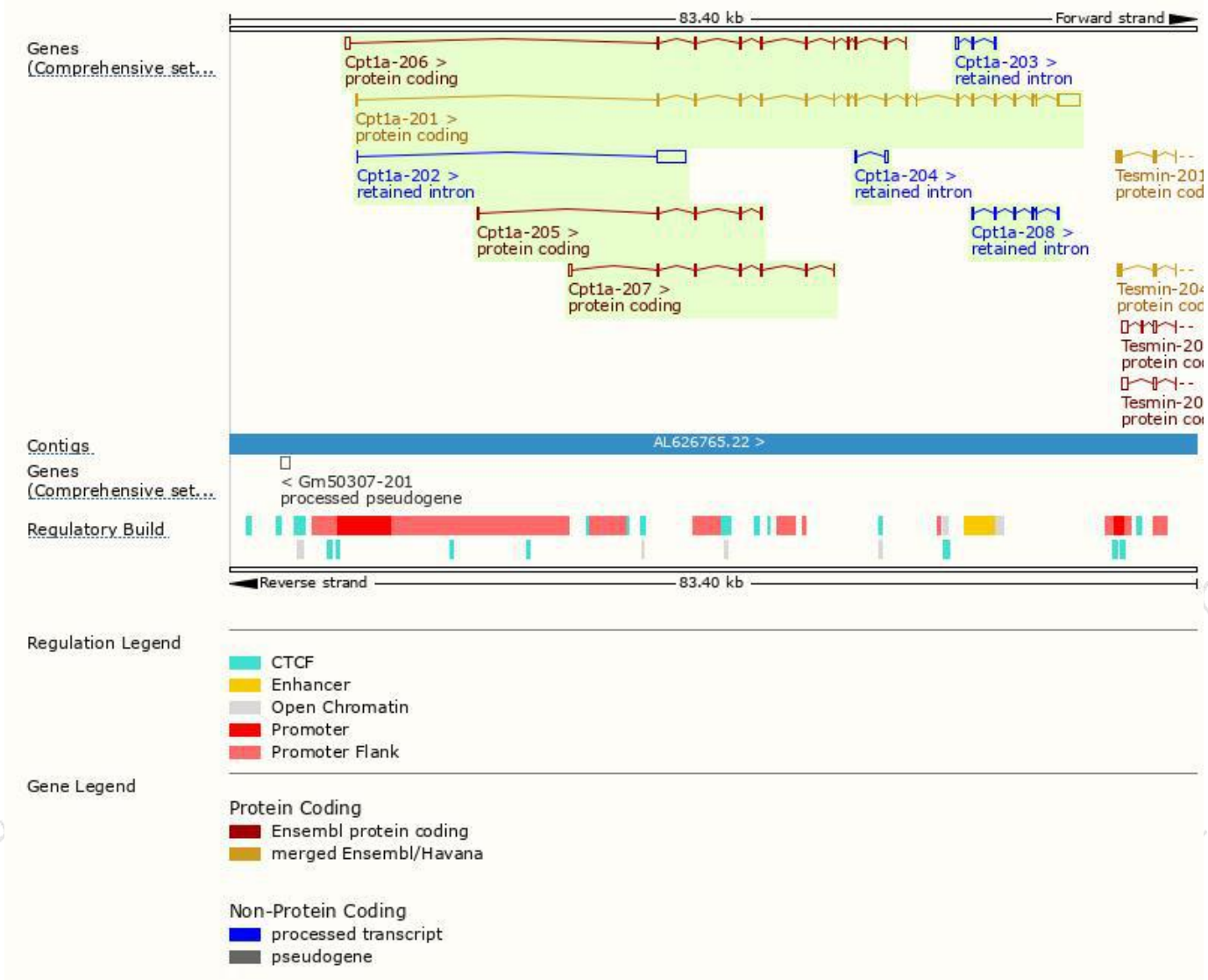
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cpt1a-201	ENSMUST00000025835.5	4317	773aa	Protein coding	CCDS29395	P97742 Q7TQD5	TSL:1 GENCODE basic APPRIS P1
Cpt1a-206	ENSMUST00000237938.1	1583	401aa	Protein coding	-	A0A494BAD3	CDS 3' incomplete
Cpt1a-207	ENSMUST00000238015.1	1012	251aa	Protein coding	-	A0A494BAC7	CDS 3' incomplete
Cpt1a-205	ENSMUST00000237562.1	659	185aa	Protein coding	-	A0A494BBF2	CDS 3' incomplete
Cpt1a-202	ENSMUST00000235968.1	2513	No protein	Retained intron	-	-	
Cpt1a-208	ENSMUST00000238027.1	839	No protein	Retained intron	-	-	
Cpt1a-203	ENSMUST00000237444.1	466	No protein	Retained intron	-	-	
Cpt1a-204	ENSMUST00000237457.1	451	No protein	Retained intron	-	-	

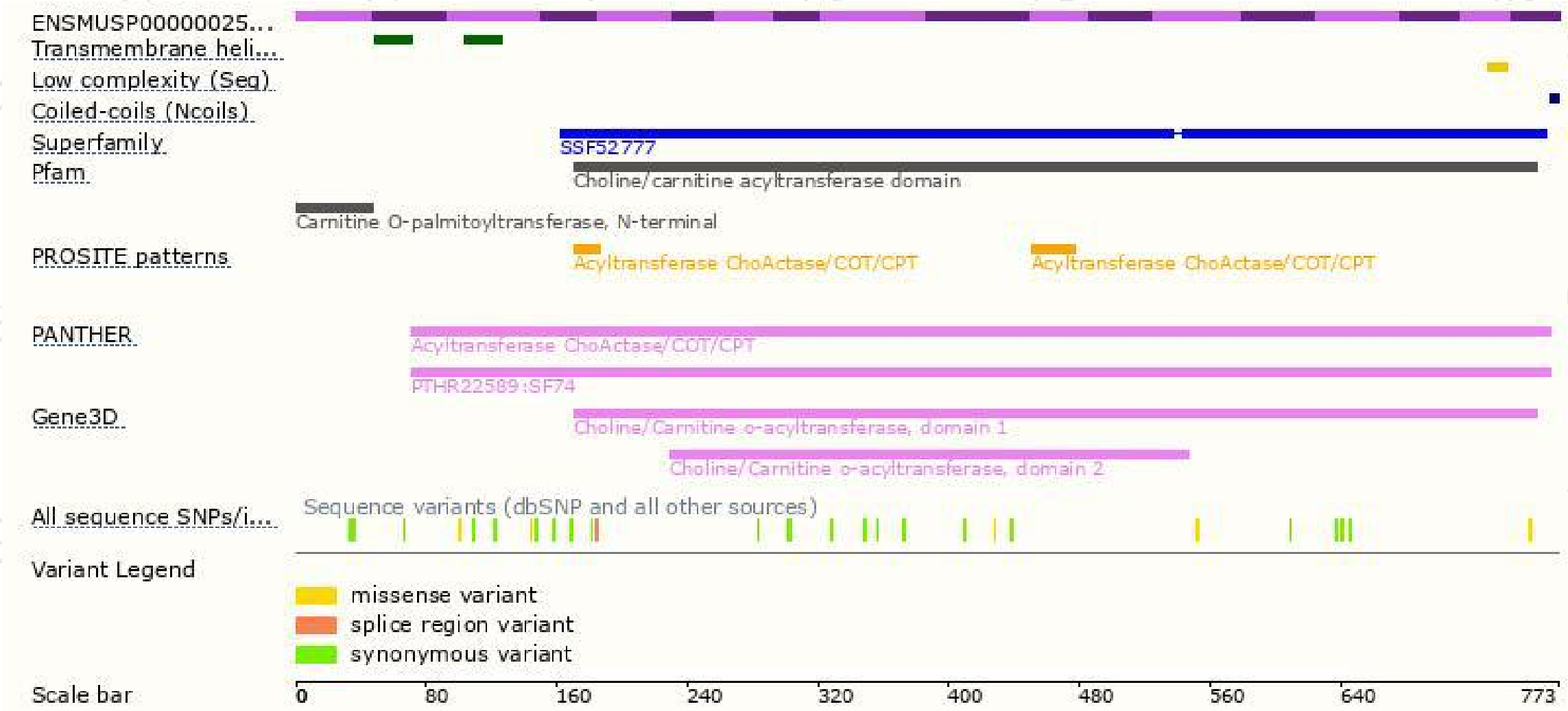
The strategy is based on the design of *Cpt1a-201* transcript,the transcription is shown below:



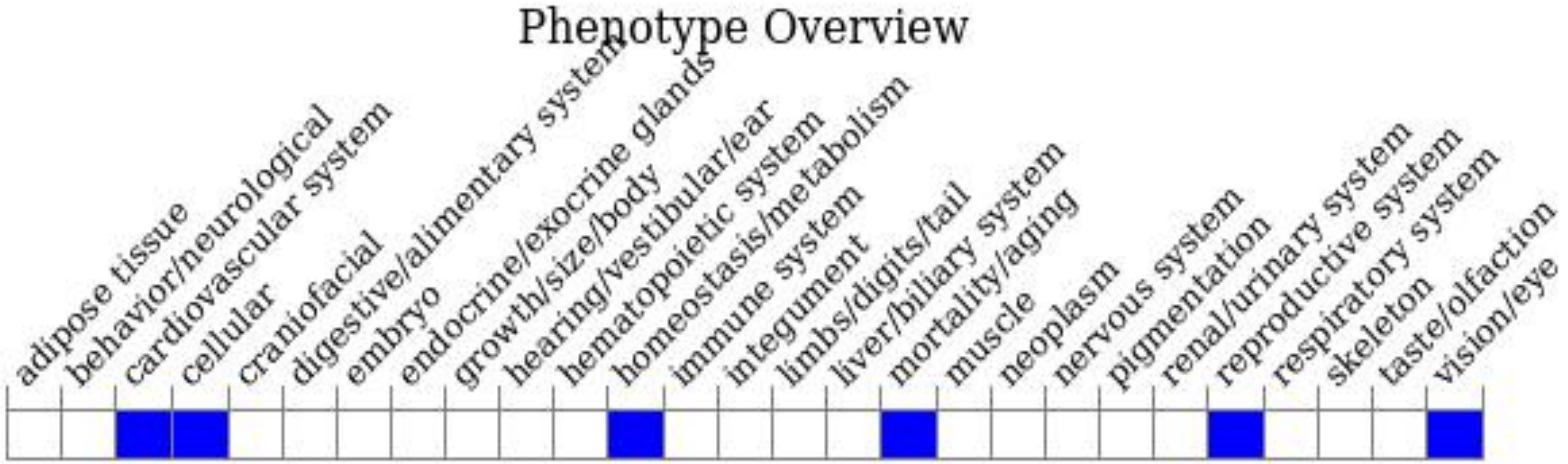
Genomic location distribution



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/).

According to the existing MGI data,homozygous null mice display embryonic lethality. Heterozygous null mice display decreased serum glucose and increased serum free fatty acid levels after fasting.

If you have any questions, you are welcome to inquire.

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