Mlst8 Cas9-CKO Strategy

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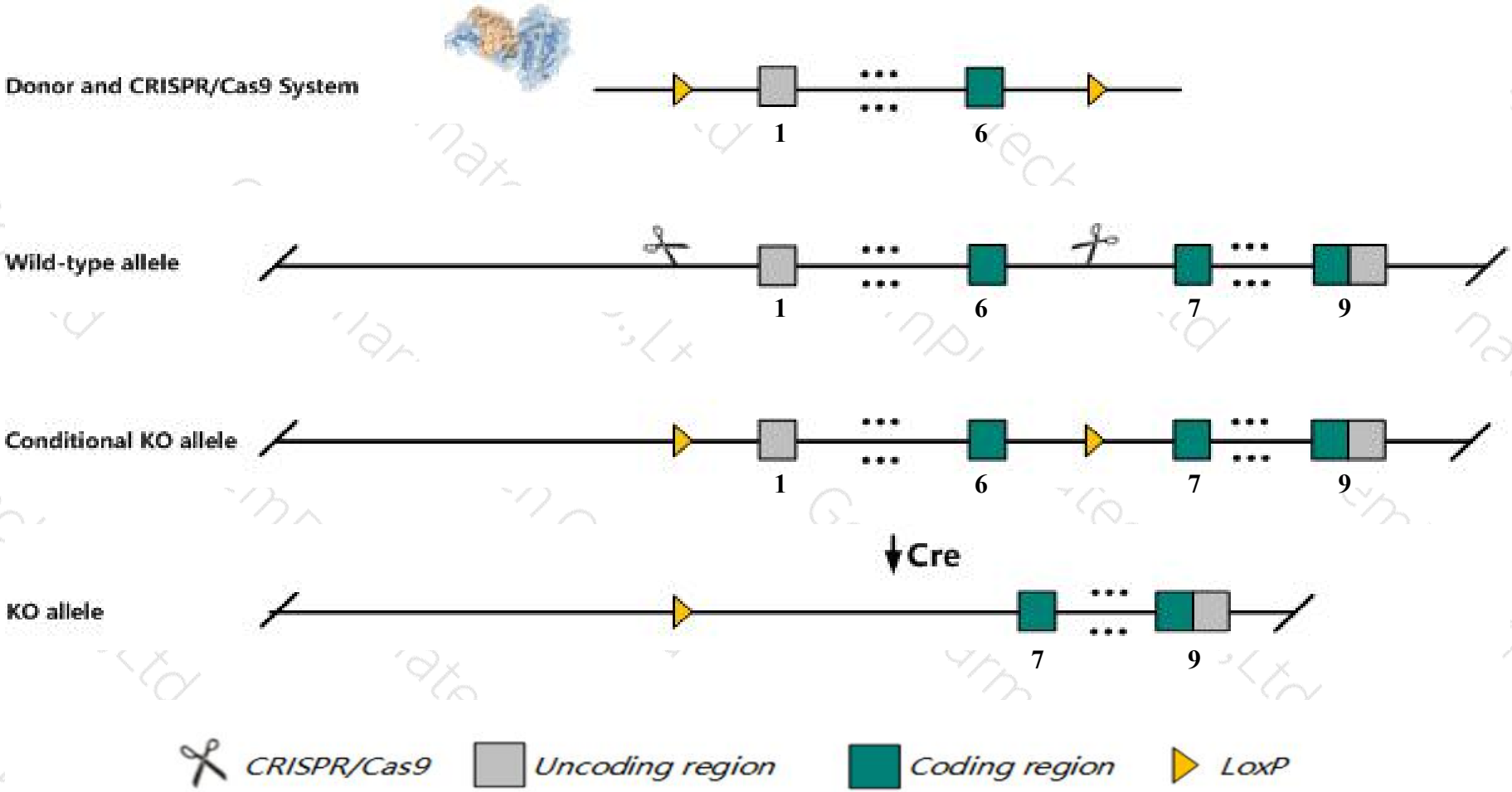
Project Overview

Project Name	***Mlst8***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mlst8* gene. The schematic diagram is as follows:



Technical routes

- The *Mlst8* gene has 10 transcripts. According to the structure of *Mlst8* gene, exon1-exon6 of *Mlst8-201* (ENSMUST00000070888.13) 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 *Mlst8* 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,Mice homozygous for a null mutation exhibit lethality around E10.5 and abnormal yolk sac vasculature, brain development and heart development.

➢This strategy may affect the 5-terminal regulation of the gene of interest and *Gm50062* .

➢ The *Mlst8* gene is located on the Chr17. 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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Mlst8 MTOR associated protein, LST8 homolog (S. cerevisiae) [Mus musculus (house mouse)]

Gene ID: 56716, updated on 19-Feb-2019

Summary

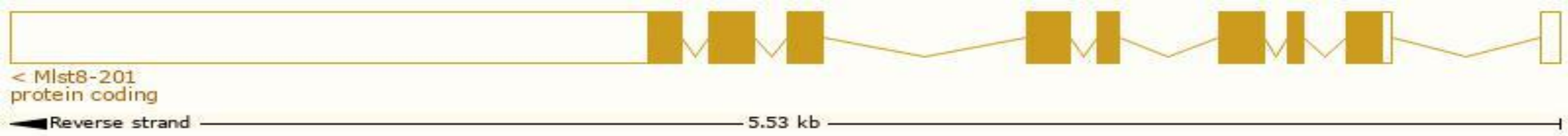
Official Symbol	Mlst8 provided by MGI
Official Full Name	MTOR associated protein, LST8 homolog (S. cerevisiae) provided by MGI
Primary source	MGI:MGI:1929514
See related	Ensembl:ENSMUSG00000024142
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	0610033N12Rik, AA409454, AI505104, AI851821, Gbl
Expression	Ubiquitous expression in thymus adult (RPKM 14.9), large intestine adult (RPKM 14.2) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

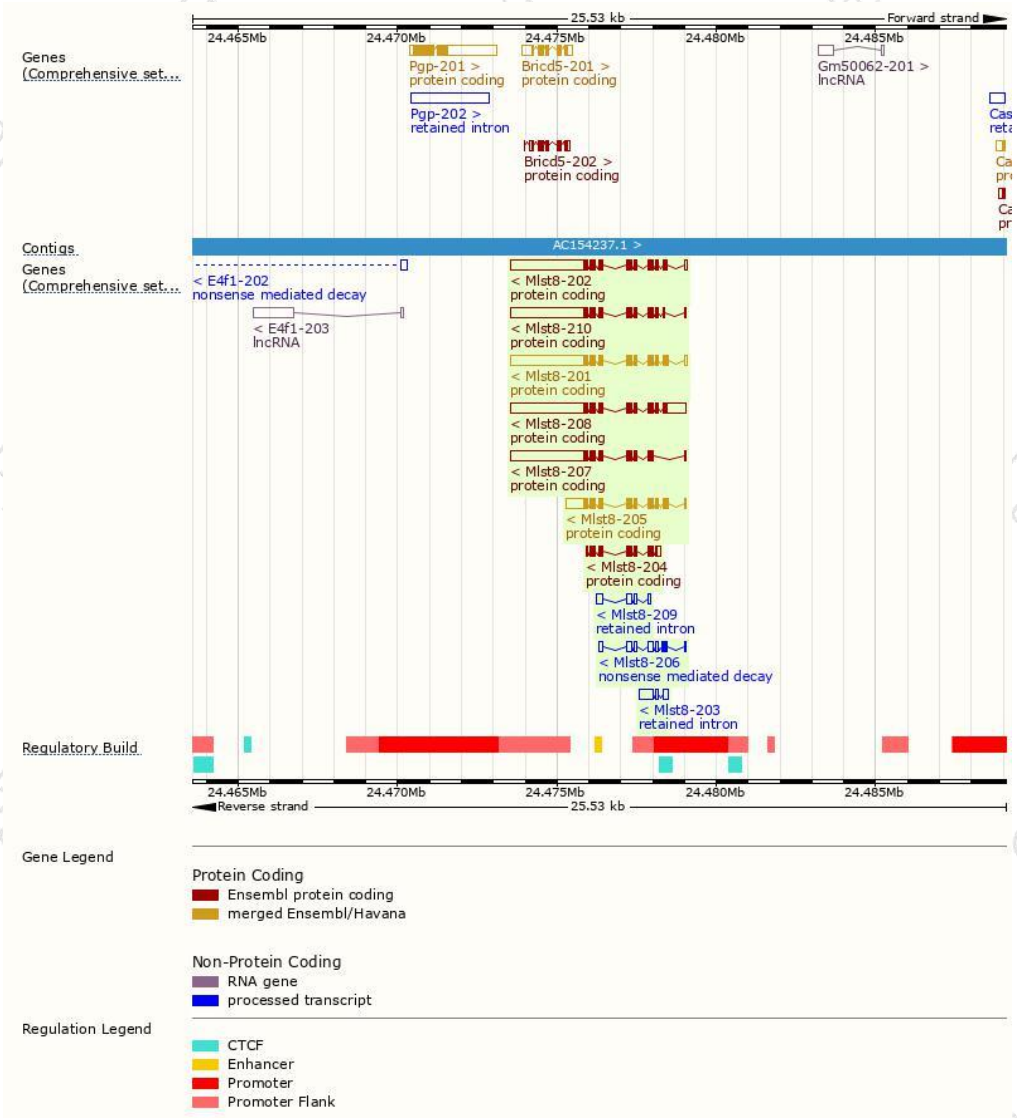
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mlst8-208	ENSMUST00000234686.1	3855	326aa	Protein coding	CCDS28484	-	GENCODE basic APPRIS P1
Mlst8-201	ENSMUST00000070888.13	3358	326aa	Protein coding	CCDS28484	Q9DCJ1	TSL:1 GENCODE basic APPRIS P1
Mlst8-202	ENSMUST00000179163.2	3340	326aa	Protein coding	CCDS28484	Q9DCJ1	TSL:1 GENCODE basic APPRIS P1
Mlst8-205	ENSMUST00000234335.1	1574	326aa	Protein coding	CCDS28484	-	GENCODE basic APPRIS P1
Mlst8-210	ENSMUST00000234941.1	3223	271aa	Protein coding	-	-	GENCODE basic
Mlst8-207	ENSMUST00000234543.1	3127	260aa	Protein coding	-	-	GENCODE basic
Mlst8-204	ENSMUST00000234147.1	850	238aa	Protein coding	-	-	CDS 3' incomplete
Mlst8-206	ENSMUST00000234516.1	786	56aa	Nonsense mediated decay	-	-	
Mlst8-203	ENSMUST00000234032.1	668	No protein	Retained intron	-	-	
Mlst8-209	ENSMUST00000234892.1	519	No protein	Retained intron	-	-	

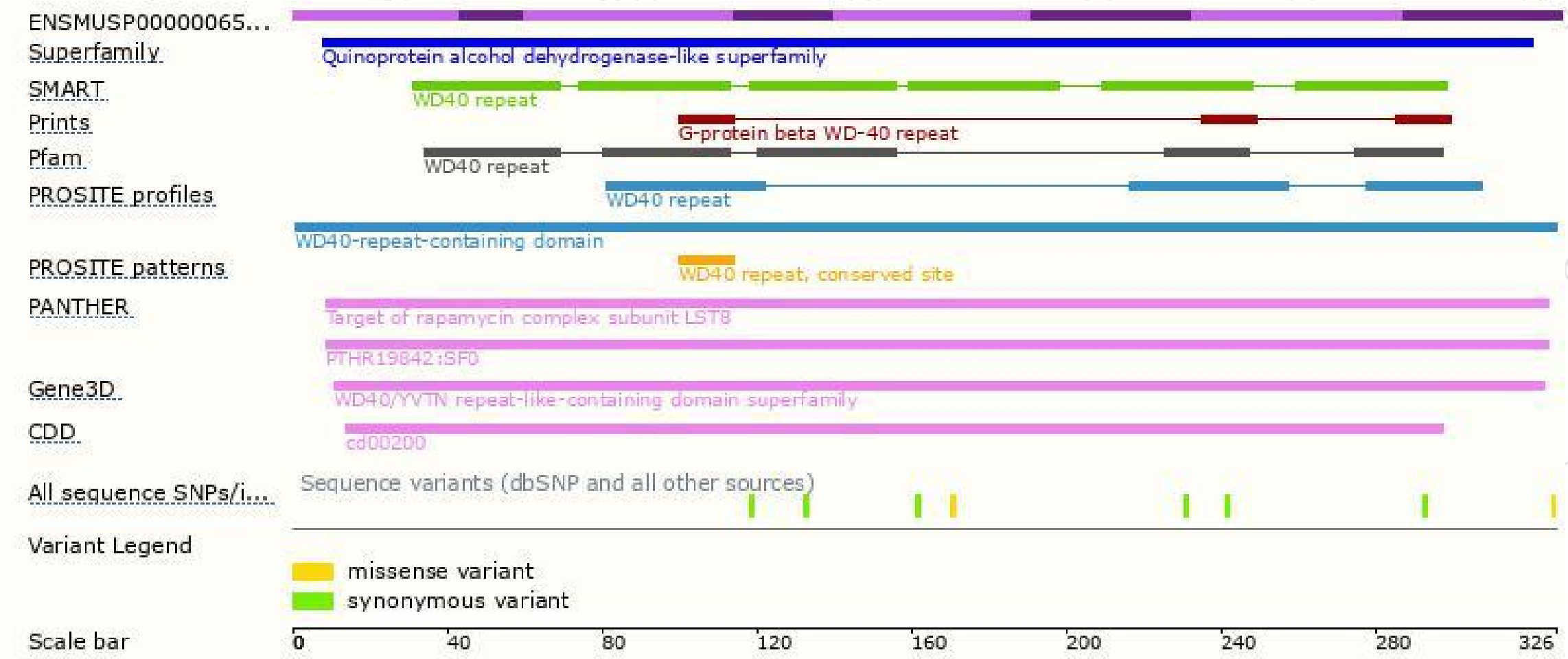
The strategy is based on the design of *Mlst8-201* transcript,The transcription is shown below



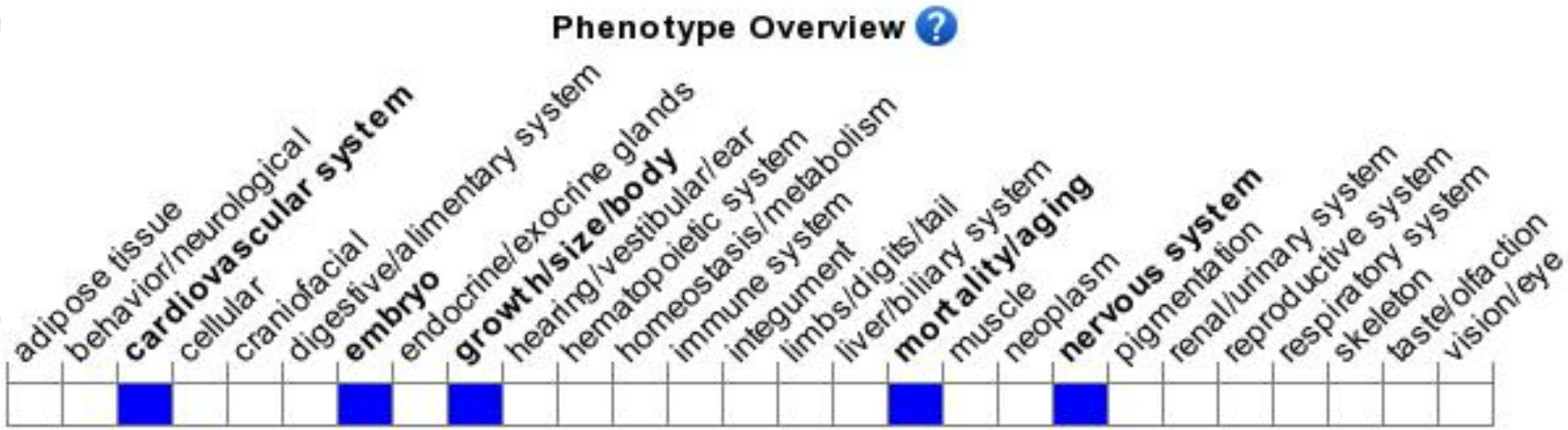
Genomic location distribution



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,Mice homozygous for a null mutation exhibit lethality around E10.5 and abnormal yolk sac vasculature, brain development and heart development.

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

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