Tmem67 Cas9-CKO Strategy

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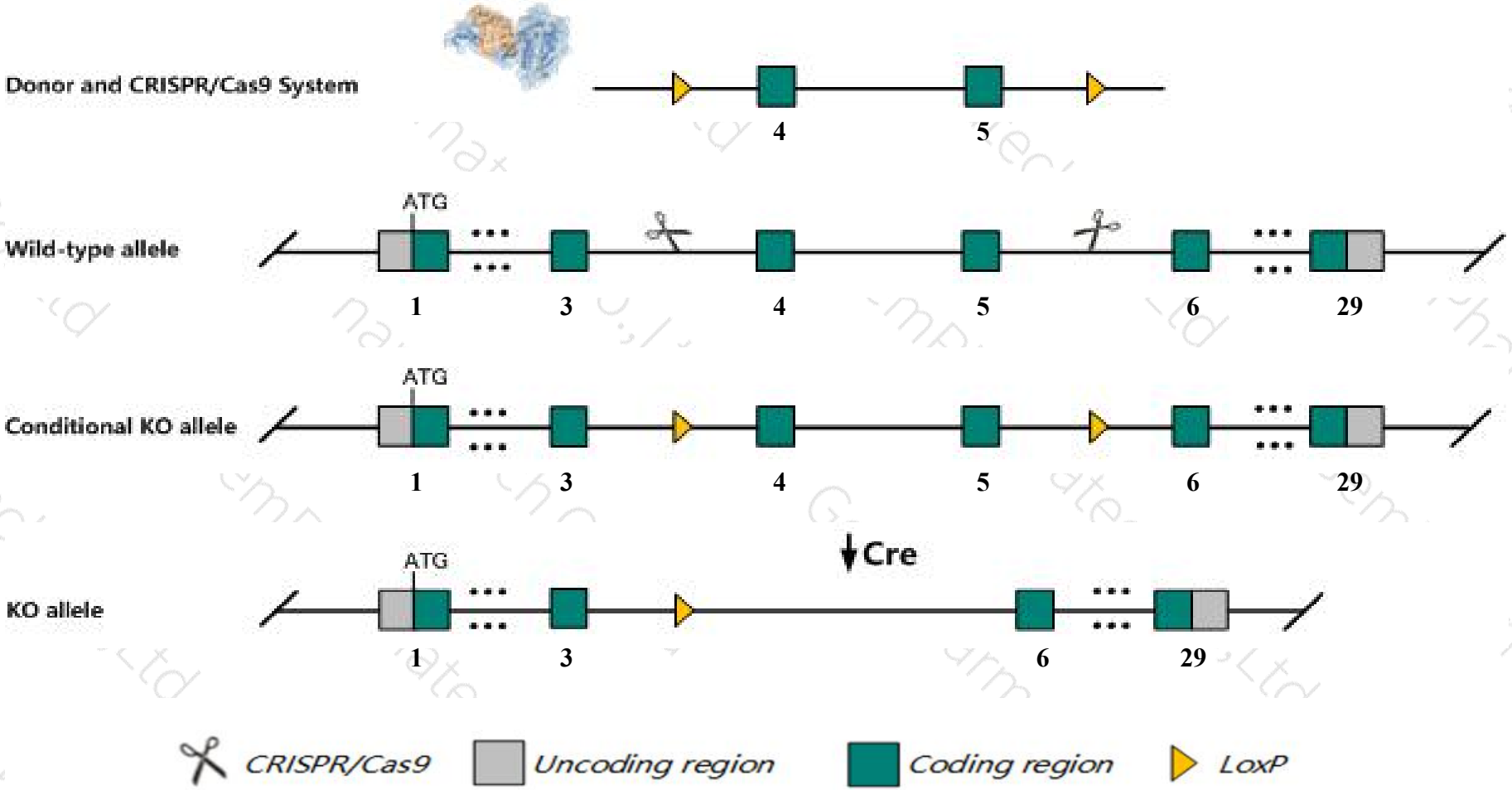
Project Overview

Project Name	***Tmem67***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Tmem67* gene. The schematic diagram is as follows:



Technical routes

➢ The *Tmem67* gene has 6 transcripts. According to the structure of *Tmem67* gene, exon4-exon5 of *Tmem67-202*(ENSMUST00000108293.2) transcript is recommended as the knockout region.The region contains 194bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Tmem67* 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 targeted allele exhibit neonatal/postanal lethality, kidney cysts, and Meckel-Gruber or Joubert syndrome-like phenotypes depending on the filial generation of the backcross to C57BL/6J. Mice homozygous for an ENU-induced allele exhibit cardiovascular defects and cystic kidney.

➢ The *Tmem67* gene is located on the Chr4. 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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Tmem67 transmembrane protein 67 [Mus musculus (house mouse)]

Gene ID: 329795, updated on 13-Mar-2020

Summary

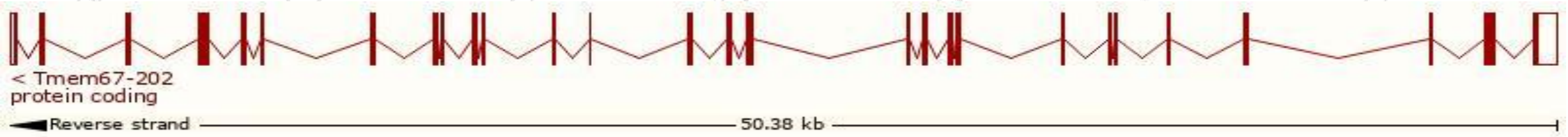
Official Symbol	Tmem67 provided by MGI
Official Full Name	transmembrane protein 67 provided byMGI
Primary source	MGI:MGI:1923928
See related	Ensembl:ENSMUSG00000049488
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	5330408M12Rik, B230117O07, b2b1163.1Clo, b2b1291.1Clo
Expression	Broad expression in testis adult (RPKM 9.1), CNS E14 (RPKM 4.8) and 24 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

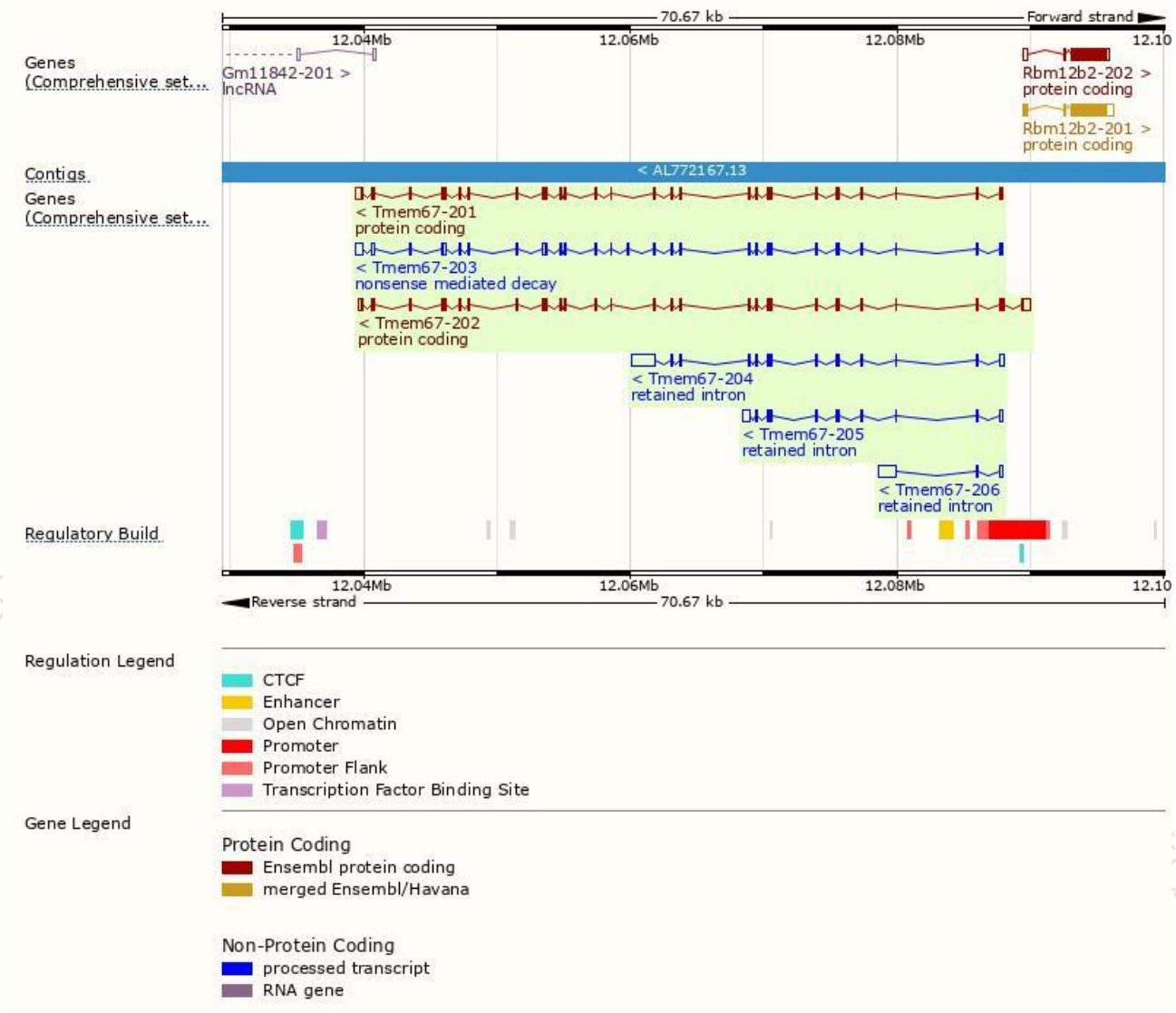
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tmem67-201	ENSMUST00000050686.9	3456	995aa	Protein coding	CCDS17974	E9QNI1	TSL:1 GENCODE basic APPRIS P2
Tmem67-202	ENSMUST00000108293.2	3945	1061aa	Protein coding	-	A2AJP5	TSL:5 GENCODE basic APPRIS ALT2
Tmem67-203	ENSMUST00000131145.8	3533	207aa	Nonsense mediated decay	-	D6RGQ2	CDS 5' incomplete TSL:1
Tmem67-204	ENSMUST00000146140.7	3278	No protein	Retained intron	-	-	TSL:1
Tmem67-206	ENSMUST00000151859.1	1688	No protein	Retained intron	-	-	TSL:1
Tmem67-205	ENSMUST00000147746.7	1517	No protein	Retained intron	-	-	TSL:2

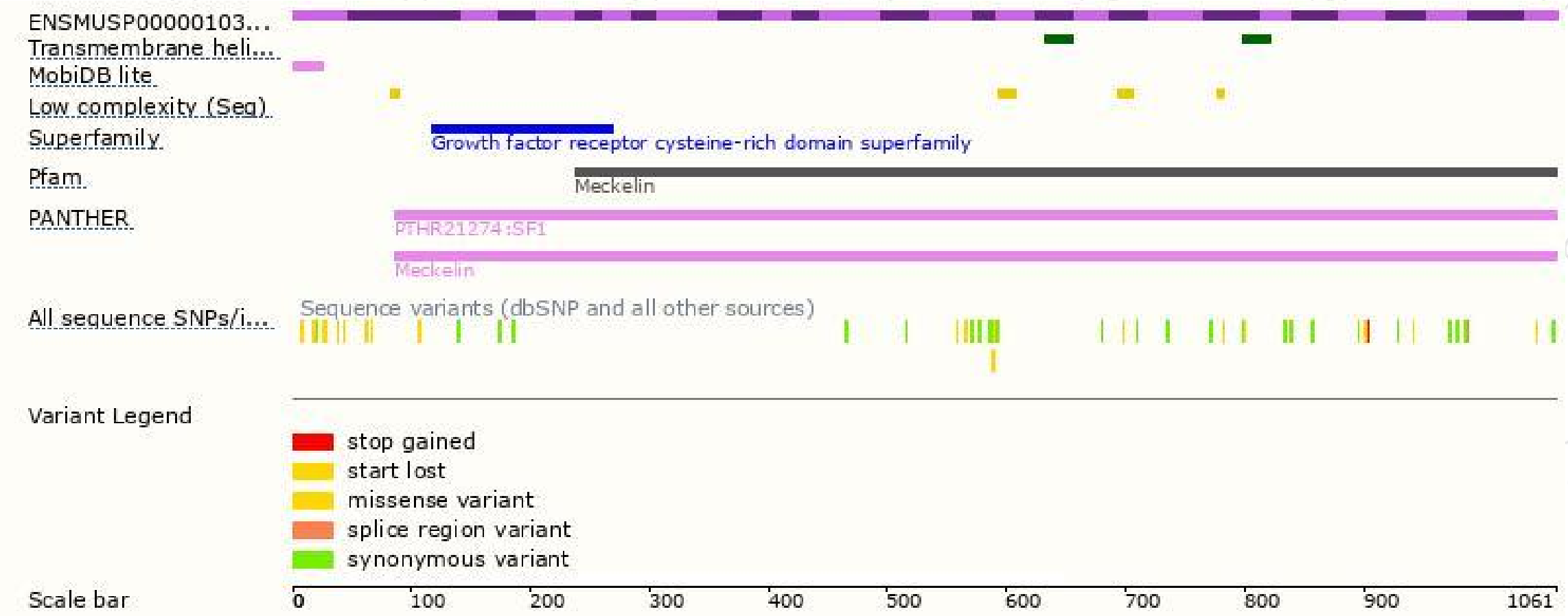
The strategy is based on the design of *Tmem67-202* transcript,the transcription is shown below:



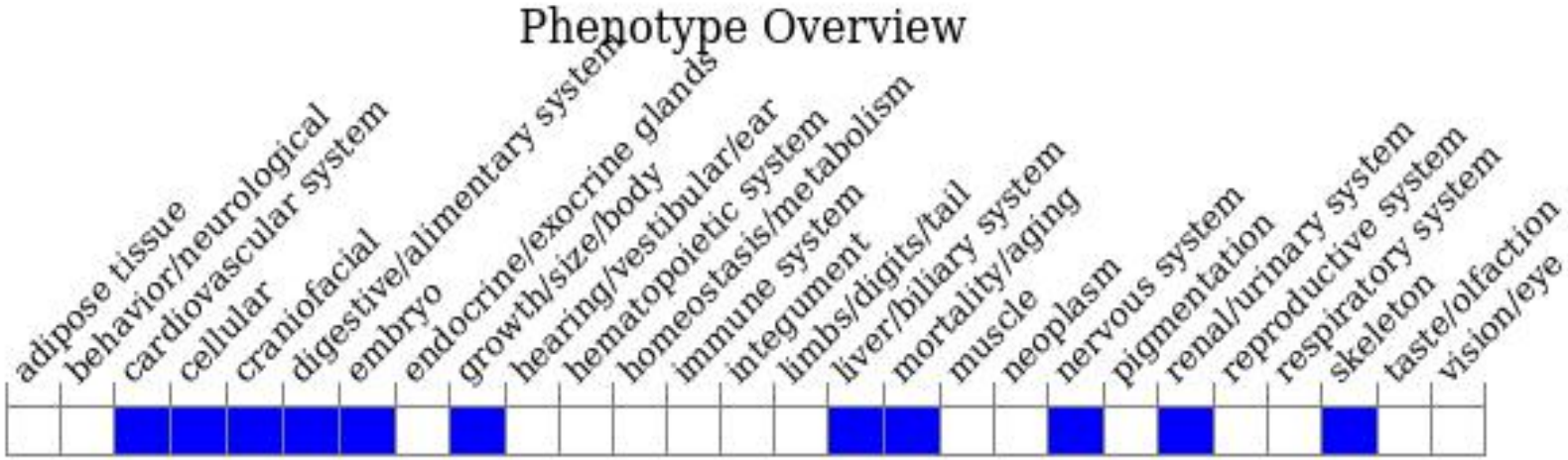
Genomic location distribution



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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