

# *Myo1e* Cas9-CKO Strategy

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**Reviewer: Lingyan Wu**

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# Project Overview

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**Project Name**

*Myole*

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**Project type**

**Cas9-CKO**

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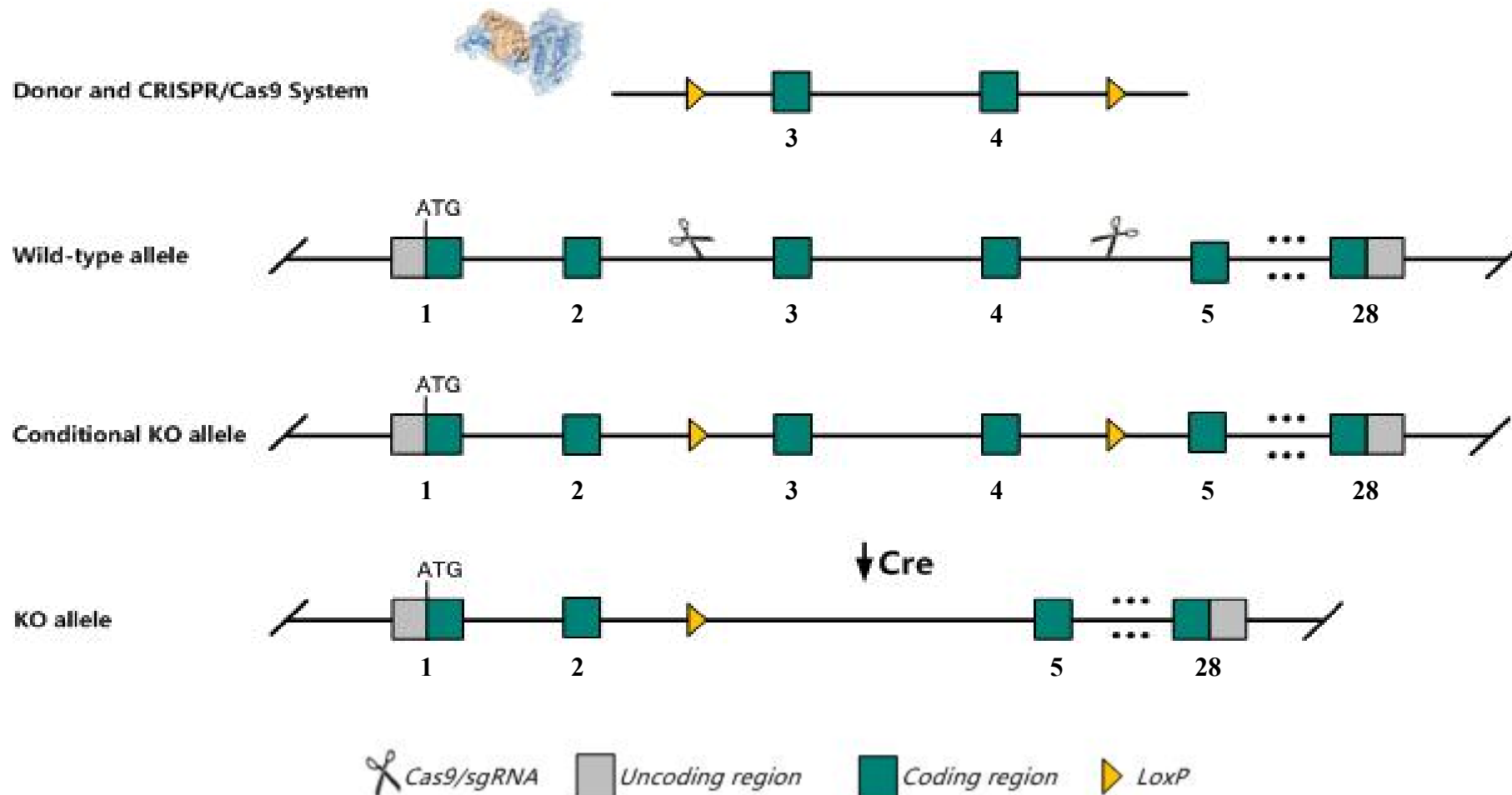
**Strain background**

**C57BL/6JGpt**

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# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Myo1e* gene. The schematic diagram is as follows:



The *Myo1e* gene has 4 transcripts. According to the structure of *Myo1e* gene, exon3-exon4 of *Myo1e-201*(ENSMUST00000034745.9) transcript is recommended as the knockout region. The region contains 185bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Myo1e* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor 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.

According to the existing MGI data, homozygotes for a gene trapped allele exhibit embryonic lethality, embryonic hemorrhaging and hematopoietic defects. Homozygotes for a knock-out allele show proteinuria, chronic renal injury, kidney inflammation, and defects in renal filtration and podocyte organization.

The *Myo1e* 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.

**Myo1e myosin IE [Mus musculus (house mouse)]**

Gene ID: 71602, updated on 26-Jan-2021

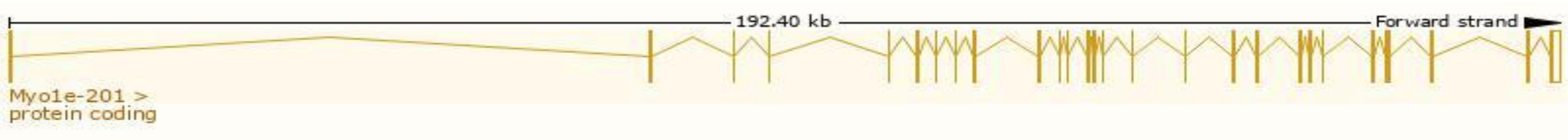
**Summary****Official Symbol** Myo1e provided by [MGI](#)**Official Full Name** myosin IE provided by [MGI](#)**Primary source** [MGI:MGI:106621](#)**See related** [Ensembl:ENSMUSG00000032220](#)**Gene type** protein coding**RefSeq status** VALIDATED**Organism** [Mus musculus](#)**Lineage** Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus**Also known as** 2310020N23Rik, 9130023P14Rik, AA407778, myosin-1e, myr 3**Expression** Ubiquitous expression in colon adult (RPKM 9.1), lung adult (RPKM 7.2) and 28 other tissues [See more](#)**Orthologs** [human](#) [all](#)

# Transcript information      Ensembl

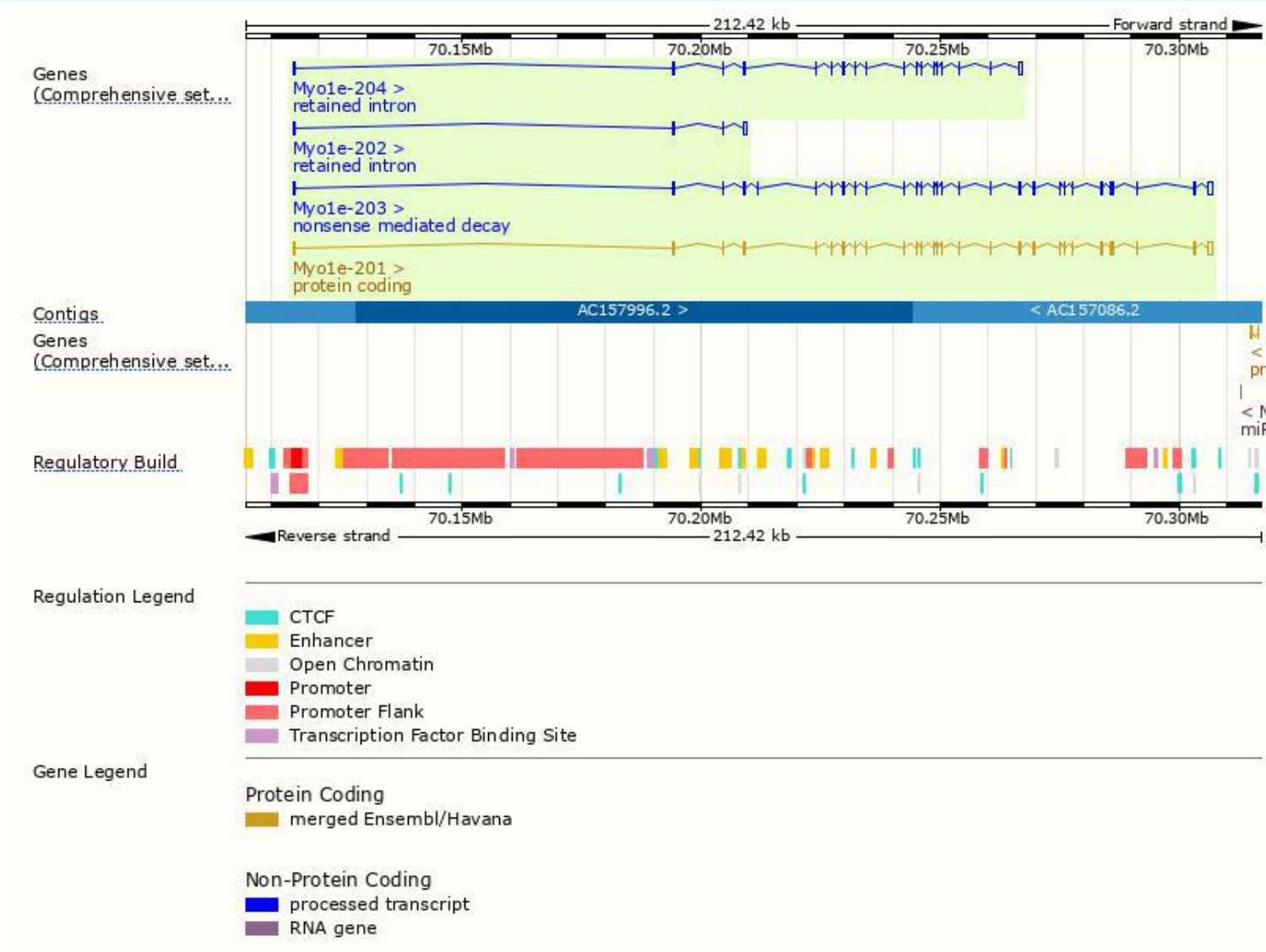
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Myo1e-201	<a href="#">ENSMUST00000034745.9</a>	4625	<a href="#">1107aa</a>	Protein coding	<a href="#">CCDS40680</a>		TSL:1 , GENCODE basic , APPRIS P1 ,
Myo1e-203	<a href="#">ENSMUST00000214042.2</a>	4699	<a href="#">116aa</a>	Nonsense mediated decay	-		TSL:1 ,
Myo1e-204	<a href="#">ENSMUST00000214767.2</a>	2671	No protein	Retained intron	-		TSL:1 ,
Myo1e-202	<a href="#">ENSMUST00000213863.2</a>	1260	No protein	Retained intron	-		TSL:1 ,

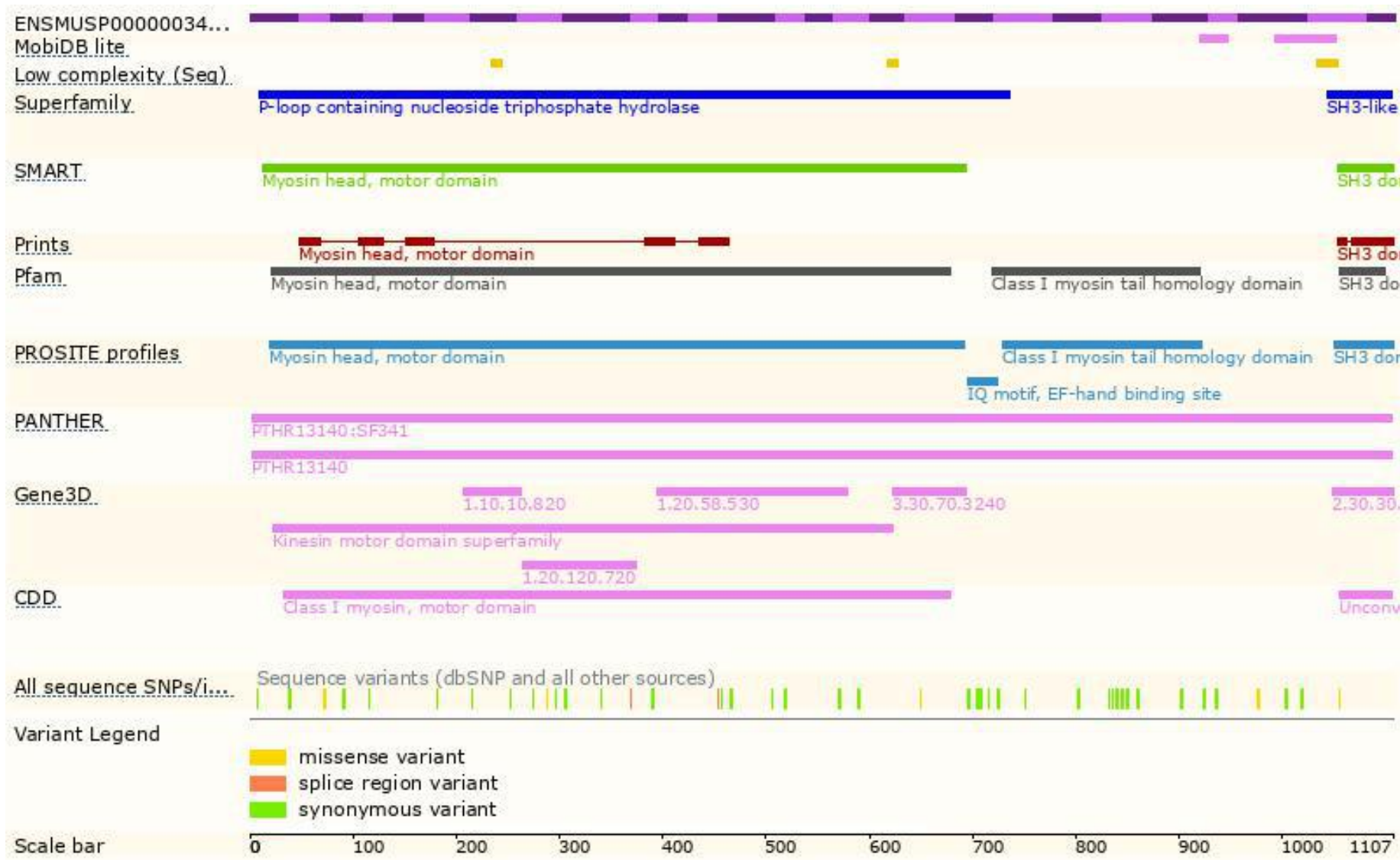
The strategy is based on the design of *Myo1e-201* transcript,the transcription is shown below:



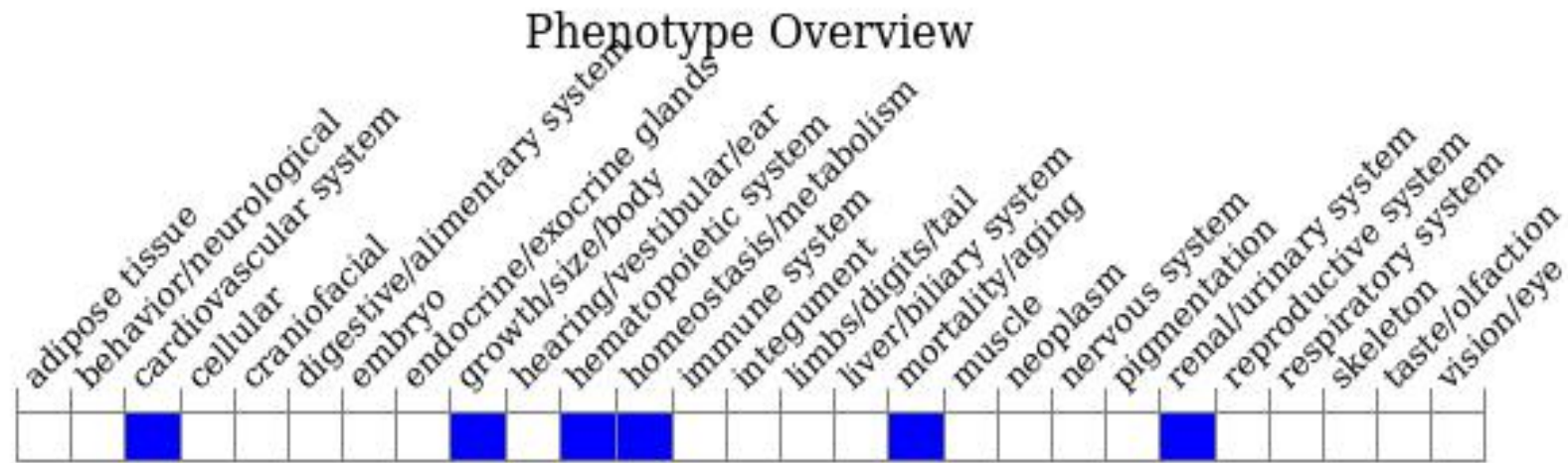
# Genomic location distribution



# 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, homozygotes for a gene trapped allele exhibit embryonic lethality, embryonic hemorrhaging and hematopoietic defects. Homozygotes for a knock-out allele show proteinuria, chronic renal injury, kidney inflammation, and defects in renal filtration and podocyte organization.

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

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