

***Rhobtb3* Cas9-CKO Strategy**

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Project Overview

Project Name

Rhobtb3

Project type

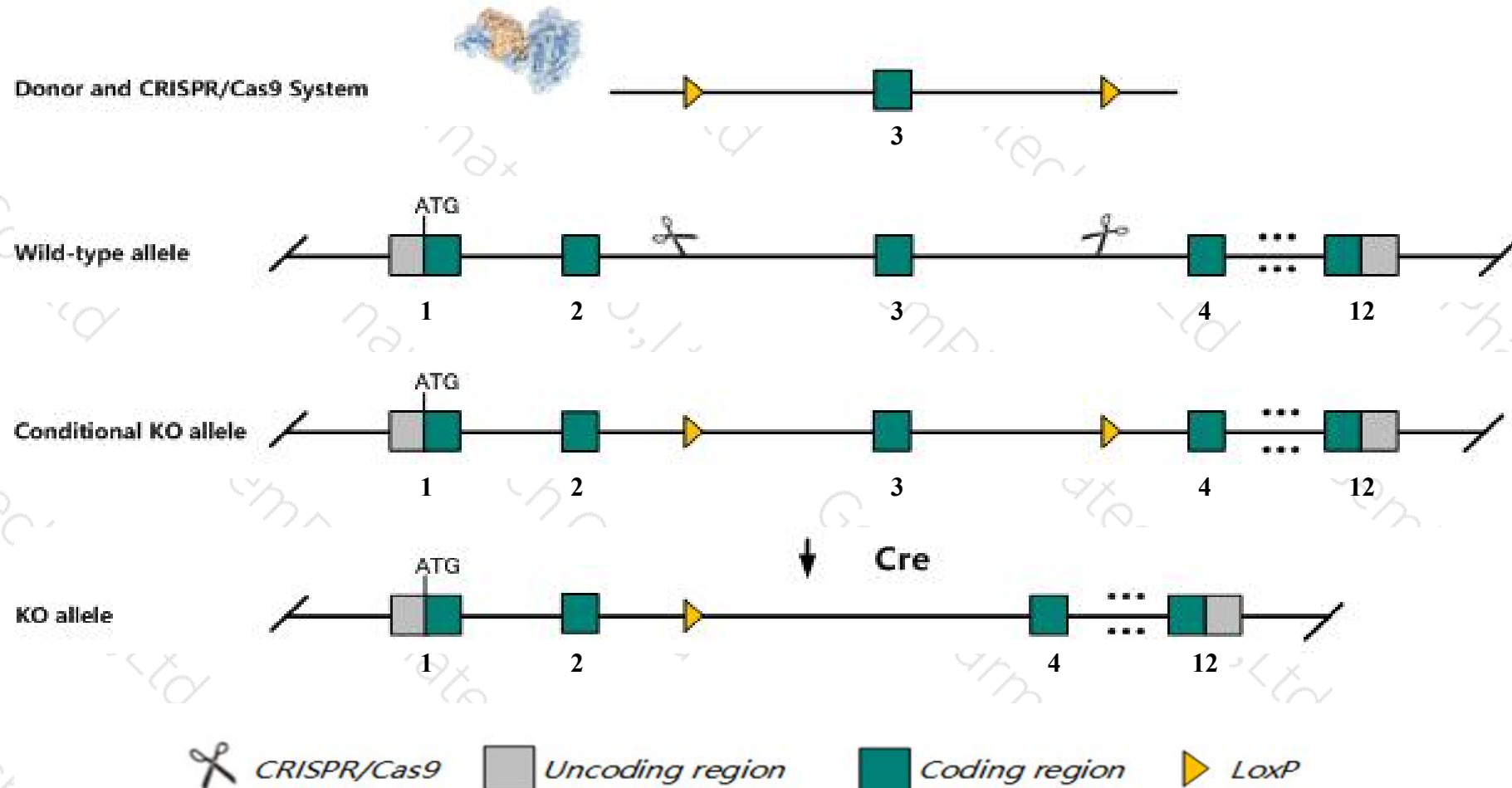
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rhobtb3* gene. The schematic diagram is as follows:



Technical routes

- The *Rhobtb3* gene has 5 transcripts. According to the structure of *Rhobtb3* gene, exon3 of *Rhobtb3*-201(ENSMUST00000022078.11) transcript is recommended as the knockout region. The region contains 187bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rhobtb3* 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.

- According to the existing MGI data, mice homozygous for a knock-out allele exhibit preweaning lethality, reduced body weight and slightly reduced organ weights that varies by sex.
- The *Rhobtb3* gene is located on the Chr13. 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)

Rhobtb3 Rho-related BTB domain containing 3 [Mus musculus (house mouse)]

Gene ID: 73296, updated on 13-Mar-2020

Summary



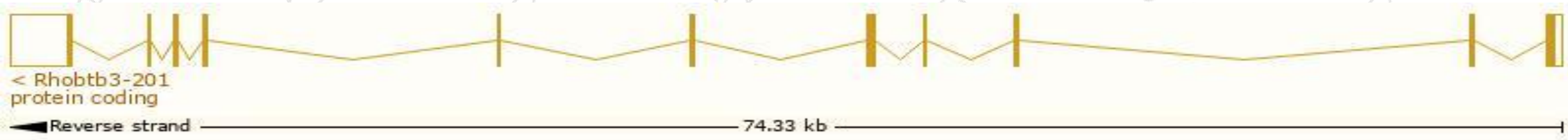
Official Symbol	Rhobtb3 provided by MGI
Official Full Name	Rho-related BTB domain containing 3 provided by MGI
Primary source	MGI:MGI:1920546
See related	Ensembl:ENSMUSG00000021589
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1700040C17Rik, 2610033K01Rik, 4930503C18Rik, AI317148, AW208826, mKIAA0878
Expression	Broad expression in CNS E11.5 (RPKM 11.7), limb E14.5 (RPKM 9.8) and 23 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

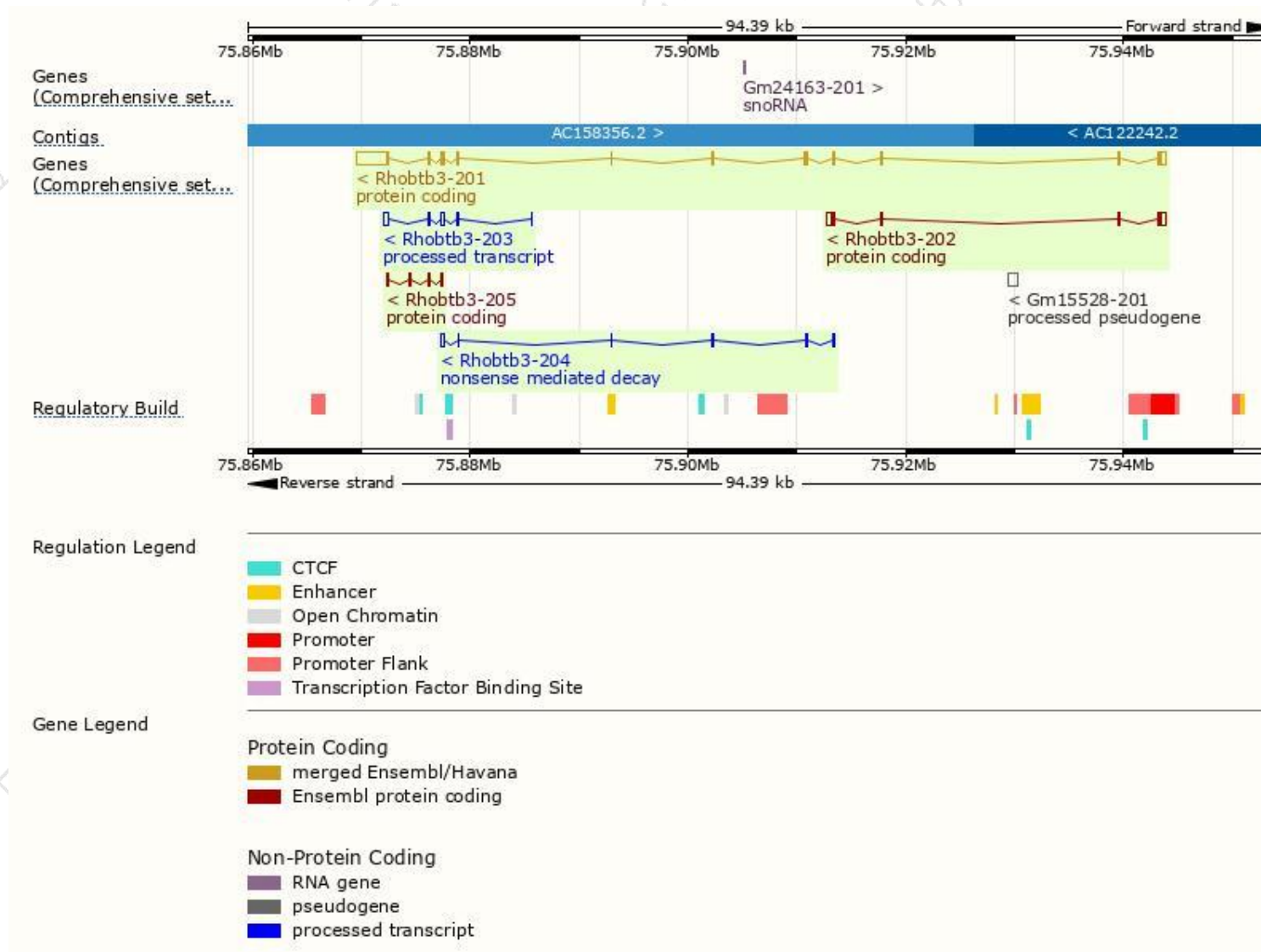
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rhobtb3-201	ENSMUST00000022078.11	4980	611aa	Protein coding	CCDS26652	Q9CTN4	TSL:1 GENCODE basic APPRIS P1
Rhobtb3-202	ENSMUST00000109606.2	1676	260aa	Protein coding	-	Q8BV11	TSL:1 GENCODE basic
Rhobtb3-205	ENSMUST00000222923.1	303	88aa	Protein coding	-	A0A1Y7VJQ5	CDS 5' incomplete TSL:1
Rhobtb3-204	ENSMUST00000220939.1	712	100aa	Nonsense mediated decay	-	A0A1Y7VMM2	CDS 5' incomplete TSL:3
Rhobtb3-203	ENSMUST00000123353.1	937	No protein	Processed transcript	-	-	TSL:1

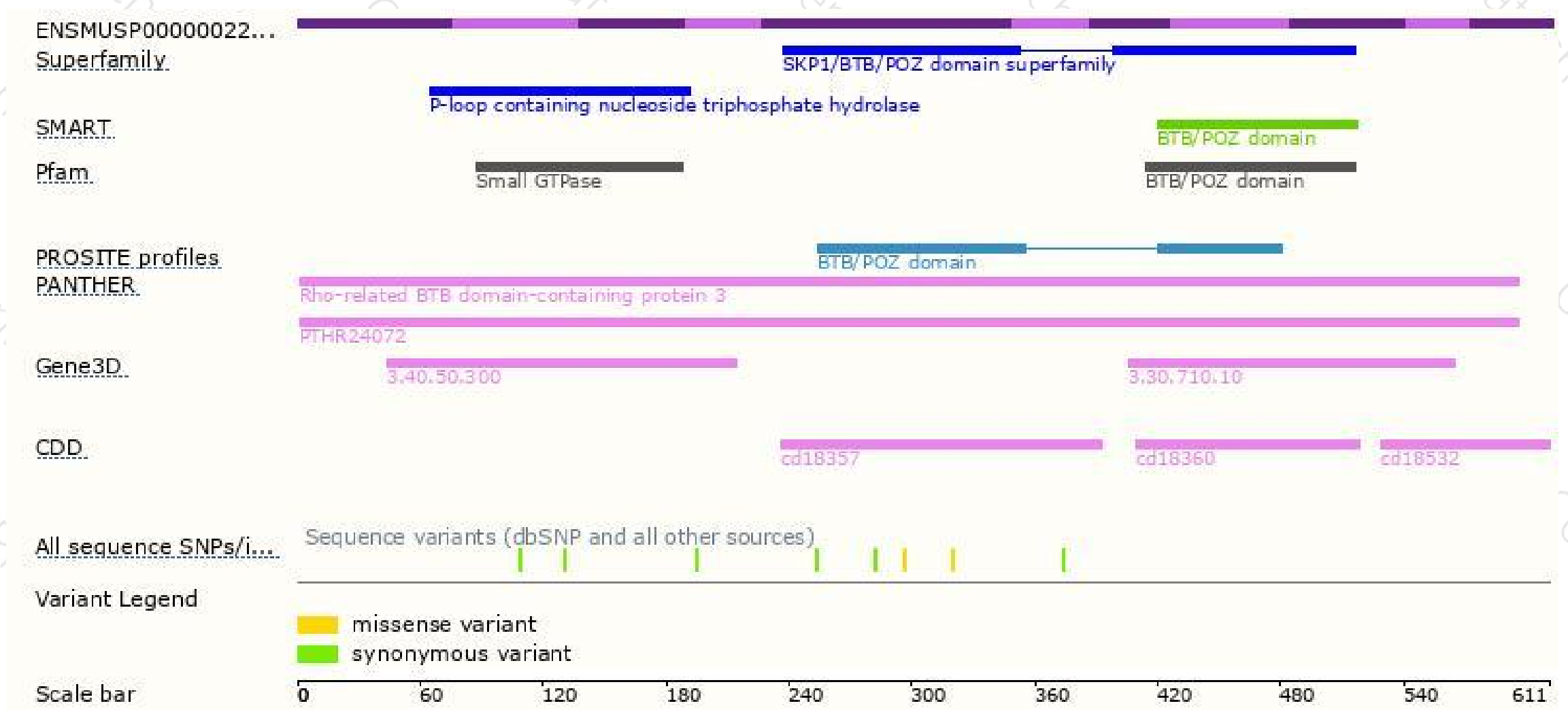
The strategy is based on the design of *Rhobtb3-201* transcript,the transcription is shown below:



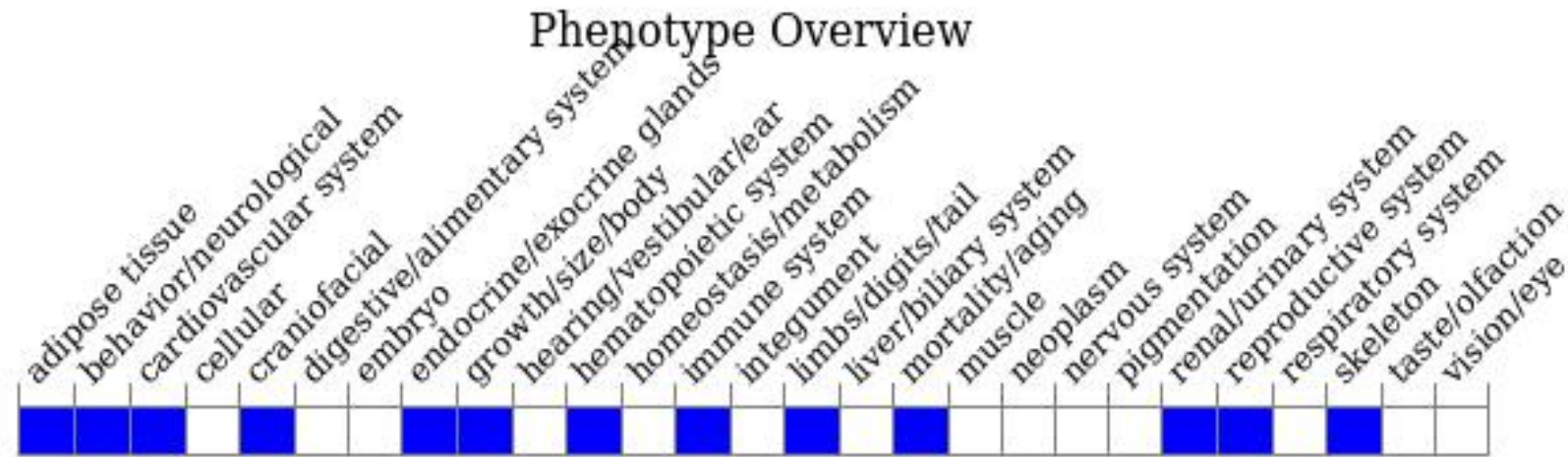
Genomic location distribution



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 knock-out allele exhibit preweaning lethality, reduced body weight and slightly reduced organ weights that varies by sex.

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

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