

***Rab6a* Cas9-CKO Strategy**

Designer: Bingxuan Li

Reviewer: Ruirui Zhang

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Project Overview

Project Name

Rab6a

Project type

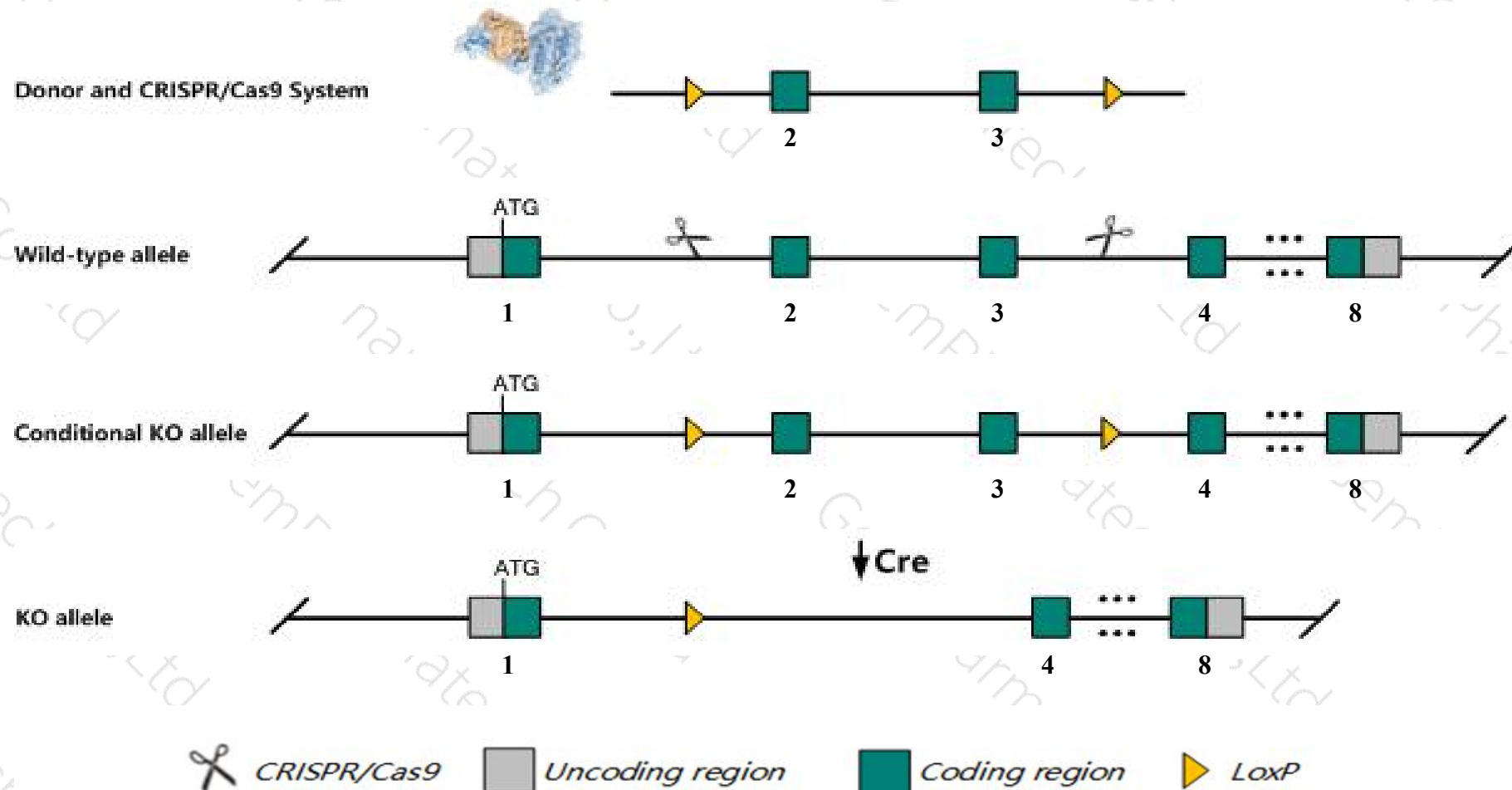
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rab6a* gene. The schematic diagram is as follows:



- The *Rab6a* gene has 4 transcripts. According to the structure of *Rab6a* gene, exon2-exon3 of *Rab6a-201* (ENSMUST00000032946.9) transcript is recommended as the knockout region. The region contains 113bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rab6a* 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 die around e6 with disorganized epiblast.
- The *Rab6a* gene is located on the Chr7. 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)

Rab6a RAB6A, member RAS oncogene family [Mus musculus (house mouse)]

Gene ID: 19346, updated on 13-Mar-2020

Summary

Official Symbol Rab6a provided by [MGI](#)

Official Full Name RAB6A, member RAS oncogene family provided by [MGI](#)

Primary source [MGI:MGI:894313](#)

See related [Ensembl:ENSMUSG00000030704](#)

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 2610028L11Rik, AA419671, Rab6

Expression Ubiquitous expression in cortex adult (RPKM 77.2), CNS E18 (RPKM 68.4) and 28 other tissues [See more](#)

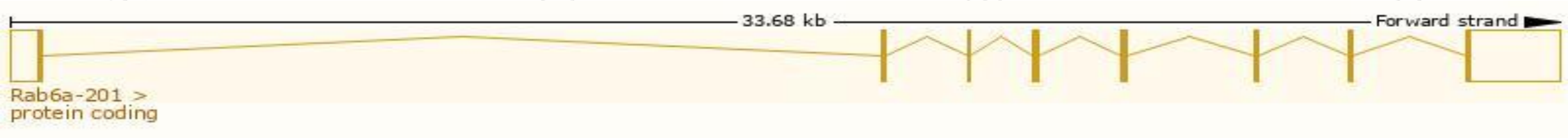
Orthologs [human](#) [all](#)

Transcript information (Ensembl)

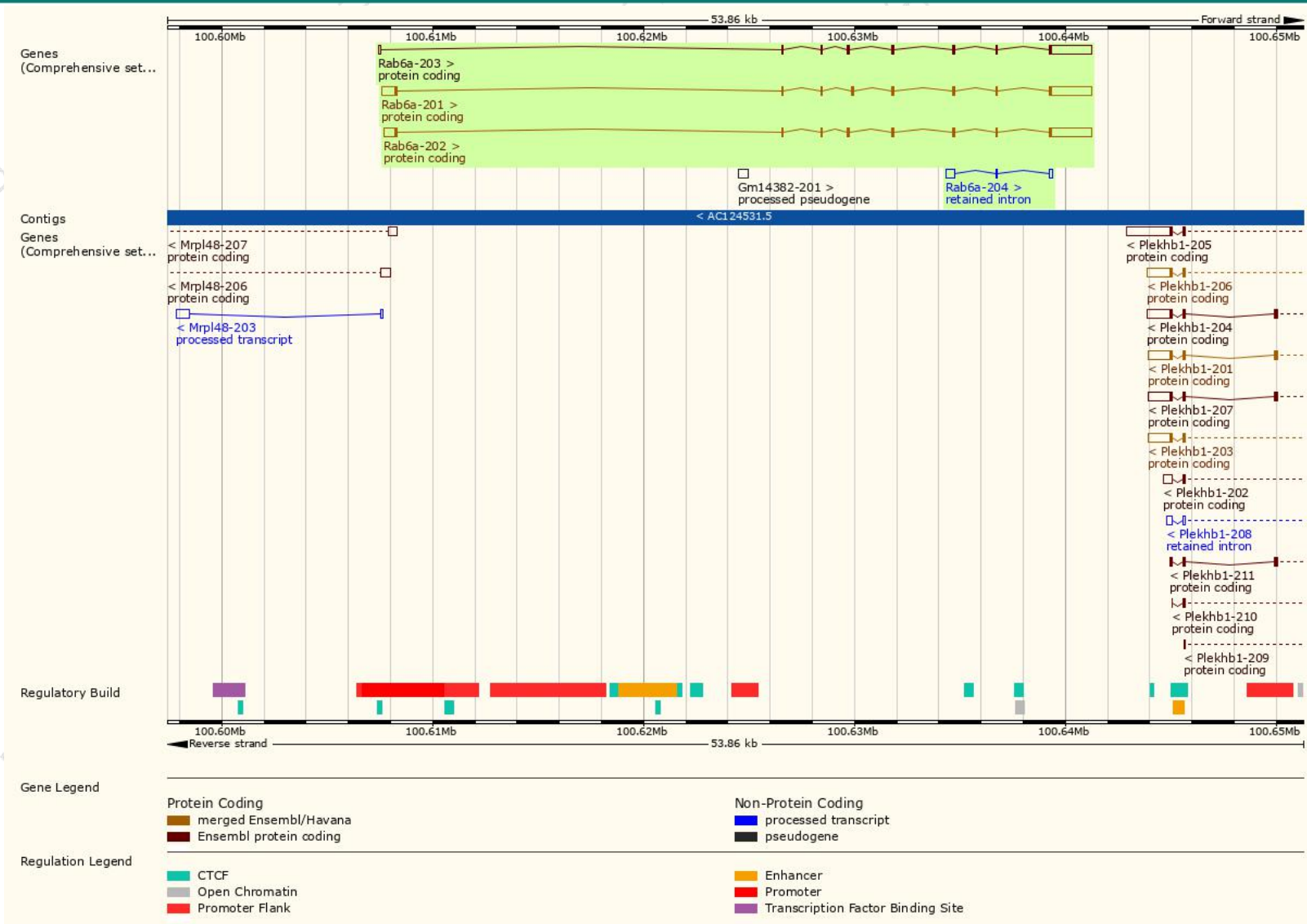
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rab6a-201	ENSMUST00000032946.9	3214	208aa	Protein coding	CCDS21503	P35279_Q0PD54	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P3
Rab6a-202	ENSMUST00000098252.4	3110	208aa	Protein coding	CCDS52323	P35279_Q3U4W5	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT1
Rab6a-203	ENSMUST00000107048.7	2617	175aa	Protein coding	-	D3YV69	TSL:3 GENCODE basic
Rab6a-204	ENSMUST00000123960.1	567	No protein	Retained intron	-	-	TSL:2

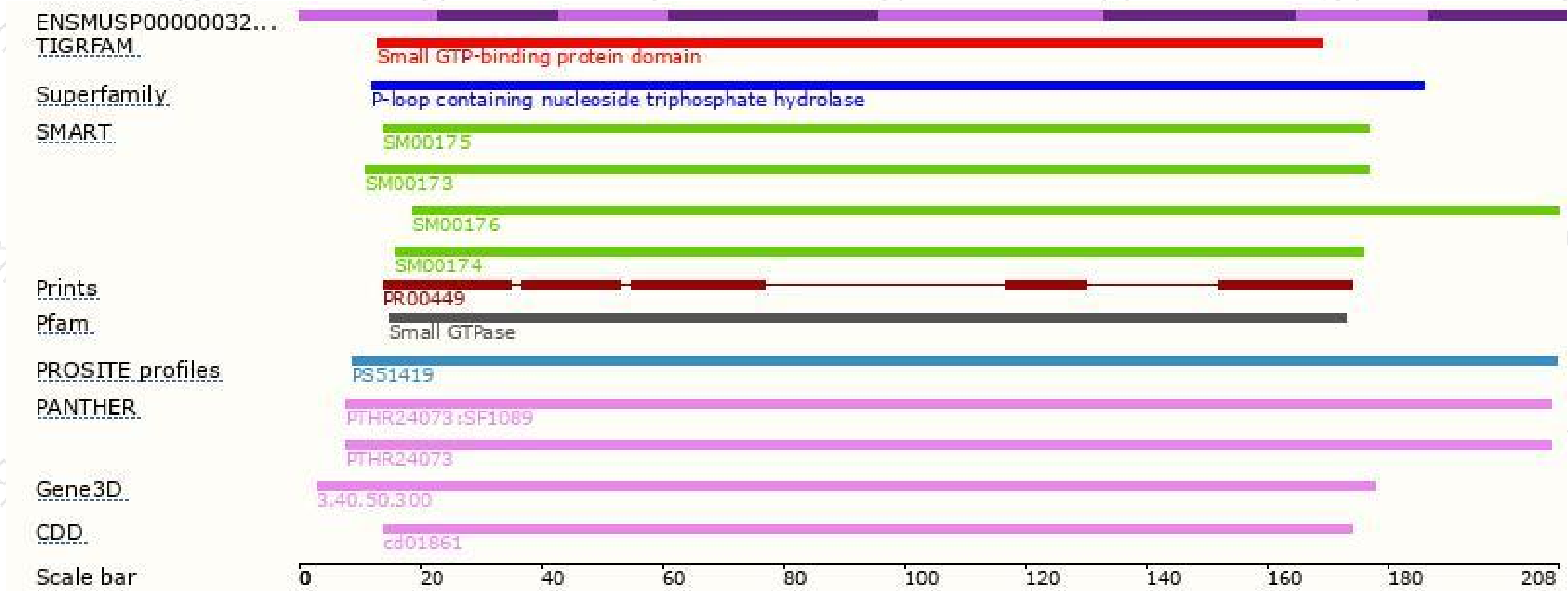
The strategy is based on the design of *Rab6a-201* transcript,the transcription is shown below:



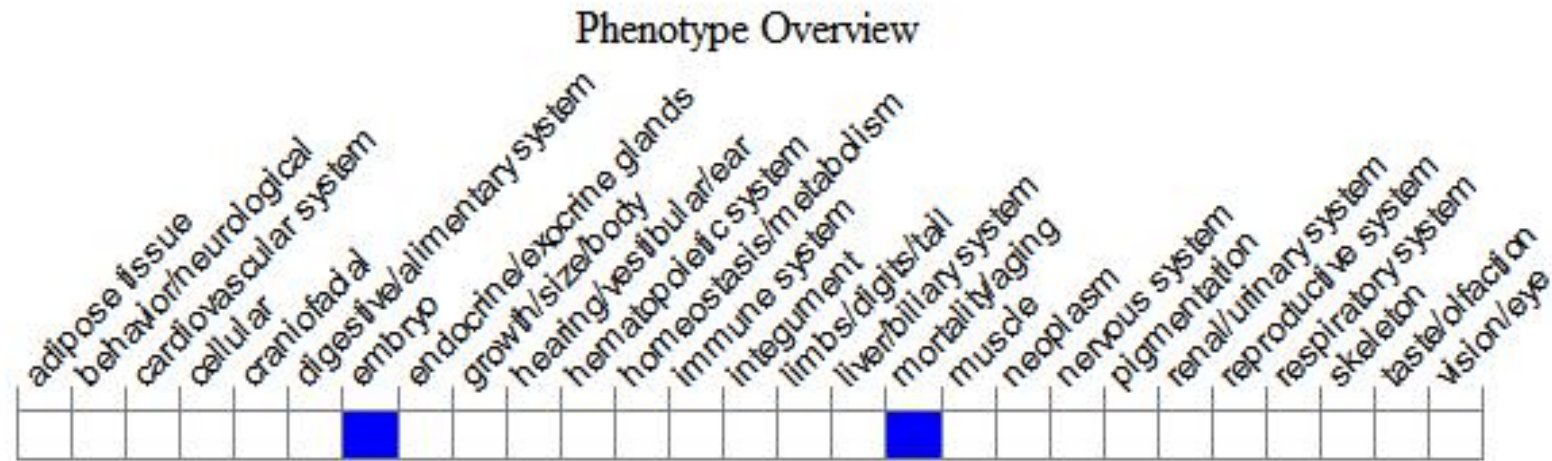
Genomic location distribution



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 die around E6 with disorganized epiblast.

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

Tel: 400-9660890

