Rab8a Cas9-KO Strategy

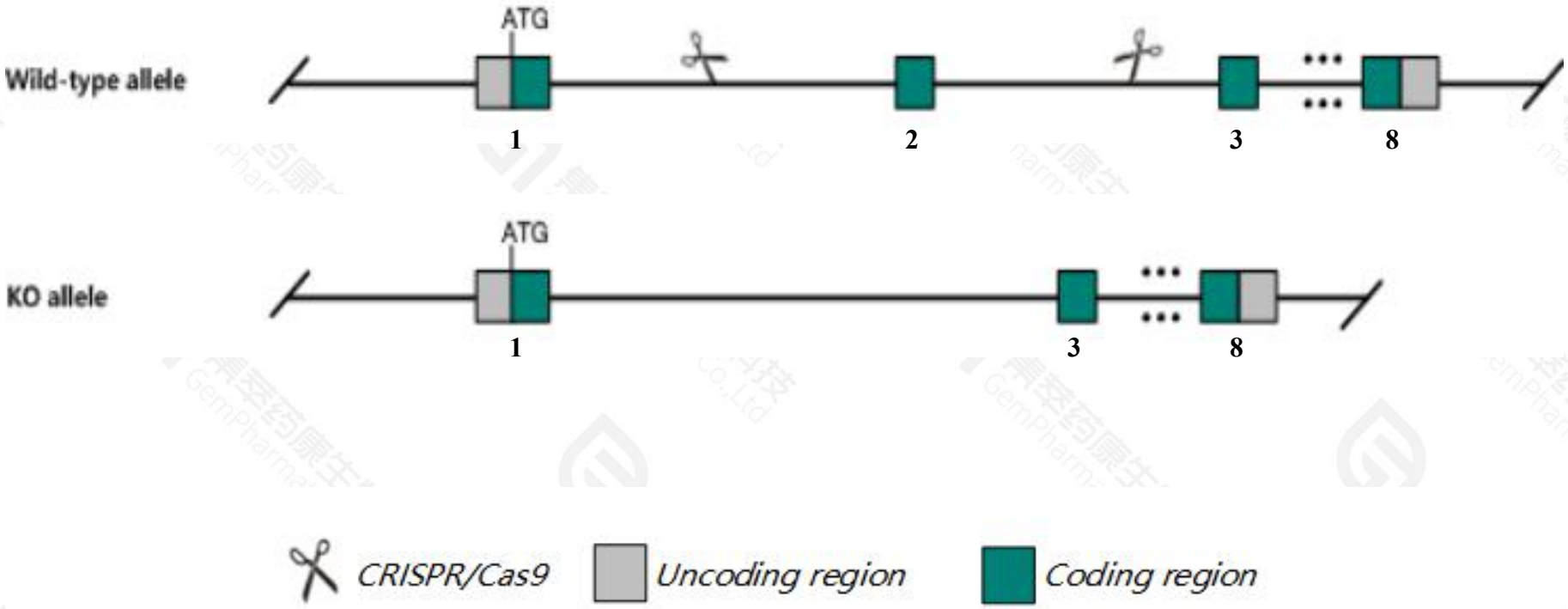
Designer: Daohua Xu

Project Overview

Project Name	***Rab8a***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rab8a* gene. The schematic diagram is as follows:



Technical routes

➢ The *Rab8a* gene has 3 transcripts. According to the structure of *Rab8a* gene, exon2 of *Rab8a-201*(ENSMUST00000003121.9) transcript is recommended as the knockout region.The region contains 61bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Rab8a* gene. The brief process is as follows: CRISPR/Cas9 system 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.

Notice

➢ According to the existing MGI data,mice homozygous for a null allele die 3 to 4 weeks after birth and exhibit cachexia, diarrhea, intestinal swelling, shortened microvilli with inclusion bodies, and a failure to absorb nutrients.

➢ The *Rab8a* gene is located on the Chr8. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

集萃药康 GemPharmatech

Rab8a RAB8A, member RAS oncogene family [Mus musculus (house mouse)]

Gene ID: 17274, updated on 13-Mar-2020

Summary

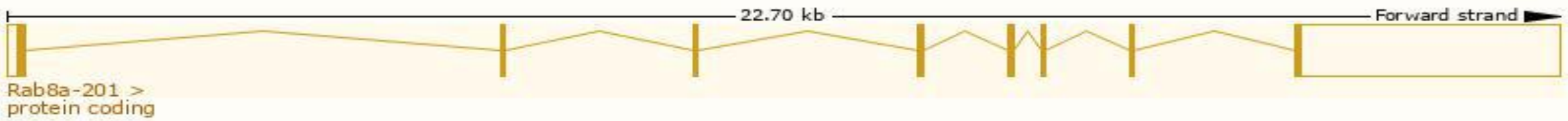
Official Symbol	Rab8a provided by MGI
Official Full Name	RAB8A, member RAS oncogene family provided by MGI
Primary source	MGI:MGI:96960
See related	Ensembl:ENSMUSG00000003037
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AA409338, Mel
Expression	Ubiquitous expression in large intestine adult (RPKM 57.7), duodenum adult (RPKM 54.3) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

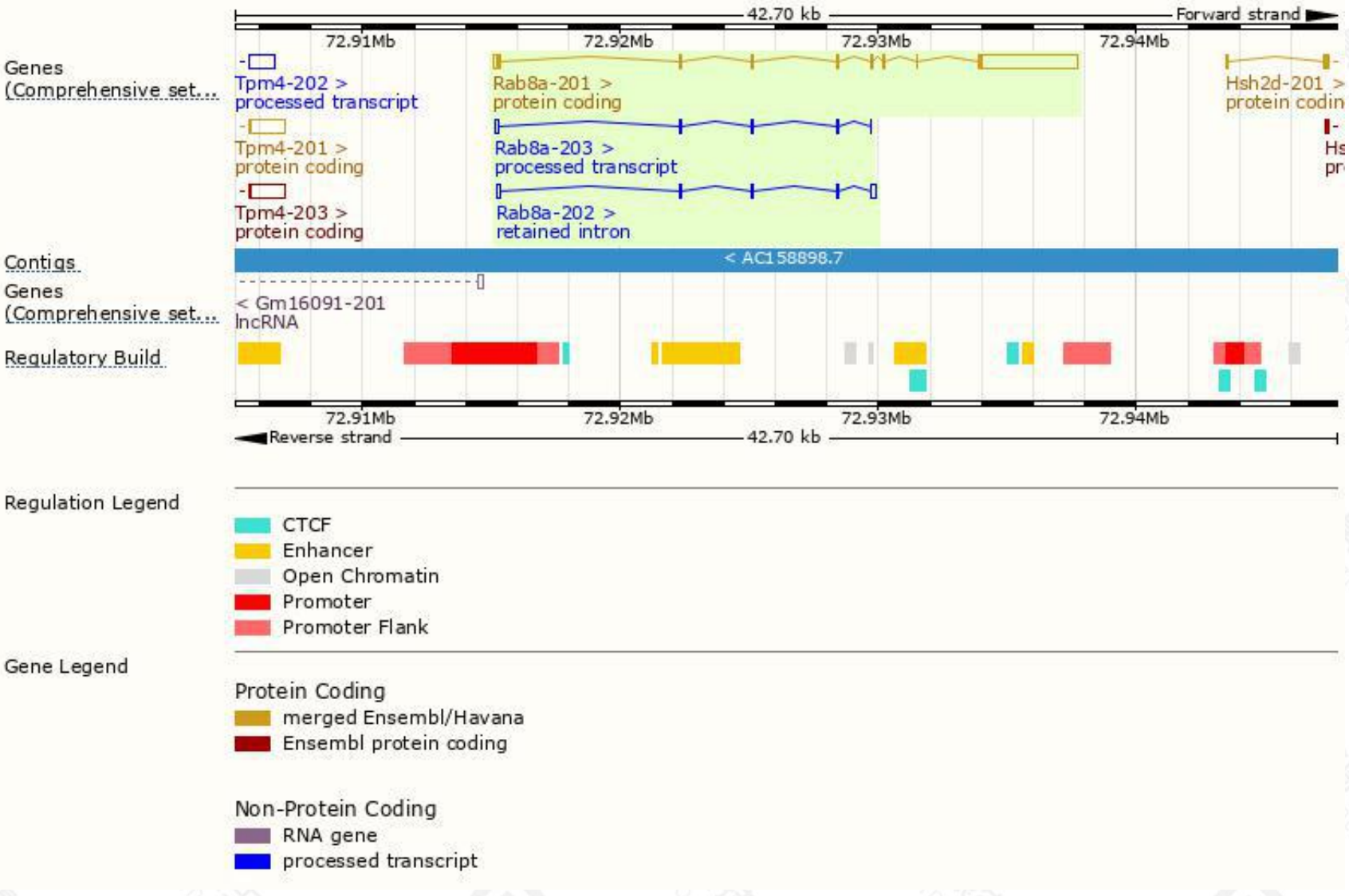
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rab8a-201	ENSMUST00000003121.8	4550	207aa	Protein coding	CCDS22409	P55258 Q0PD50	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 P1
Rab8a-203	ENSMUST00000135613.7	324	No protein	Processed transcript	-	-	TSL:5
Rab8a-202	ENSMUST00000128203.1	542	No protein	Retained intron	-	-	TSL:2

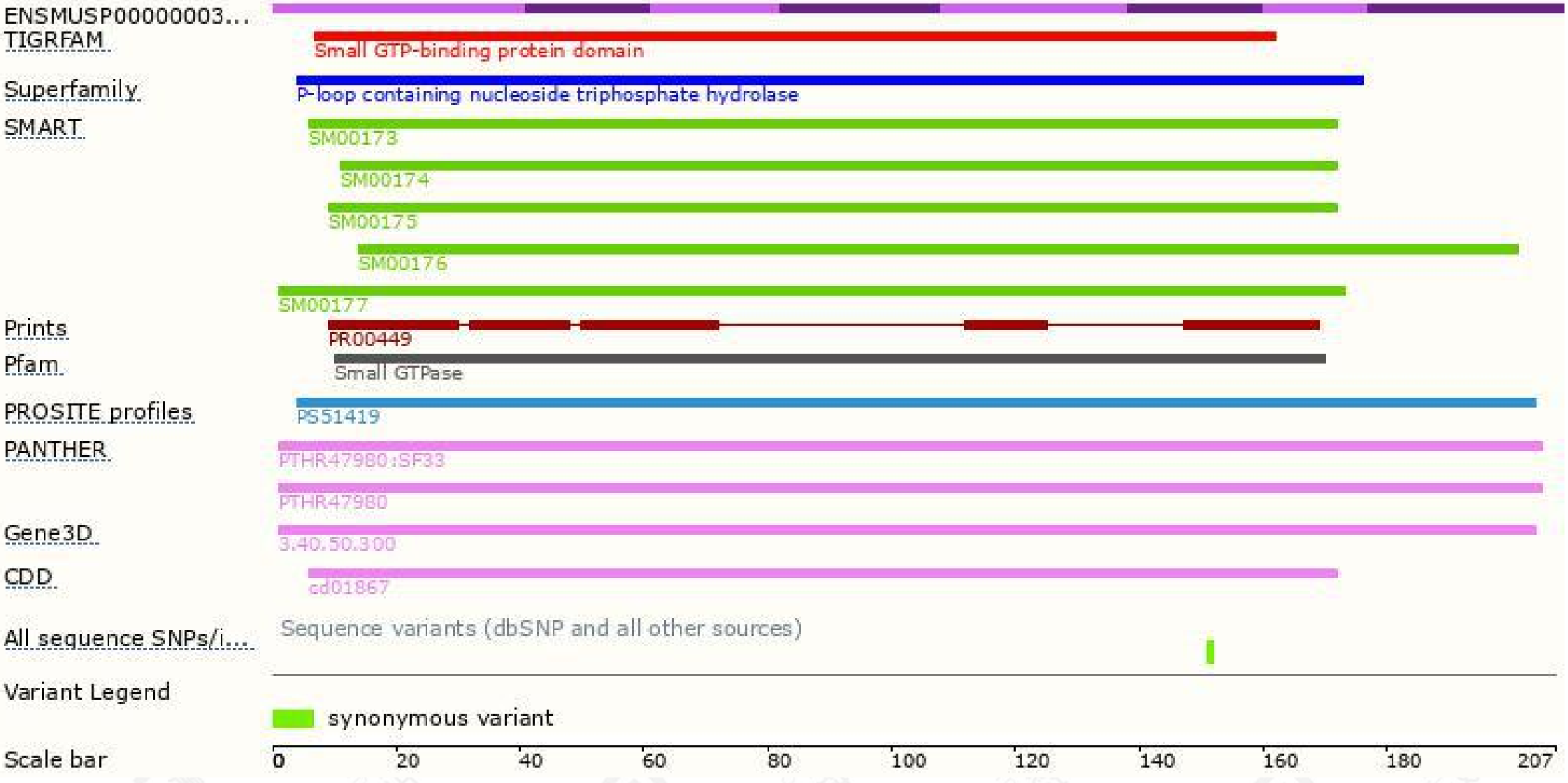
The strategy is based on the design of *Rab8a-201* transcript,the transcription is shown below:



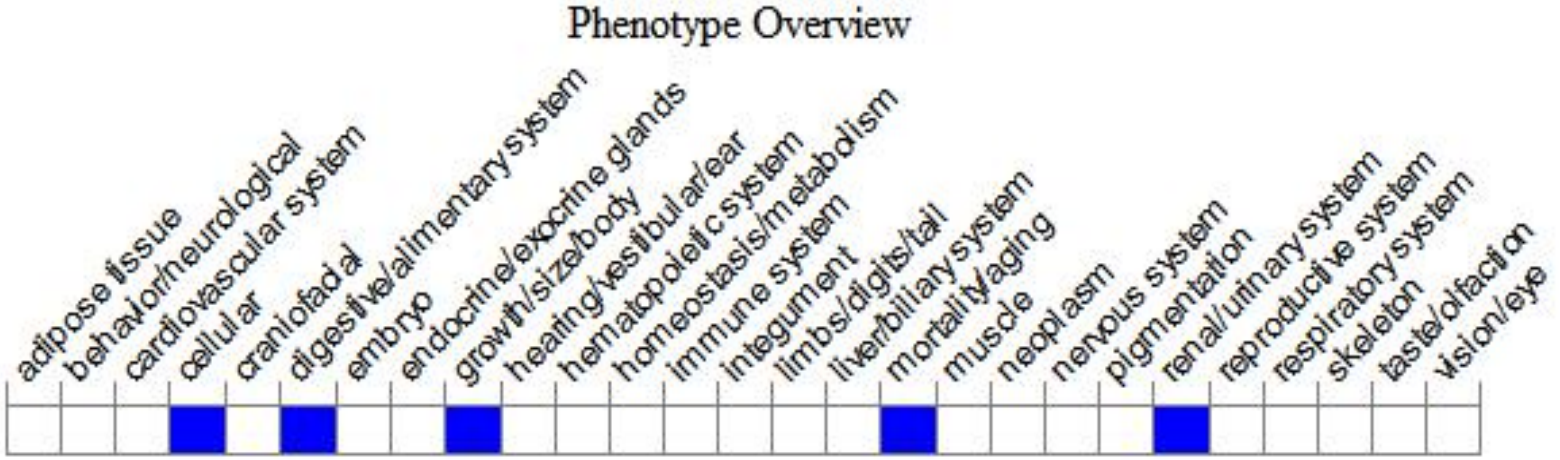
Genomic location distribution



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 allele die 3 to 4 weeks after birth and exhibit cachexia, diarrhea, intestinal swelling, shortened microvilli with inclusion bodies, and a failure to absorb nutrients.

集萃药康
GemPharmatech

If you have any questions, you are welcome to inquire.
Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd