Scn4a Cas9-KO Strategy

Designer: Rui Xiong

Reviewer: Miaomiao Cui

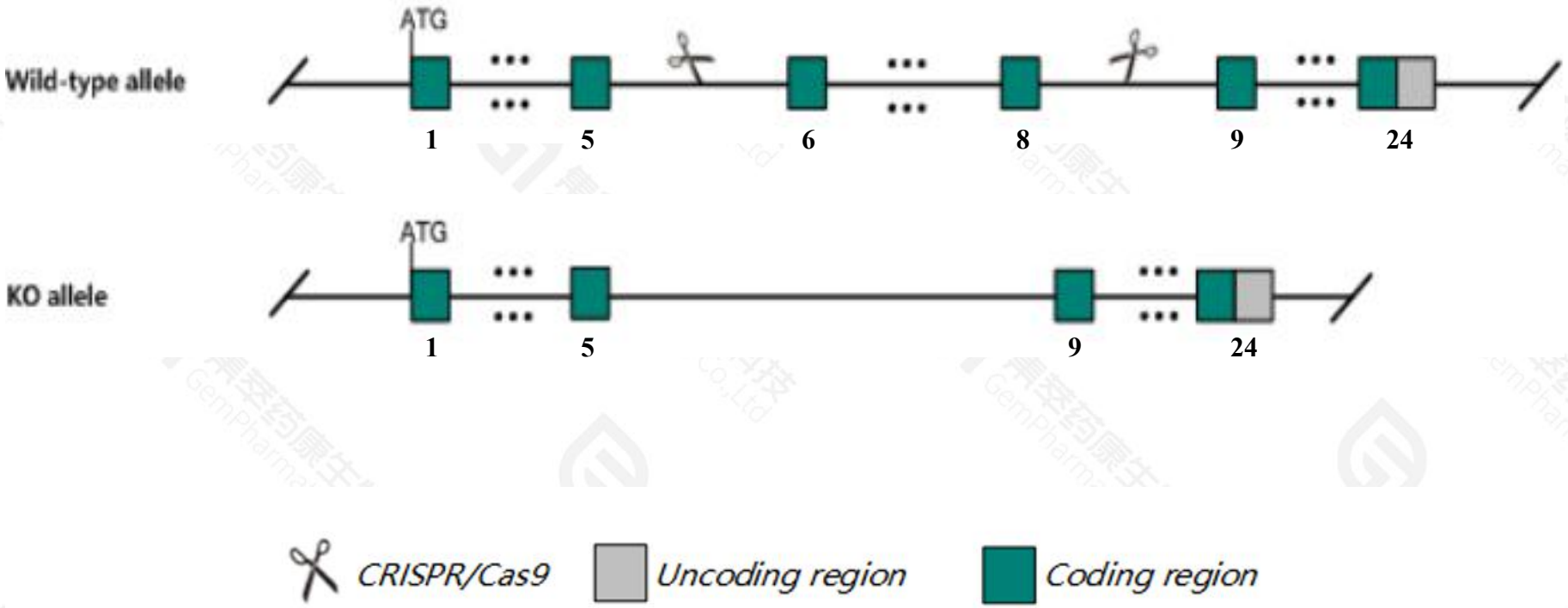
Design Date: 2021-5-27

Project Overview

Project Name	***Scn4a***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Scn4a* gene. The schematic diagram is as follows:



Technical routes

➢ The *Scn4a* gene has 2 transcripts. According to the structure of *Scn4a* gene, exon6-exon8 of *Scn4a-201*(ENSMUST00000021056.8) transcript is recommended as the knockout region.The region contains 521bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Scn4a* 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 heterozygous or homozygous for a knock-in allele develop myotonia, increased myofiber damage, K+-sensitive paralysis and susceptibility to delayed weakness during recovery from fatigue. Homozygotes show perinatal lethality, low survival rate, unusual hind-limb clasping and reduced body weight.

➢ The *Scn4a* gene is located on the Chr11. 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

Scn4a sodium channel, voltage-gated, type IV, alpha [Mus musculus (house mouse)]

Gene ID: 110880, updated on 17-Nov-2020

Summary

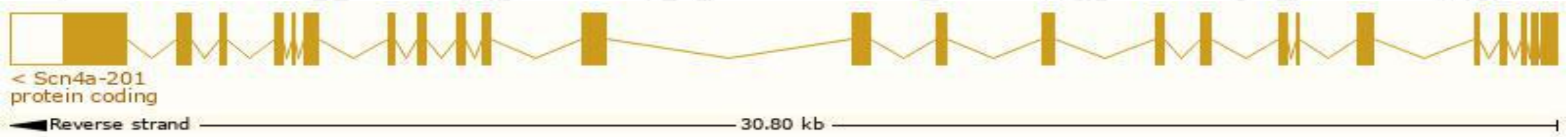
Official Symbol	Scn4a provided by MGI
Official Full Name	sodium channel, voltage-gated, type IV, alpha provided by MGI
Primary source	MGI:MGI:98250
See related	Ensembl:ENSMUSG00000001027
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Nav, Nav1.4, SkM, SkM1, mH2
Expression	Biased expression in mammary gland adult (RPKM 6.9), heart adult (RPKM 4.1) and 3 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

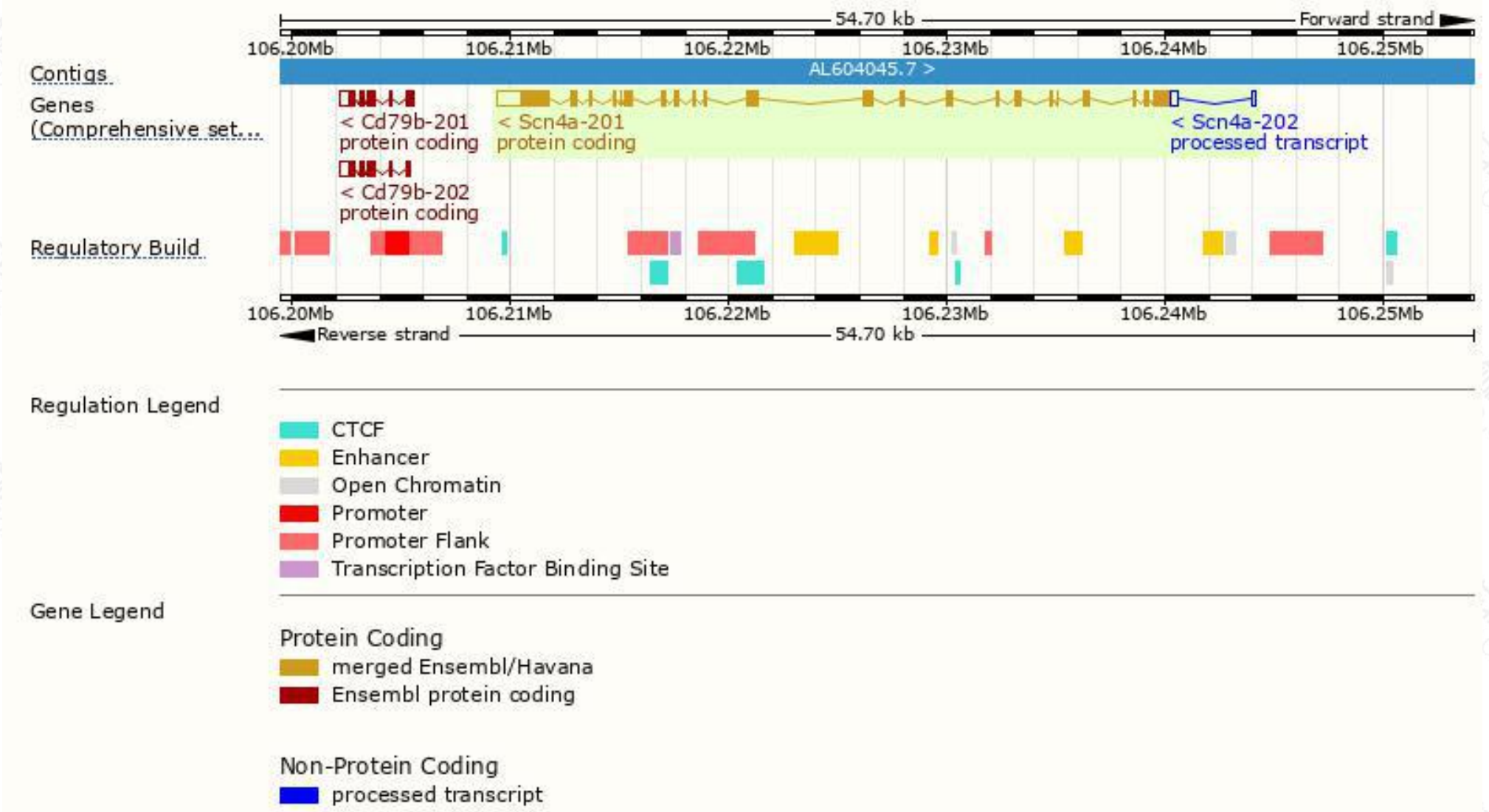
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Scn4a-201	ENSMUST00000021056.8	6598	1841aa	Protein coding	CCDS48961		TSL:1 , GENCODE basic , APPRIS P1 ,
Scn4a-202	ENSMUST00000174877.2	443	No protein	Processed transcript	-		TSL:2 ,

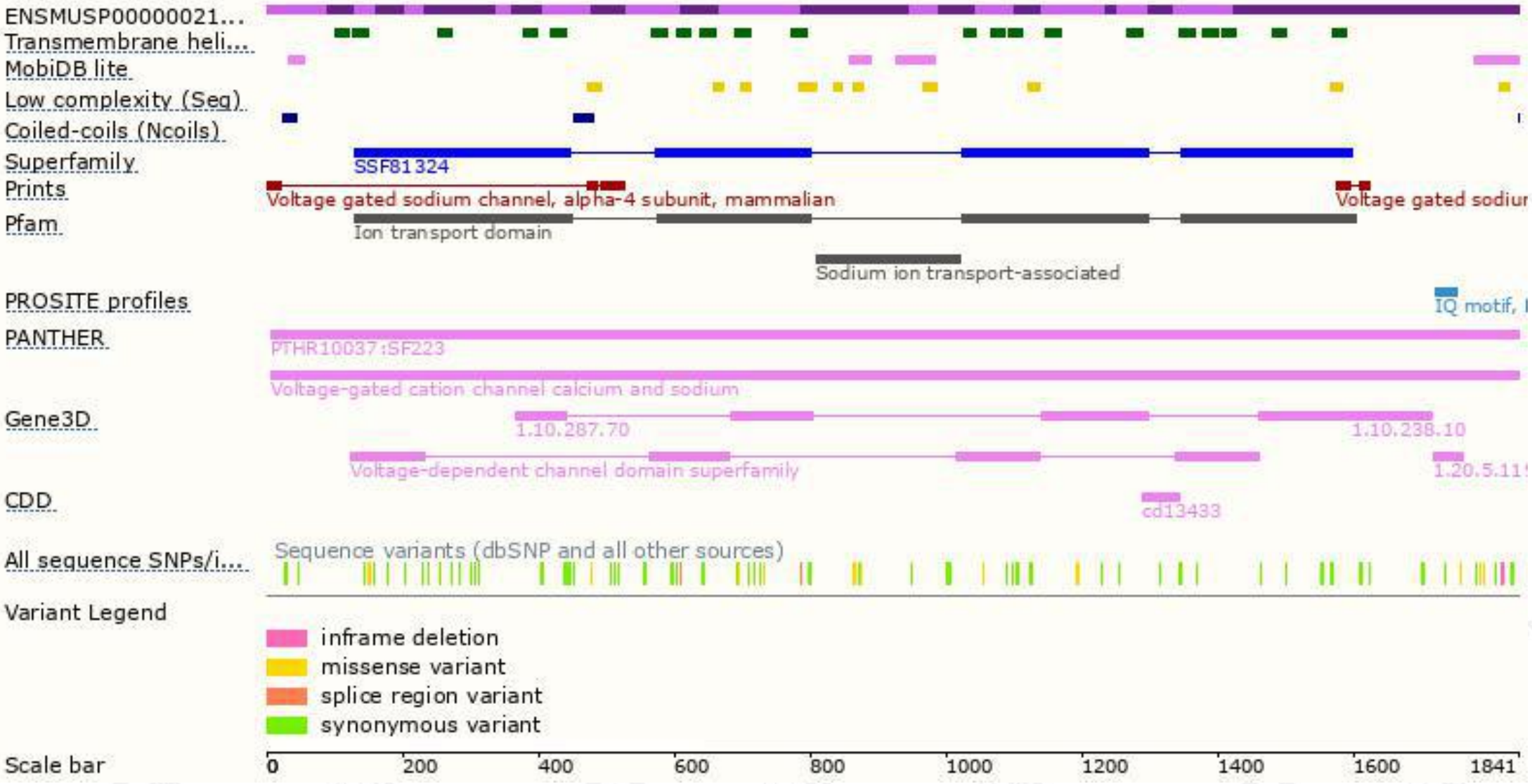
The strategy is based on the design of *Scn4a-201* transcript,the transcription is shown below:



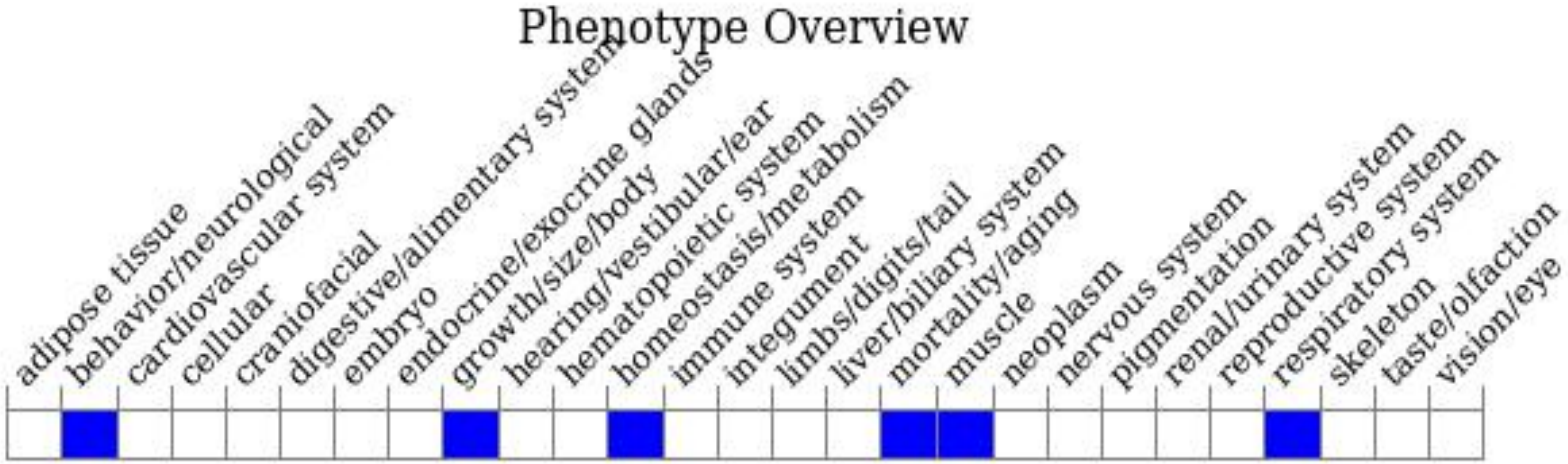
Genomic location distribution



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 heterozygous or homozygous for a knock-in allele develop myotonia, increased myofiber damage, K+-sensitive paralysis and susceptibility to delayed weakness during recovery from fatigue. Homozygotes show perinatal lethality, low survival rate, unusual hind-limb clasping and reduced body weight.

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