Kcna2 Cas9-CKO Strategy

Designer: Huan Wang

Reviewer: Yumeng Wang

Design Date: 2021-3-12

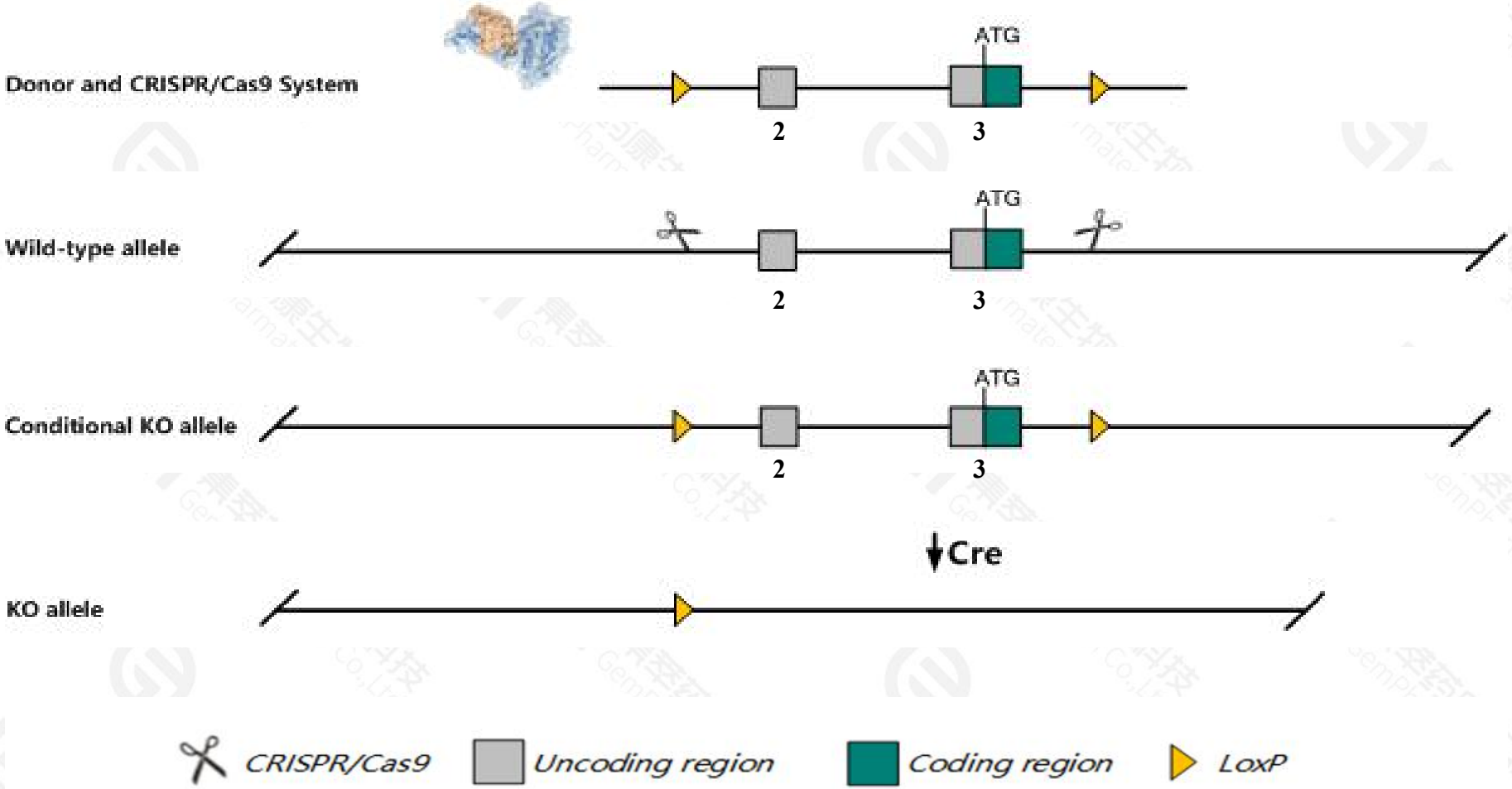
Project Overview

Project Name	***Kcna2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

集萃药康 GemPharmatech

This model will use CRISPR/Cas9 technology to edit the *Kcna2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Kcna2* gene has 3 transcripts. According to the structure of *Kcna2* gene, exon2-exon3 of *Kcna2-203*(ENSMUST00000197470.4) transcript is recommended as the knockout region.The region contains all of the coding sequence.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Kcna2* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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.

Notice

➢ According to the existing MGI data,mice homozygous for a null allele exhibit postnatal lethality, increased susceptibility to spontaneous and chemically-induced seizures and altered neuron electrophysiology. Mice homozygous for an ENU-induced allele exhibit abnormal gait, impaired coordination, and premature lethality.

➢ 5'loxp may affect the function of the *Kcna2* gene.

➢ The *Kcna2* gene is located on the Chr3. 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）

集萃药康 GemPharmatech

Kcna2 potassium voltage-gated channel, shaker-related subfamily, member 2 [Mus musculus (house mouse)]

Gene ID: 16490, updated on 13-Mar-2020

Summary

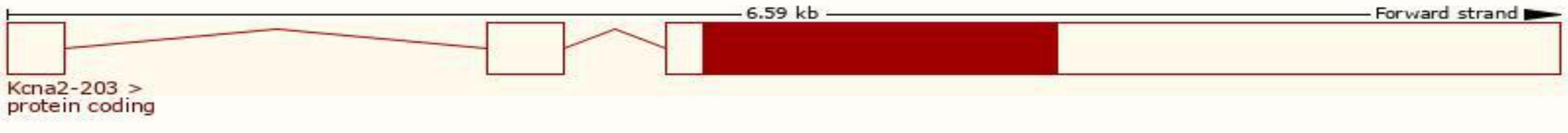
Official Symbol	Kcna2 provided by MGI
Official Full Name	potassium voltage-gated channel, shaker-related subfamily, member 2 provided by MGI
Primary source	MGI:MGI:96659
See related	Ensembl:ENSMUSG00000040724
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Akr6a4, Gm10672, Kca1-2, Kv1.2, Mk-2
Expression	Biased expression in cerebellum adult (RPKM 17.1), cortex adult (RPKM 9.8) and 5 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

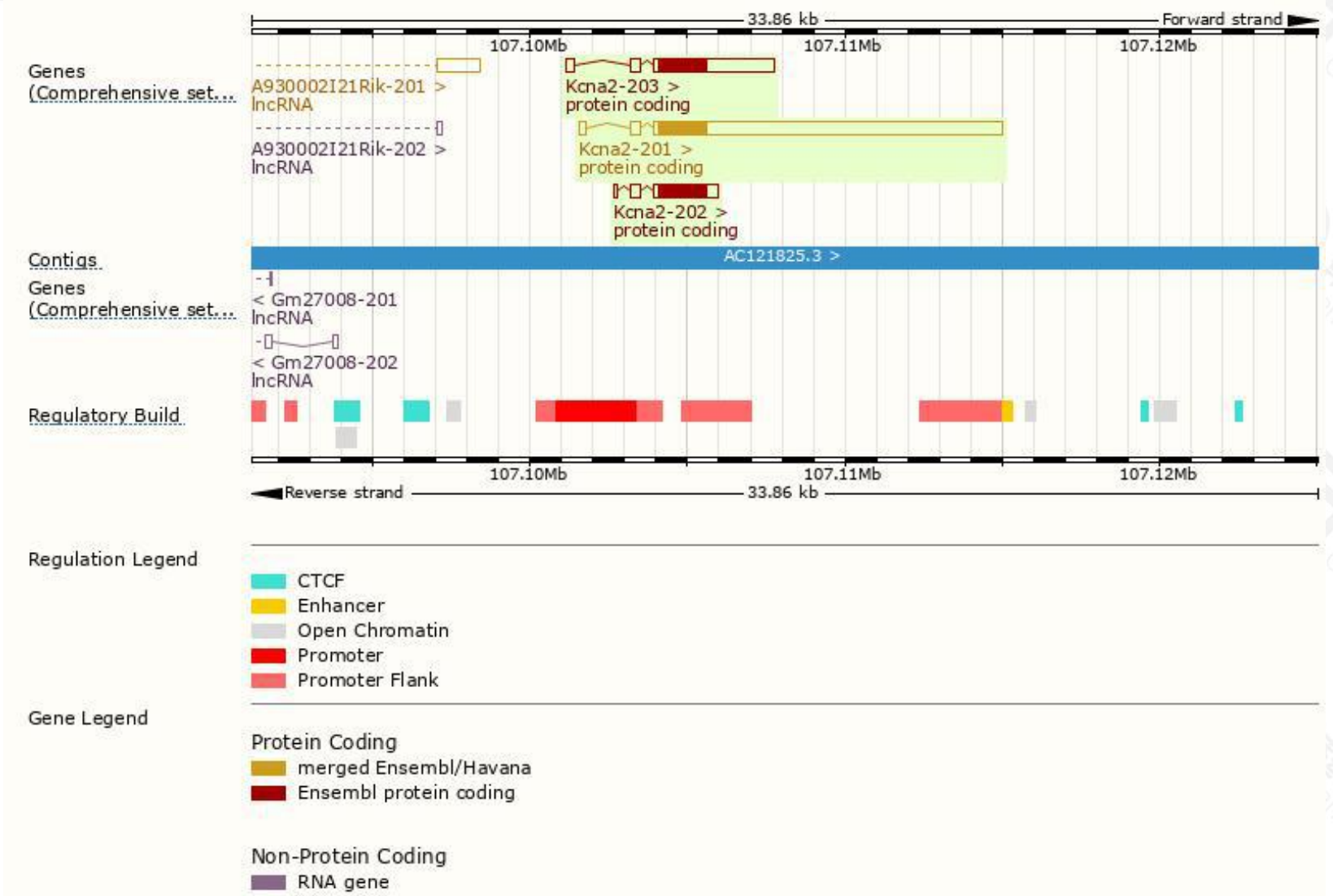
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Kcna2-201	ENSMUST00000038695.5	11582	499aa	Protein coding	CCDS17733	P63141	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
Kcna2-203	ENSMUST00000197470.4	4370	499aa	Protein coding	CCDS17733	P63141	TSL:5 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
Kcna2-202	ENSMUST00000196403.1	2430	499aa	Protein coding	CCDS17733	P63141	TSL:5 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

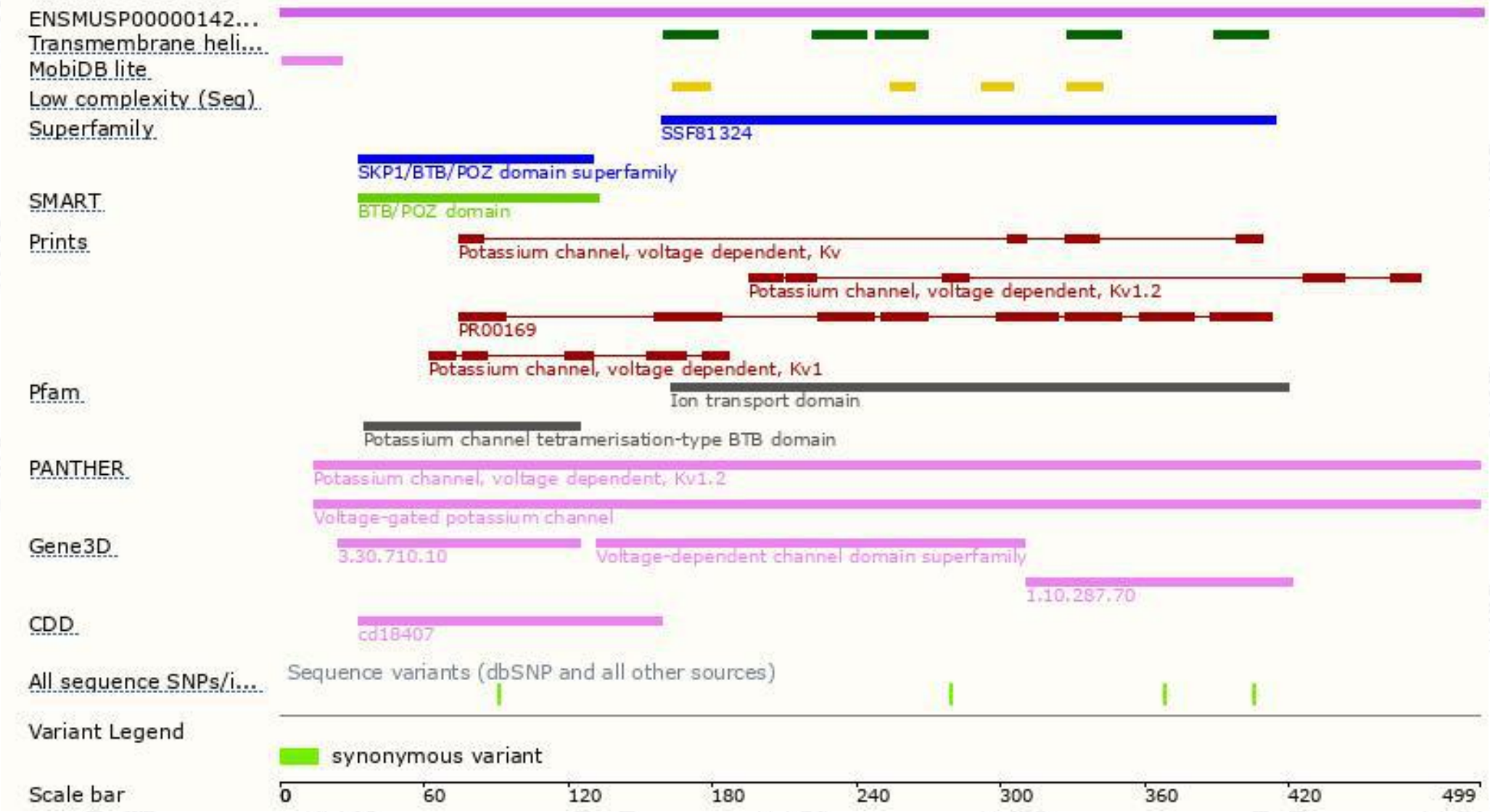
The strategy is based on the design of *Kcna2-203* transcript,the transcription is shown below:



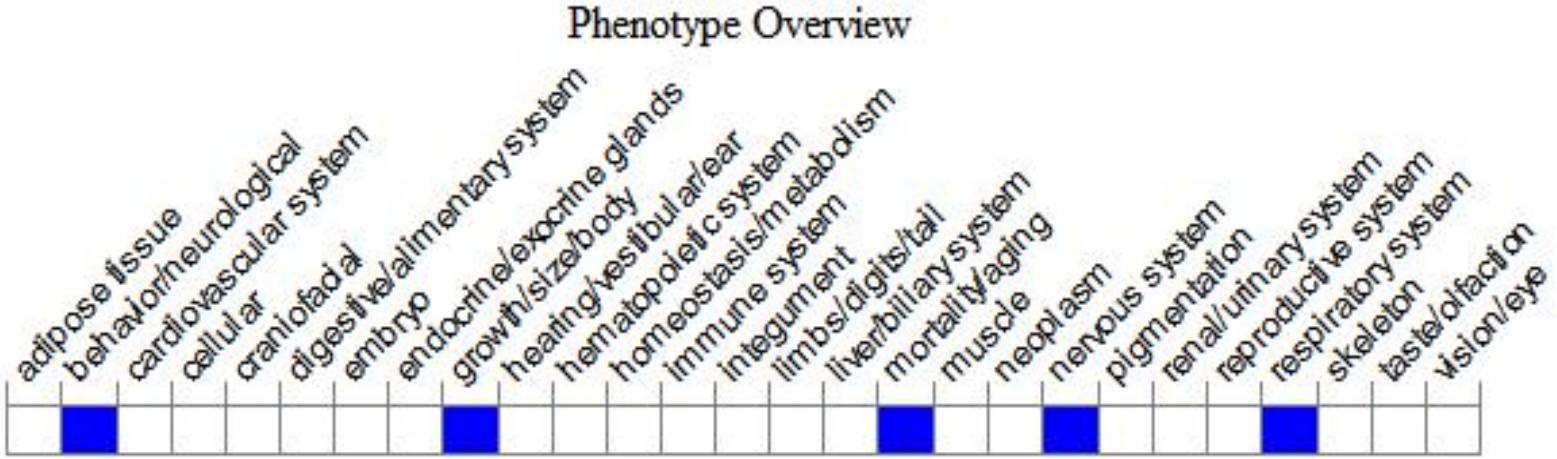
Genomic location distribution



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 exhibit postnatal lethality, increased susceptibility to spontaneous and chemically-induced seizures and altered neuron electrophysiology. Mice homozygous for an ENU-induced allele exhibit abnormal gait, impaired coordination, and premature lethality.

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