Glrb Cas9-KO Strategy

Designer: Yupeng Yang

Reviewer: Yun Li

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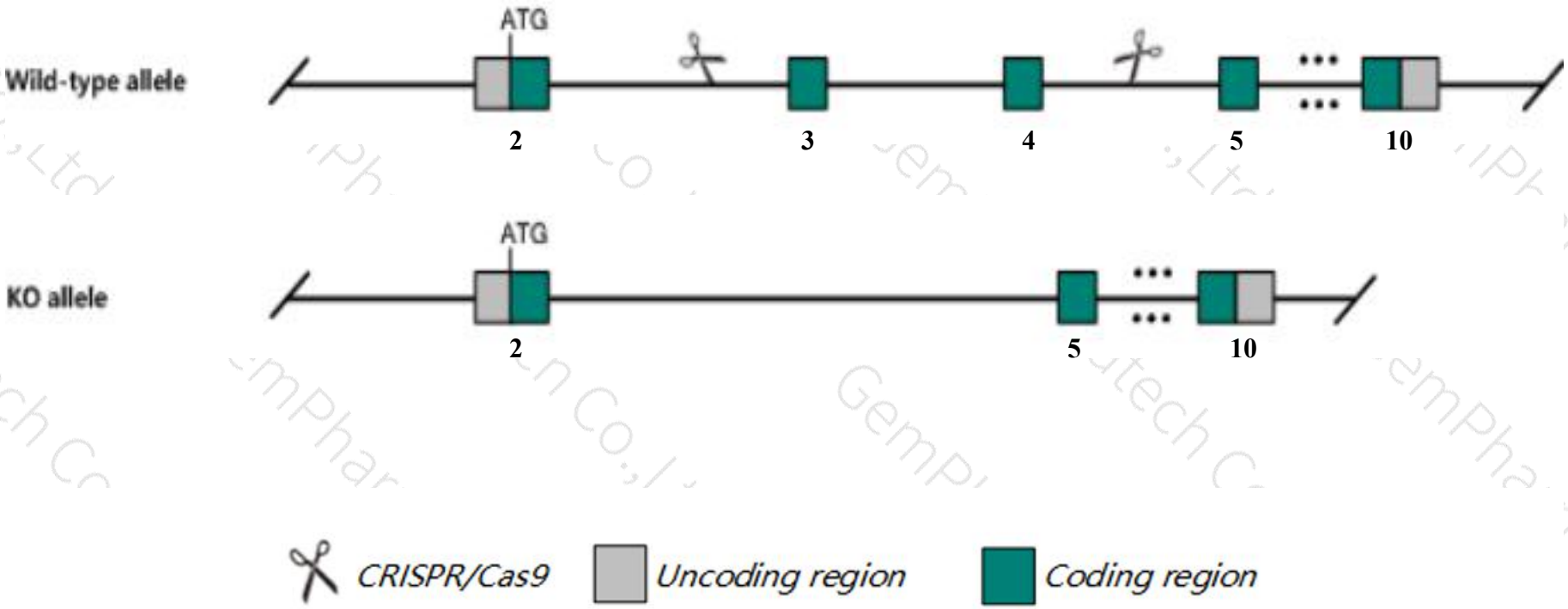
Project Overview

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

Knockout strategy

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



Technical routes

➢ The *Glrb* gene has 6 transcripts. According to the structure of *Glrb* gene, exon3-exon4 of *Glrb-201*(ENSMUST00000029654.14) transcript is recommended as the knockout region.The region contains 175bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Glrb* 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,mutations in this gene result in a neurological disorder and premature death.

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

Gene information（NCBI）

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Glrb glycine receptor, beta subunit [Mus musculus (house mouse)]

Gene ID: 14658, updated on 13-Mar-2020

Summary

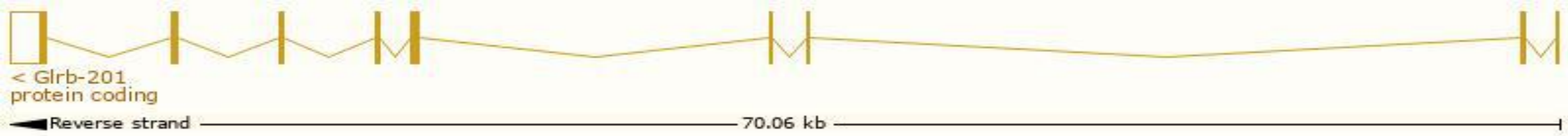
Official Symbol	Glrb provided by MGI
Official Full Name	glycine receptor, beta subunit provided by MGI
Primary source	MGI:MGI:95751
See related	Ensembl:ENSMUSG00000028020
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI853901, Glyrb, spa, spastic
Summary	This gene encodes the beta subunit of the glycine receptor, which is a pentamer composed of alpha and beta subunits. The receptor functions as a neurotransmitter-gated ion channel, which produces hyperpolarization via increased chloride conductance due to the binding of glycine to the receptor. This gene is transcribed throughout the central nervous system of neonatal and adult mice. In humans, mutations in this gene cause startle disease, also known as hereditary hyperekplexia or congenital stiff-person syndrome, a disease characterized by muscular rigidity. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Sep 2016]
Expression	Biased expression in cerebellum adult (RPKM 42.8), cortex adult (RPKM 30.7) and 5 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

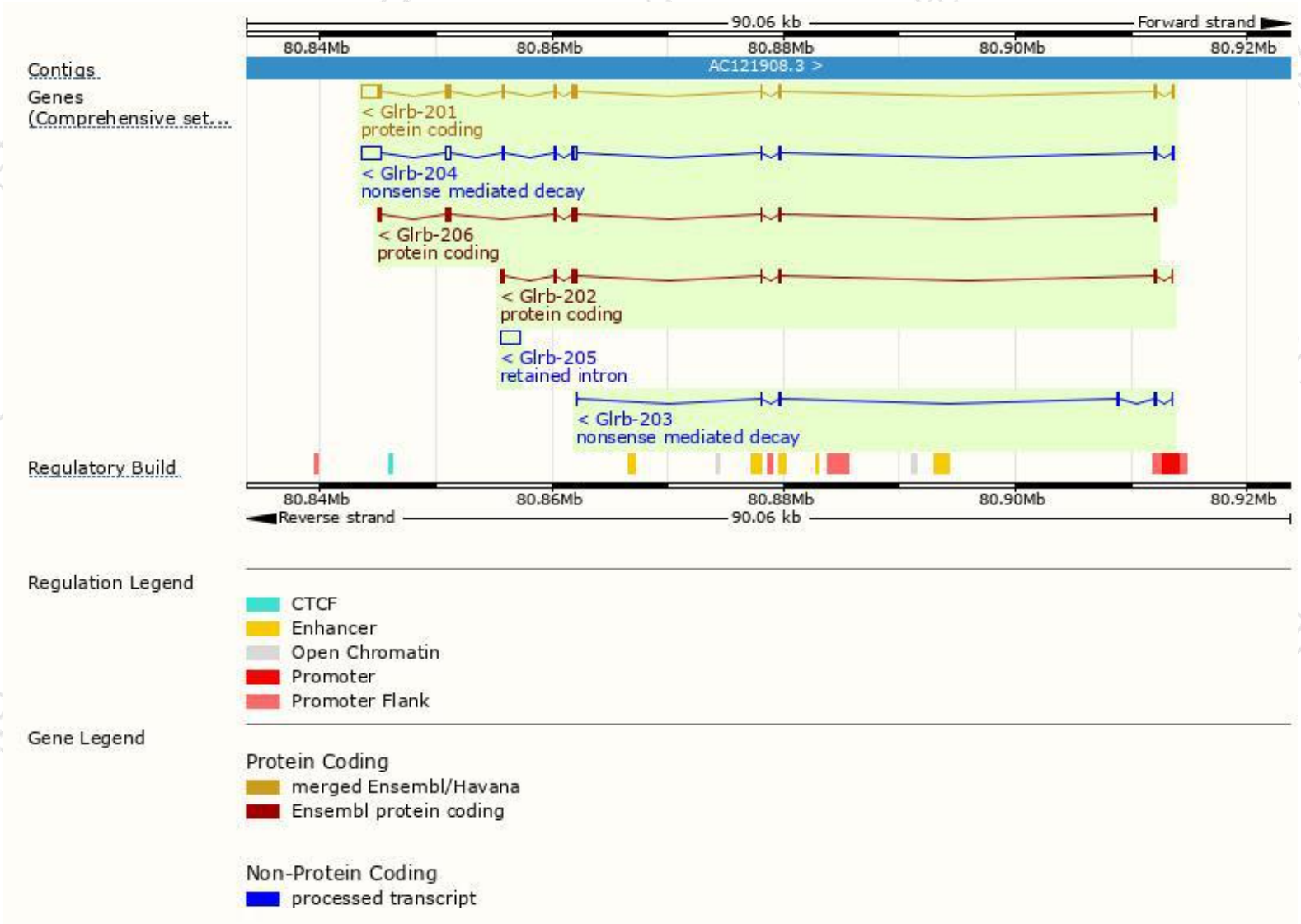
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Glrb-201	ENSMUST00000029654.14	3029	496aa	Protein coding	CCDS17424	P48168	TSL:1 GENCODE basic APPRIS P1
Glrb-206	ENSMUST00000194085.5	1341	445aa	Protein coding	-	A1KR23	TSL:1 GENCODE basic
Glrb-202	ENSMUST00000107743.7	1101	307aa	Protein coding	-	E9Q3E3	TSL:5 GENCODE basic
Glrb-204	ENSMUST00000135043.7	2979	58aa	Nonsense mediated decay	-	E9Q9K1	TSL:1
Glrb-203	ENSMUST00000132330.1	563	57aa	Nonsense mediated decay	-	E9PV81	TSL:5
Glrb-205	ENSMUST00000193031.1	1620	No protein	Retained intron	-	-	TSL:NA

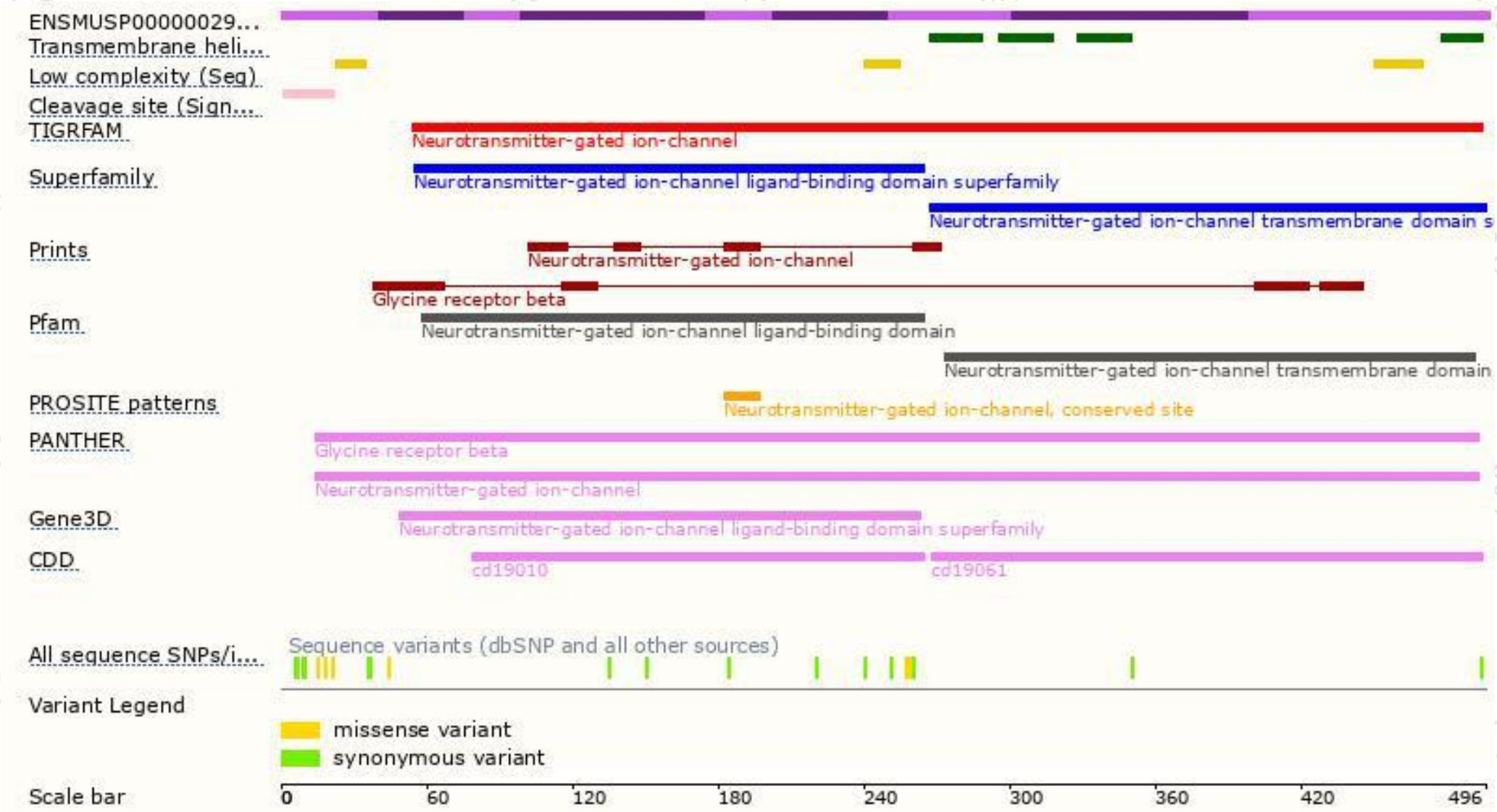
The strategy is based on the design of *Glrb-201* transcript,the transcription is shown below:



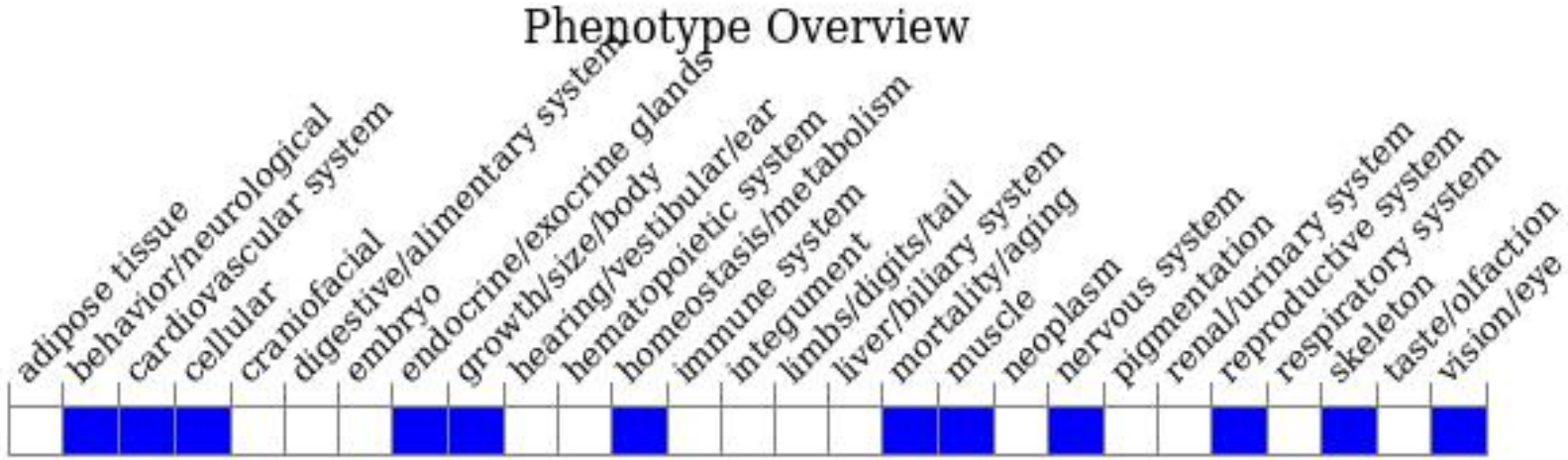
Genomic location distribution



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,mutations in this gene result in a neurological disorder and premature death.

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd