Ap3b2 Cas9-CKO Strategy

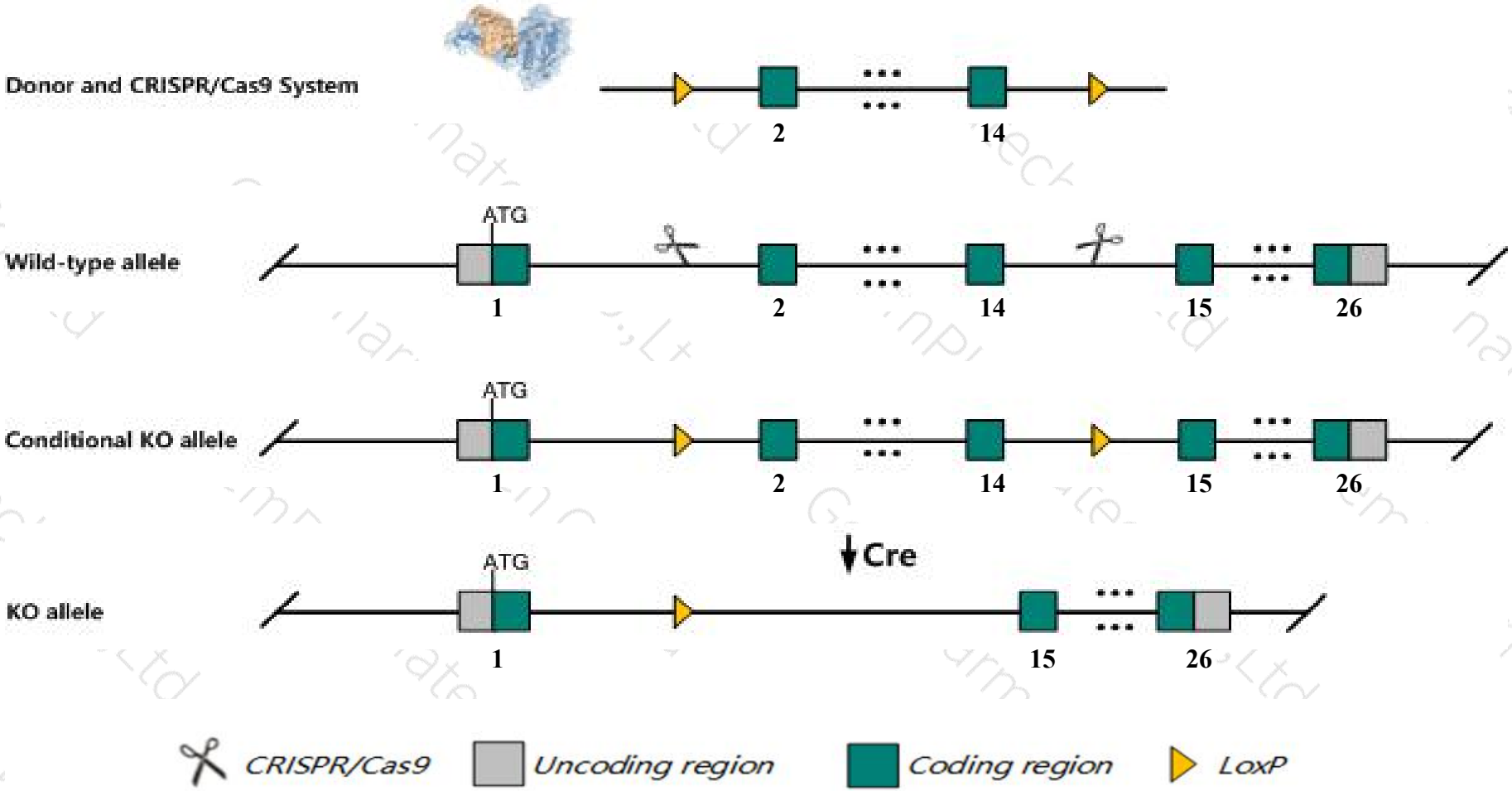
Designer: Xueting Zhang
Reviewer:Yanhua Shen
Date:2020-02-14

Project Overview

Project Name	***Ap3b2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ap3b2* gene. The schematic diagram is as follows:



Technical routes

- The *Ap3b2* gene has 9 transcripts. According to the structure of *Ap3b2* gene, exon2-exon14 of *Ap3b2-201* (ENSMUST00000082090.14) transcript is recommended as the knockout region.The region contains 1552bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Ap3b2* 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.

Notice

- According to the existing MGI data,Disruption does not alter pigmentation, but causes hyperactivity and tonic-clonic seizures and mice homozygous for a knock-out allele were found to have significantly reduced synaptic zinc levels throughout the brain, with the largest reduction observed in the CA1 stratum oriens.
- Transcript *Ap3b2*-208 may not be affected.
- The effect on transcript *Ap3b2*-204&206 is unknown.
- The *Ap3b2* 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）

集萃药康 GemPharmatech

Ap3b2 adaptor-related protein complex 3, beta 2 subunit [*Mus musculus* (house mouse)]

Gene ID: 11775, updated on 9-Feb-2020

Summary

Official Symbol	Ap3b2 provided by MGI
Official Full Name	adaptor-related protein complex 3, beta 2 subunit provided by MGI
Primary source	MGI:MGI:1100869
See related	Ensembl:ENSMUSG00000062444
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	Naptb; beta3B; [b]-NAP; AI549966; AU042881; beta-NAP
Expression	Biased expression in CNS E18 (RPKM 43.3), whole brain E14.5 (RPKM 36.7) and 9 other tissues See more
Orthologs	human all

Genomic context

Location: 7 D3; 7 45.71 cM

See Ap3b2 in Genome Data Viewer

Exon count: 27

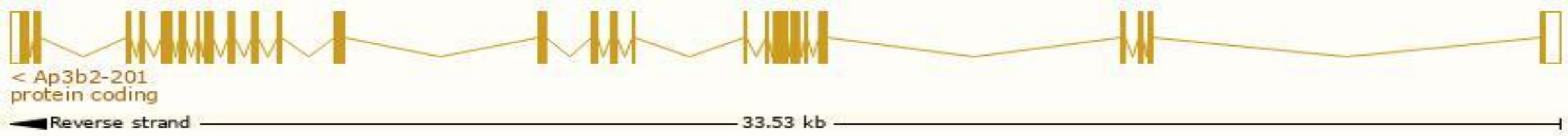
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	7	NC_000073.6 (81460399..81493997, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	7	NC_000073.5 (88605285..88638811, complement)

Transcript information（Ensembl）

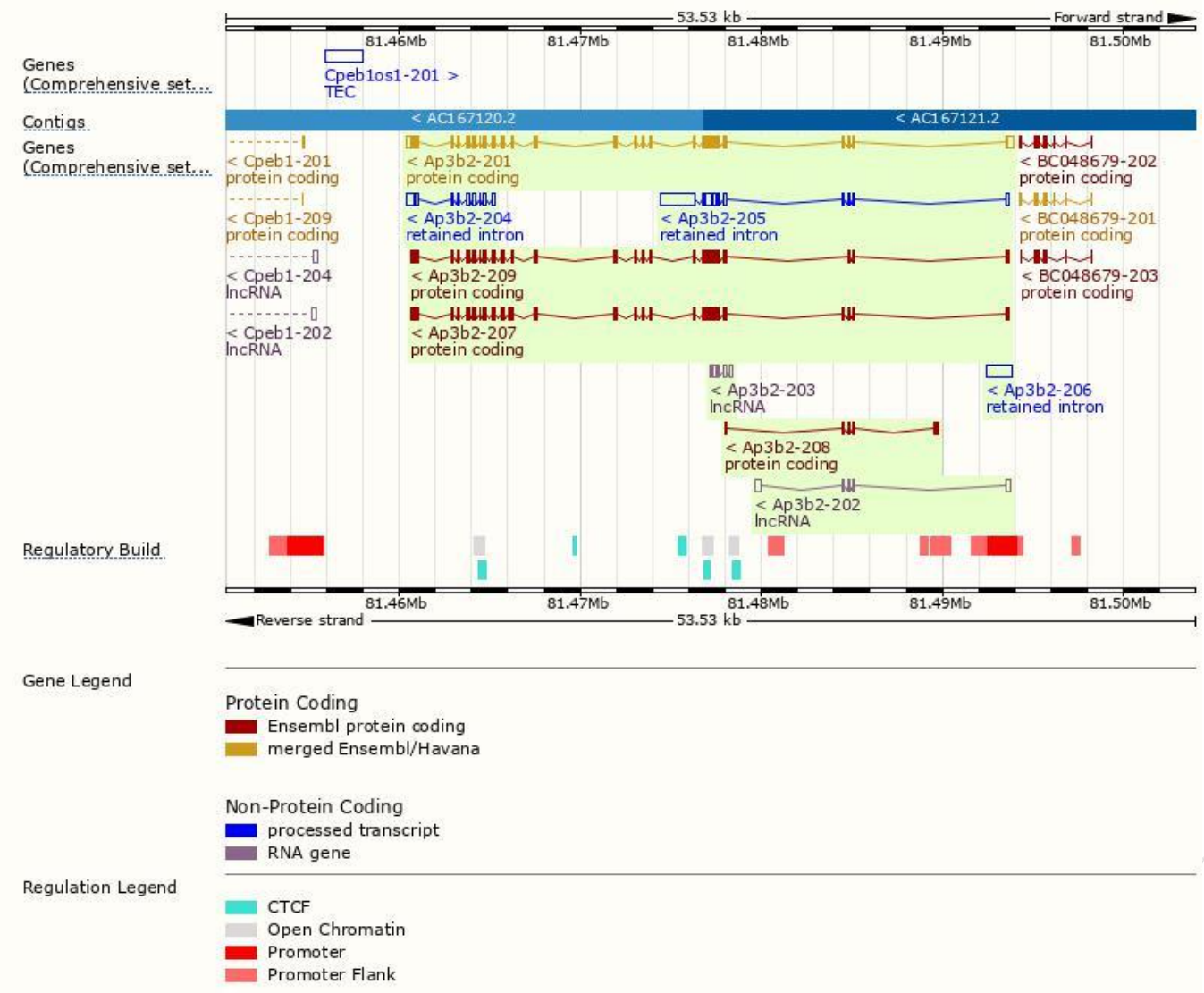
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ap3b2-201	ENSMUST00000082090.14	3806	1082aa	Protein coding	CCDS21403	Q9JME5	TSL:1 GENCODE basic APPRIS P2
Ap3b2-207	ENSMUST00000238438.1	3383	1101aa	Protein coding	-	-	GENCODE basic APPRIS ALT2
Ap3b2-209	ENSMUST00000238711.1	3230	1050aa	Protein coding	-	-	GENCODE basic
Ap3b2-208	ENSMUST00000238692.1	629	199aa	Protein coding	-	-	CDS 3' incomplete
Ap3b2-205	ENSMUST00000152355.8	3161	No protein	Retained intron	-	-	TSL:1
Ap3b2-204	ENSMUST00000147624.1	1459	No protein	Retained intron	-	-	TSL:1
Ap3b2-206	ENSMUST00000208911.1	1430	No protein	Retained intron	-	-	TSL:NA
Ap3b2-202	ENSMUST00000119121.1	875	No protein	lncRNA	-	-	TSL:1
Ap3b2-203	ENSMUST00000125634.1	715	No protein	lncRNA	-	-	TSL:3

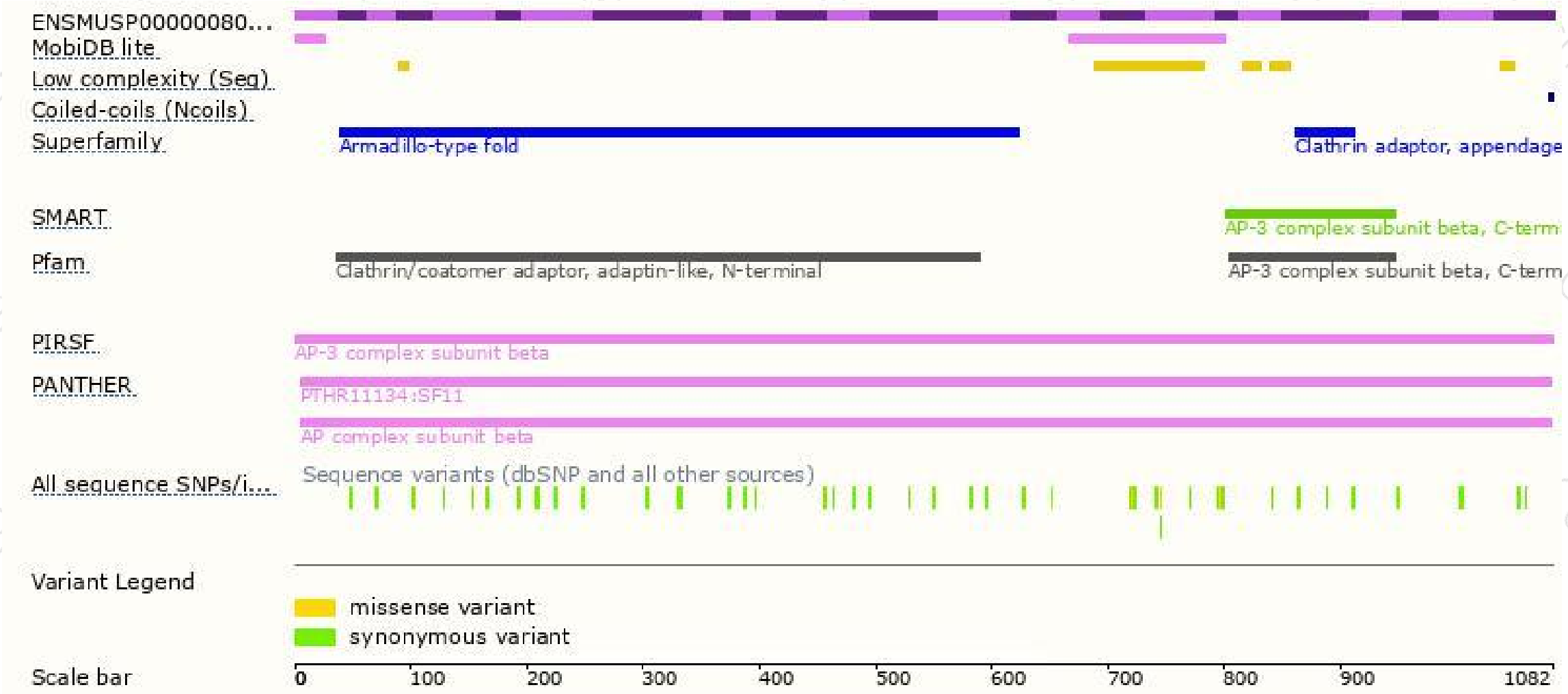
The strategy is based on the design of *Ap3b2-201* transcript,The transcription is shown below



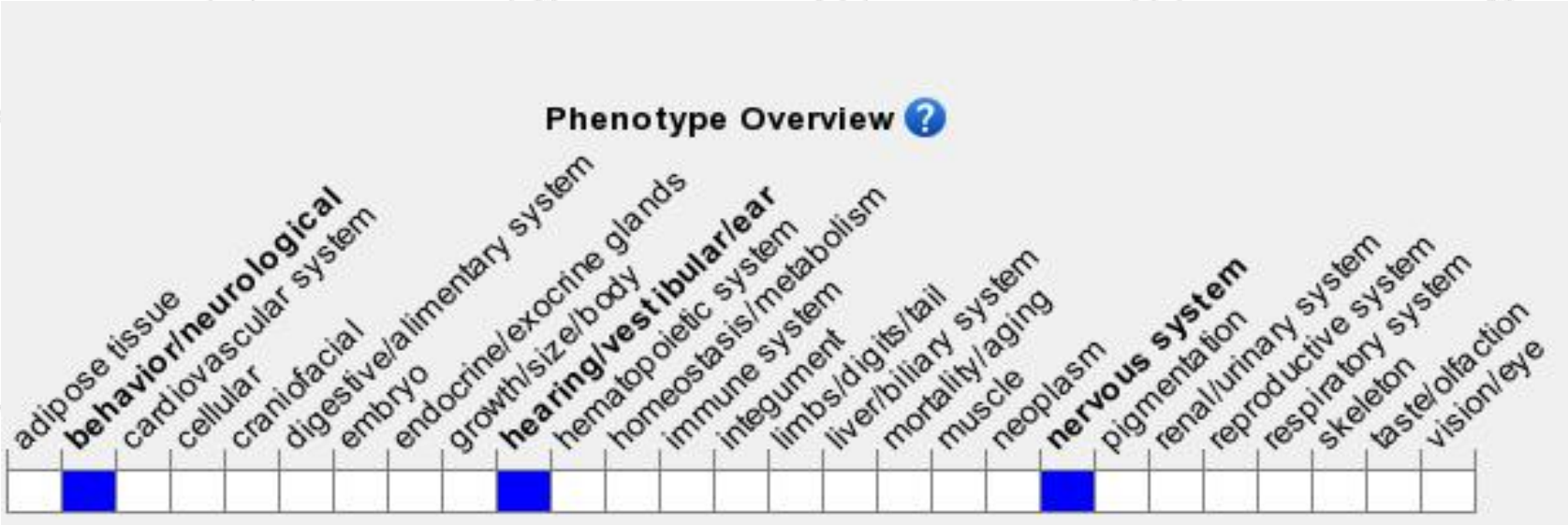
Genomic location distribution



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,Disruption does not alter pigmentation, but causes hyperactivity and tonic-clonic seizures and mice homozygous for a knock-out allele were found to have significantly reduced synaptic zinc levels throughout the brain, with the largest reduction observed in the CA1 stratum oriens.

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