Clcn3 Cas9-CKO Strategy

Designer: Huimin Su

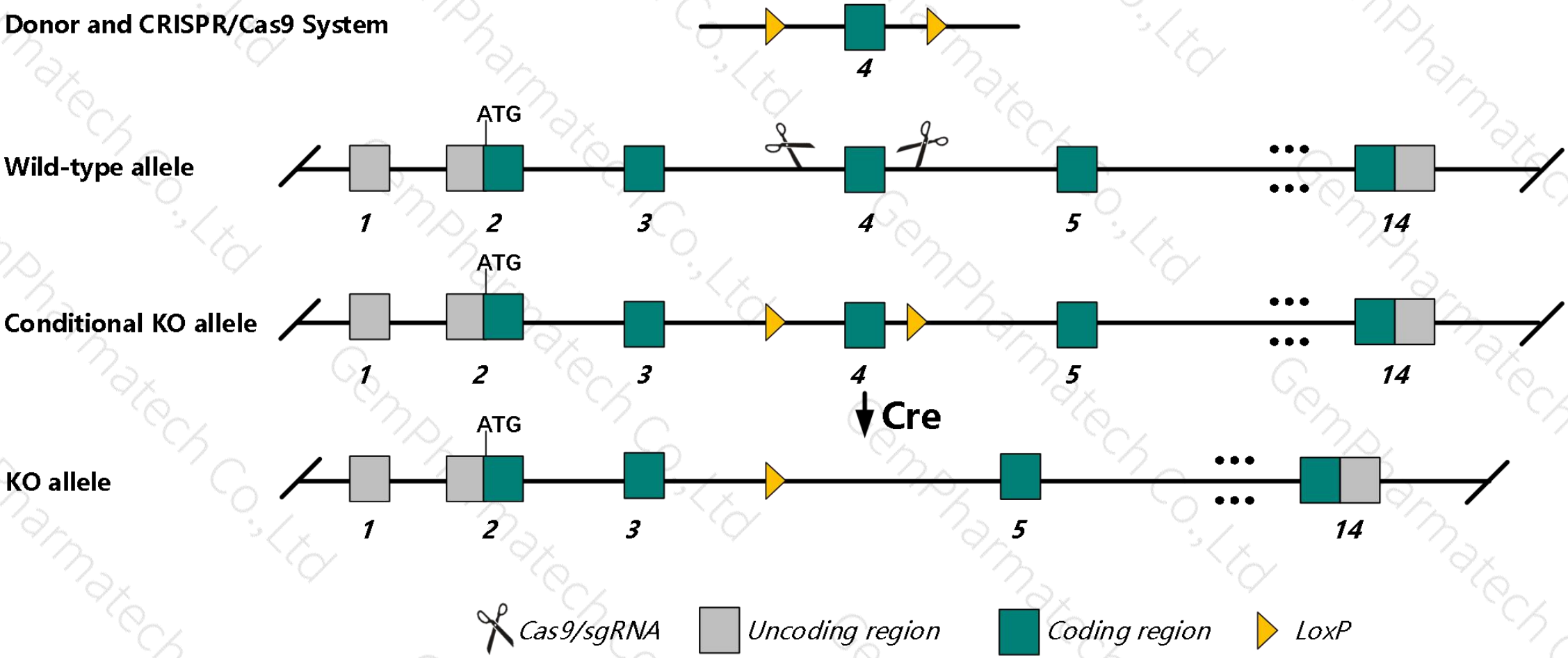
Project Overview

Project Name	***Clcn3***
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 *Clcn3* gene. The schematic diagram is as follows:



Technical routes

- The *Clcn3* gene has 10 transcripts. According to the structure of *Clcn3* gene, exon4 of *Clcn3-201* (ENSMUST00000004430.13) transcript is recommended as the knockout region.The region contains 100bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Clcn3* 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,Nullizygous mutations cause degeneration of hippocampal neurons and retinal photoreceptors, reduced body weight, behavioral deficits, gliosis, kyphosis and premature death, and may alter male fertility, ileum morphology, liver physiology, seizure susceptibility, and behavioral response to drugs.

- The *Clcn3* 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 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

Clcn3 chloride channel, voltage-sensitive 3 [Mus musculus (house mouse)]

Gene ID: 12725, updated on 28-Mar-2019

Summary

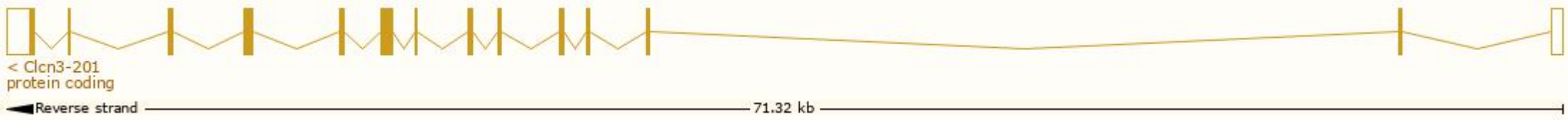
Official Symbol	Clcn3 provided by MGI
Official Full Name	chloride channel, voltage-sensitive 3 provided by MGI
Primary source	MGI:MGI:103555
See related	Ensembl:ENSMUSG00000004319
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Clc3
Expression	Ubiquitous expression in cerebellum adult (RPKM 11.4), frontal lobe adult (RPKM 10.6) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

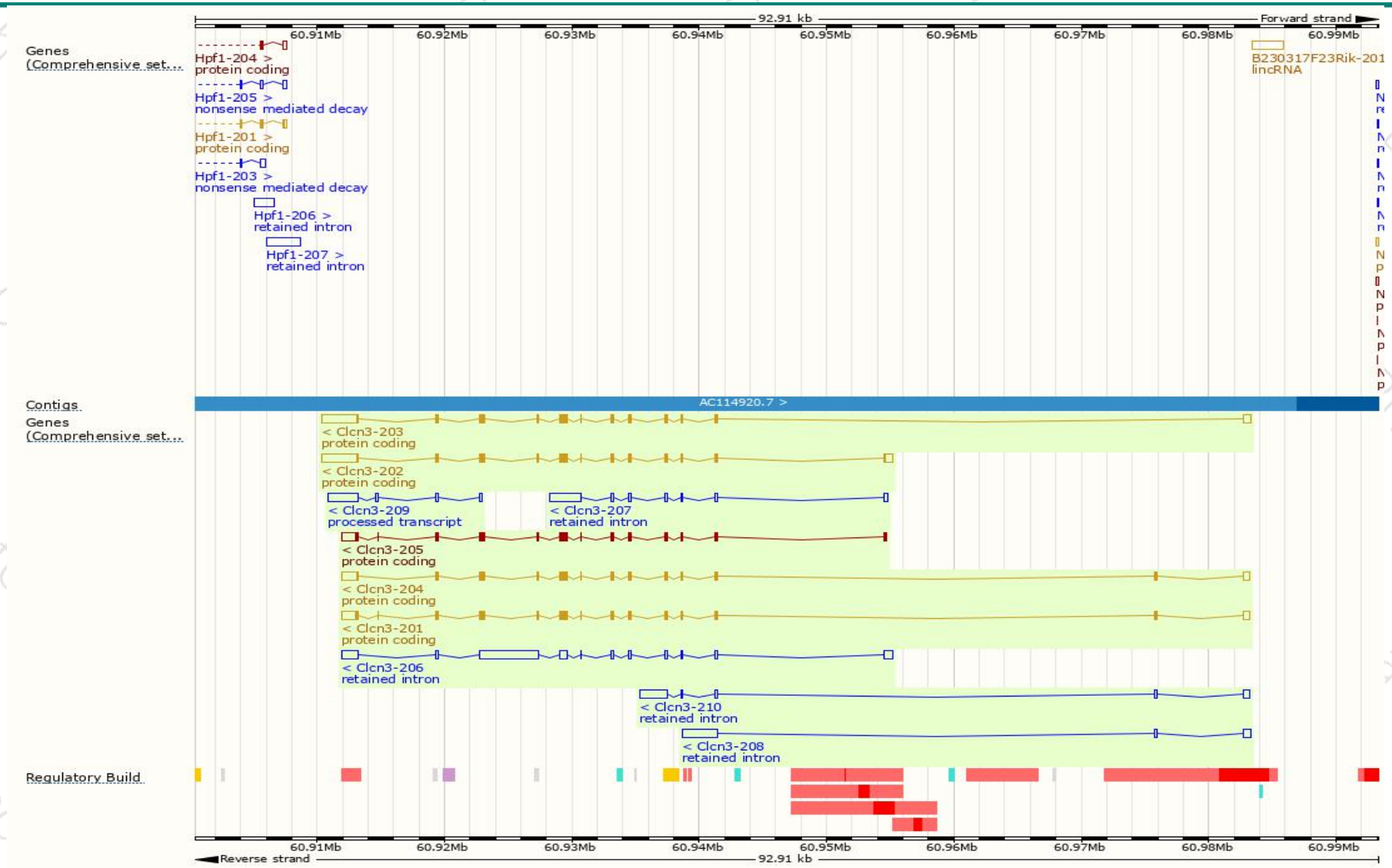
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Clcn3-202	ENSMUST00000056508.11	5684	791aa	Protein coding	CCDS22322	Q8K4W8	TSL:1 GENCODE basic APPRIS P3
Clcn3-203	ENSMUST00000093490.8	5518	760aa	Protein coding	CCDS22323	P51791 Q790S0	TSL:1 GENCODE basic
Clcn3-201	ENSMUST00000004430.13	4175	866aa	Protein coding	CCDS52555	Q3TF45	TSL:1 GENCODE basic APPRIS ALT1
Clcn3-204	ENSMUST00000110301.1	4099	818aa	Protein coding	CCDS52554	P51791	TSL:1 GENCODE basic APPRIS ALT1
Clcn3-205	ENSMUST00000110302.7	3768	839aa	Protein coding	-	E9Q2I1	TSL:5 GENCODE basic APPRIS ALT1
Clcn3-209	ENSMUST00000145741.7	2954	No protein	Processed transcript	-	-	TSL:3
Clcn3-206	ENSMUST00000129672.7	8193	No protein	Retained intron	-	-	TSL:1
Clcn3-207	ENSMUST00000132234.7	3480	No protein	Retained intron	-	-	TSL:1
Clcn3-208	ENSMUST00000145493.1	3462	No protein	Retained intron	-	-	TSL:1
Clcn3-210	ENSMUST00000147824.7	3034	No protein	Retained intron	-	-	TSL:1

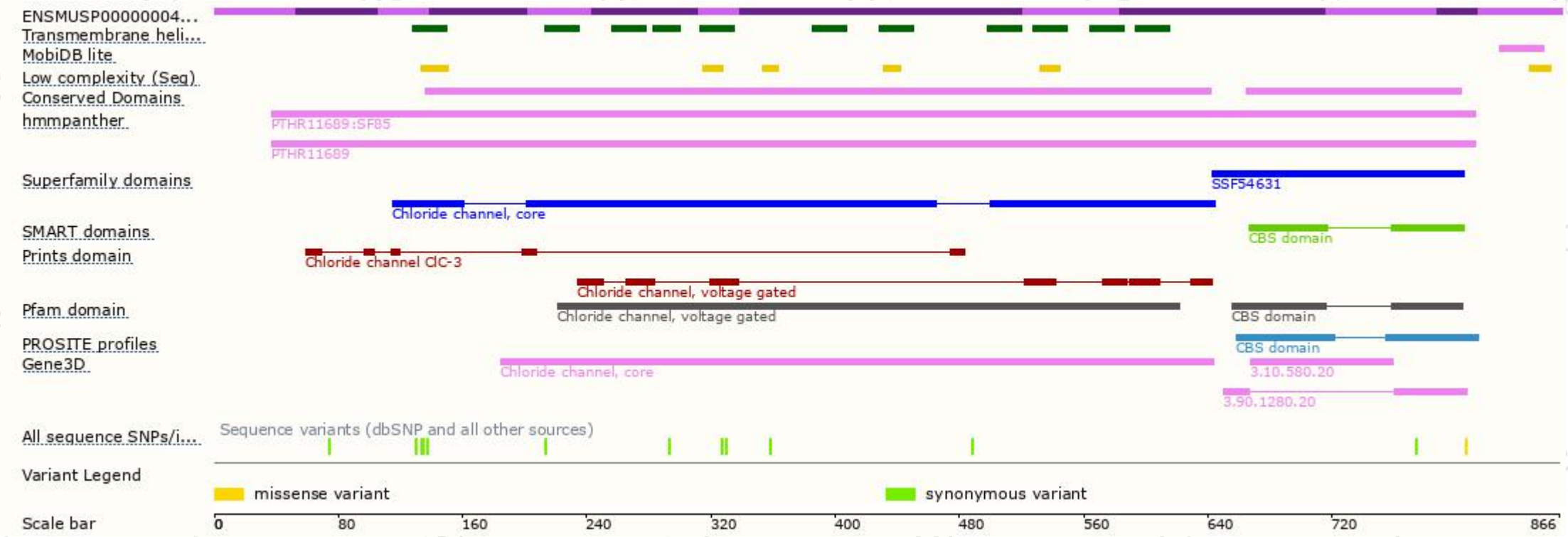
The strategy is based on the design of *Clcn3-201* transcript,The transcription is shown below



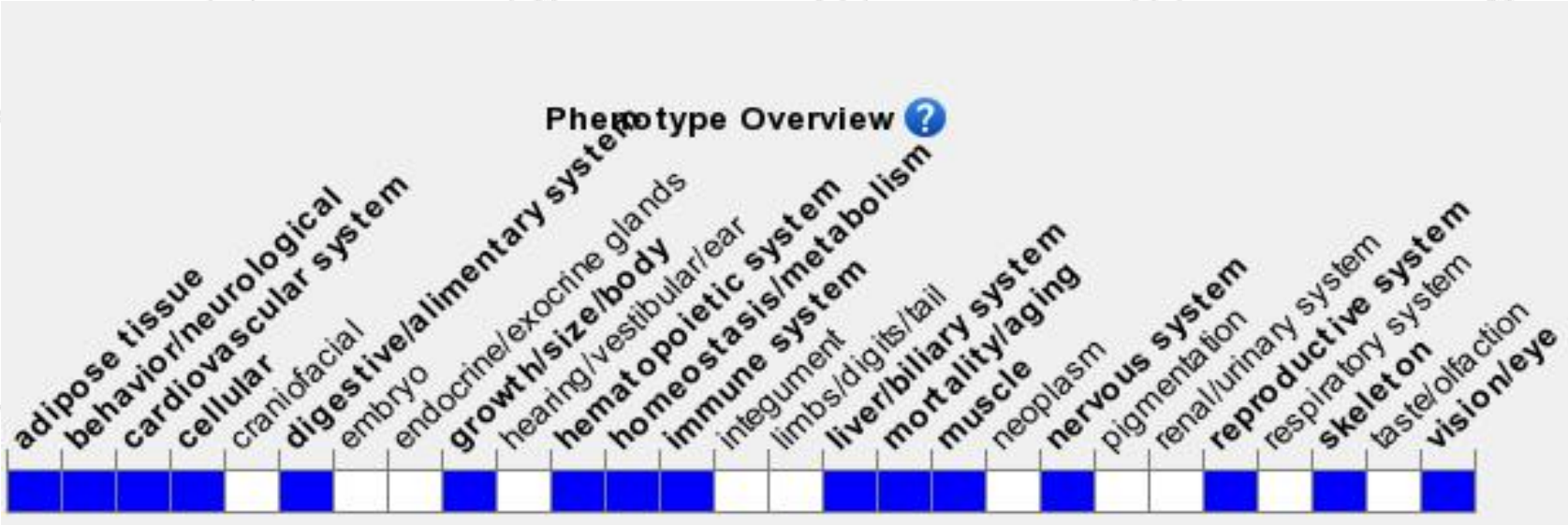
Genomic location distribution



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,Nullizygous mutations cause degeneration of hippocampal neurons and retinal photoreceptors, reduced body weight, behavioral deficits, gliosis, kyphosis and premature death, and may alter male fertility, ileum morphology, liver physiology, seizure susceptibility, and behavioral response to drugs.

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