Lhx2 Cas9-CKO Strategy

Designer:Shuang Zhang

Reviewer: Yun Li

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Project Overview

Project Name	***Lhx2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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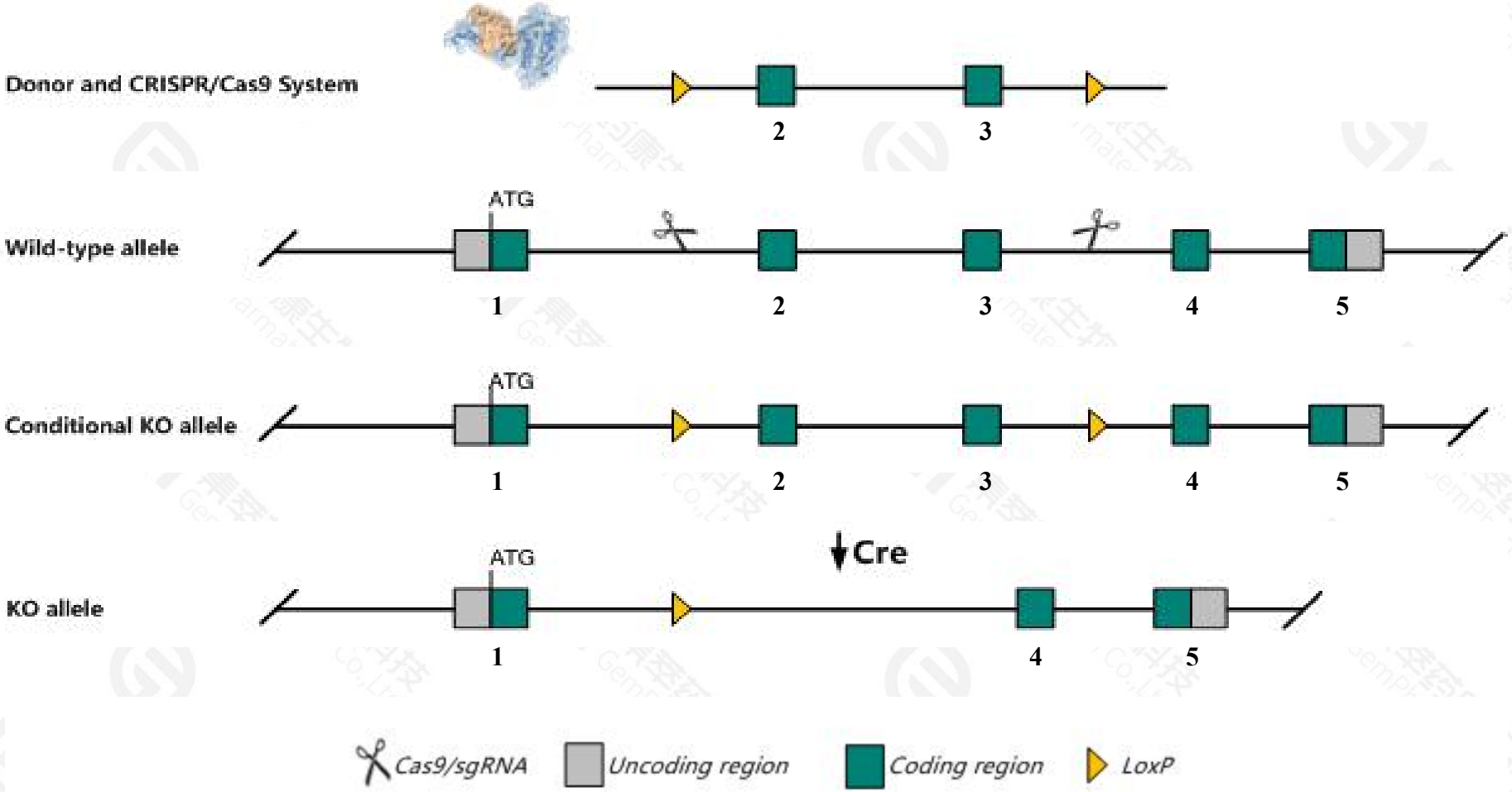
This model will use CRISPR/Cas9 technology to edit the *Lhx2* gene. The schematic diagram is as follows:



Technical routes

The *Lhx2* gene has 7 transcripts. According to the structure of *Lhx2* gene, exon2-exon3 of *Lhx2-201*(ENSMUST00000000253.6) transcript is recommended as the knockout region.The region contains 607bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Lhx2* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor was constructed.Cas9, sgRNA 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 was 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 knock-out allele exhibit lethality during fetal development and the perinatal period with abnormal liver, telencephalon, olfactory bulb, basal ganglion, and eye morphology.

The *Lhx2* gene is located on the Chr2. 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

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Lhx2 LIM homeobox protein 2 [Mus musculus (house mouse)]

Gene ID: 16870, updated on 12-Feb-2021

Summary

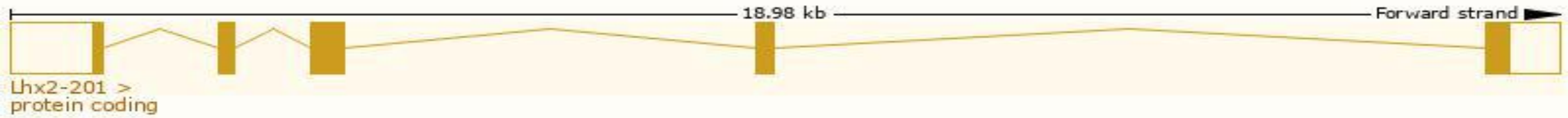
Official Symbol	Lhx2 provided by MGI
Official Full Name	LIM homeobox protein 2 provided by MGI
Primary source	MGI:MGI:96785
See related	Ensembl:ENSMUSG00000000247
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	LH, LH2A, Lh-, Lh-2, Lim2, ap, apt, apterous
Expression	Biased expression in CNS E14 (RPKM 42.9), whole brain E14.5 (RPKM 35.2) and 6 other tissues See more
Orthologs	human all

Transcript information Ensembl

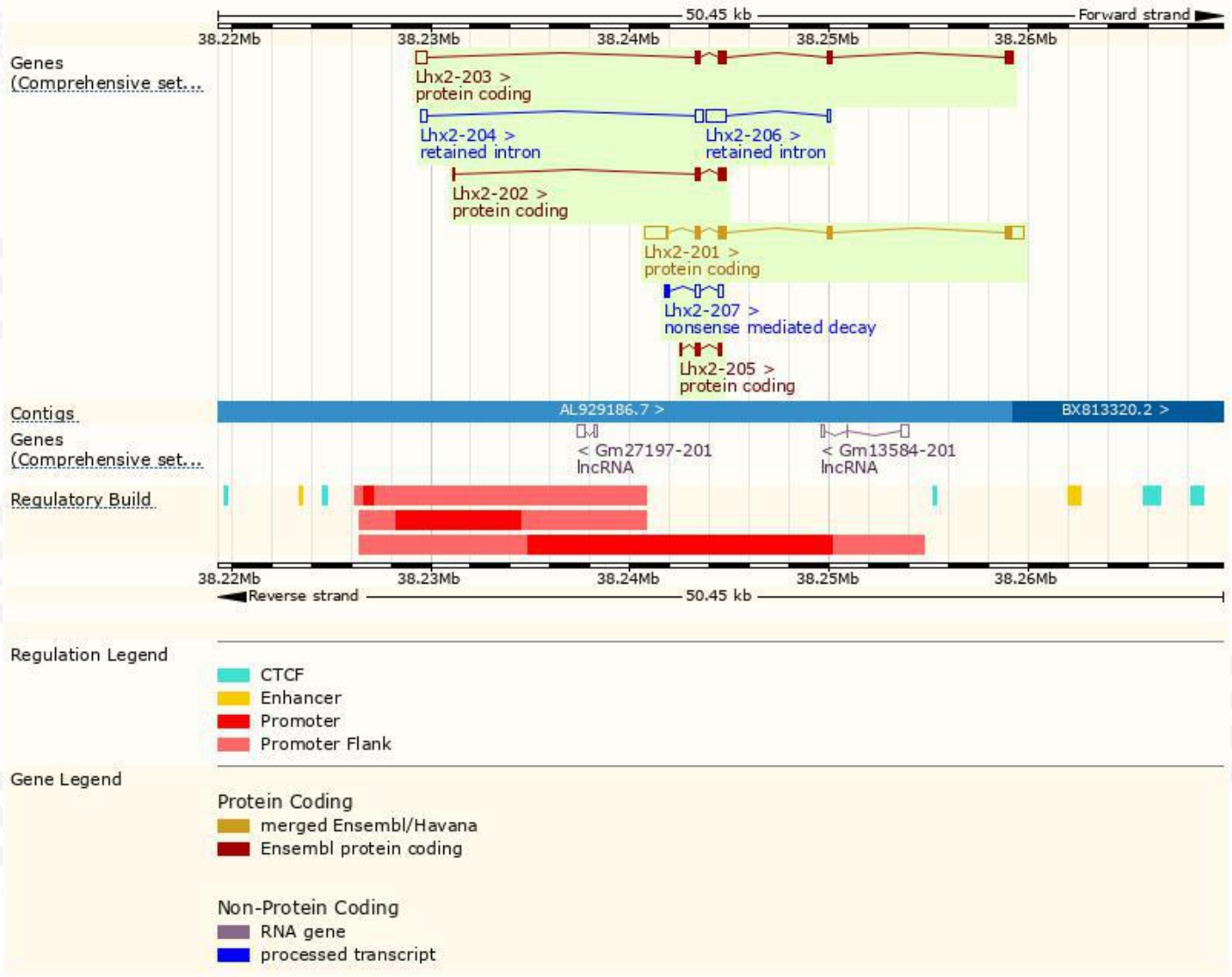
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lhx2-201	ENSMUST00000000253.6	2840	406aa	Protein coding	CCDS16008		TSL:1 , GENCODE basic , APPRIS P1 ,
Lhx2-203	ENSMUST00000143783.9	1696	365aa	Protein coding	CCDS71048		TSL:1 , GENCODE basic ,
Lhx2-202	ENSMUST00000133661.8	654	192aa	Protein coding	-		CDS 3' incomplete , TSL:2 ,
Lhx2-205	ENSMUST00000155964.3	375	108aa	Protein coding	-		CDS 3' incomplete , TSL:2 ,
Lhx2-207	ENSMUST00000176229.2	592	49aa	Nonsense mediated decay	-		TSL:3 ,
Lhx2-206	ENSMUST00000175896.2	1243	No protein	Retained intron	-		TSL:2 ,
Lhx2-204	ENSMUST00000149664.2	701	No protein	Retained intron	-		TSL:2 ,

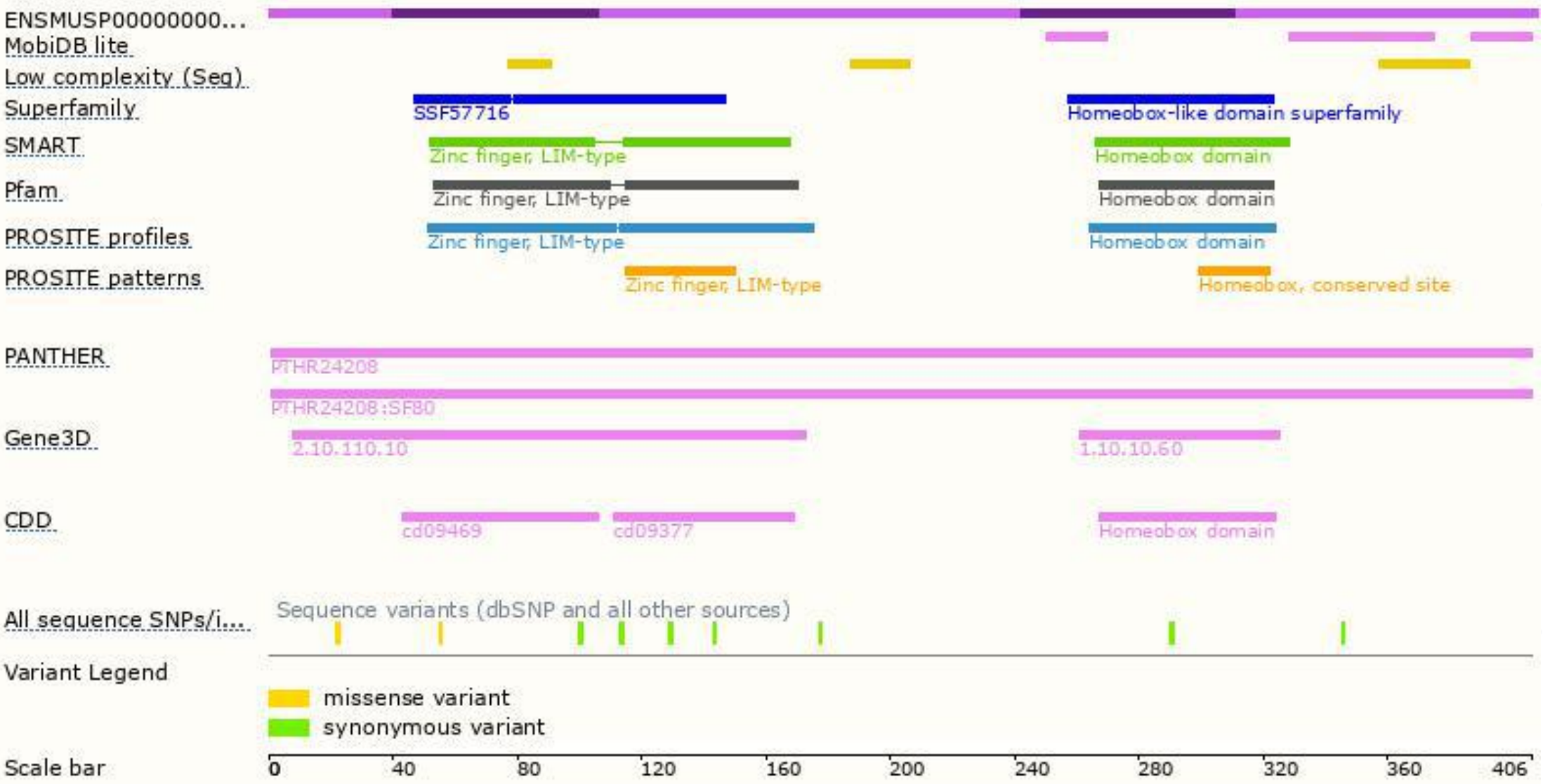
The strategy is based on the design of *Lhx2-201* transcript,the transcription is shown below:



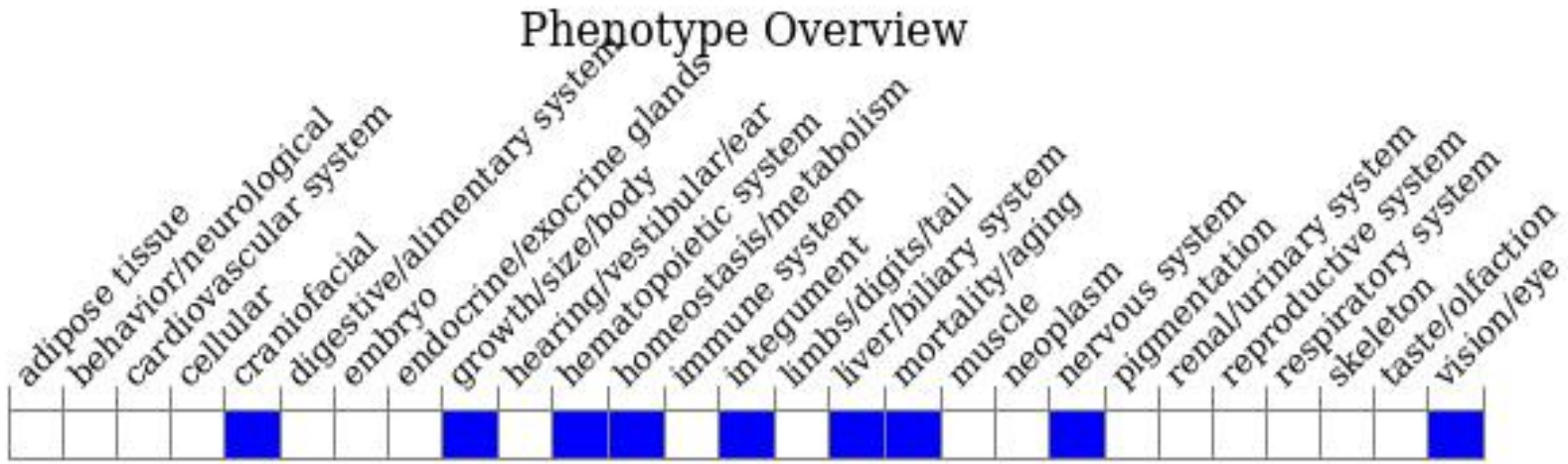
Genomic location distribution



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 homozygous for a knock-out allele exhibit lethality during fetal development and the perinatal period with abnormal liver, telencephalon, olfactory bulb, basal ganglion, and eye morphology.

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

Tel: 025-5864 1534

集萃药康生物科技
GemPharmatech Co.,Ltd