Col18a1 Cas9-CKO Strategy

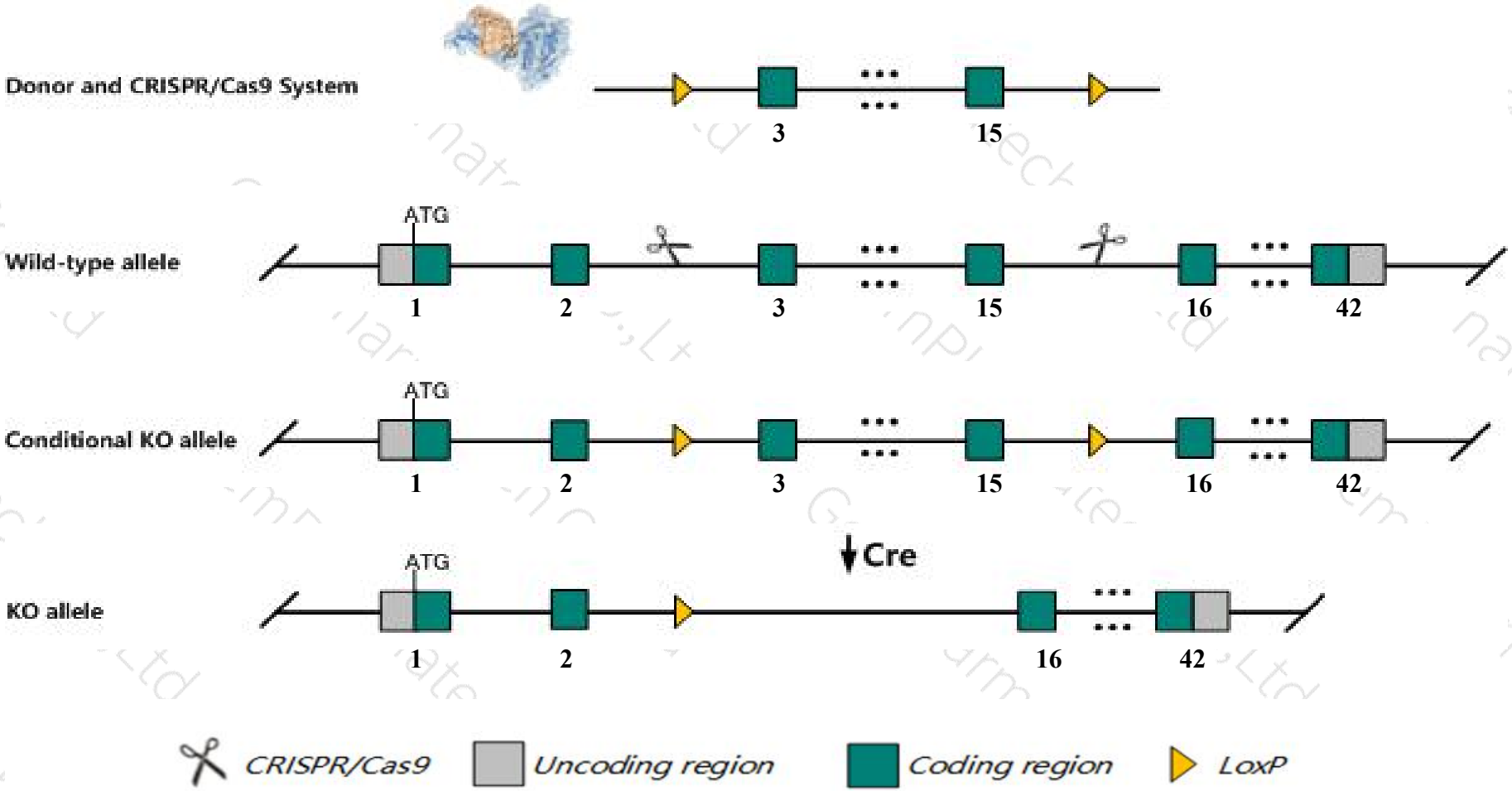
Designer:Xueting Zhang
Reviewer:Yanhua Shen
Date:2019-10-27

Project Overview

Project Name	***Col18a1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Col18a1* gene. The schematic diagram is as follows:



Technical routes

- The *Col18a1* gene has 5 transcripts. According to the structure of *Col18a1* gene, exon3-exon15 of *Col18a1-202* (ENSMUST00000081654.12) transcript is recommended as the knockout region.The region contains 1550bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Col18a1* 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,Mice homozygous for a knock-out allele exhibit defects in hyaloid vessel regression, attenuated visual function, abnormal electroretinograms, broad proximal tubule basement membrane, podocyte effacement, and softened glomeruli.
- The effect on transcript *Col18a1*-204&205 is unknown.
- The strategy may remain the partial function of N-terminal of transcript *Ccna2*-201&203.
- The *Col18a1* gene is located on the Chr10. 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

Col18a1 collagen, type XVIII, alpha 1 [*Mus musculus* (house mouse)]

Gene ID: 12822, updated on 10-Oct-2019

Summary

Official Symbol	Col18a1 provided by MGI
Official Full Name	collagen, type XVIII, alpha 1 provided by MGI
Primary source	MGI:MGI:88451
See related	Ensembl:ENSMUSG00000001435
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
Expression	Broad expression in liver adult (RPKM 128.4), liver E18 (RPKM 40.0) and 21 other tissues See more
Orthologs	human all

Genomic context

Location: 10 C1; 10 39.72 cM

See Col18a1 in Genome Data Viewer

Exon count: 44

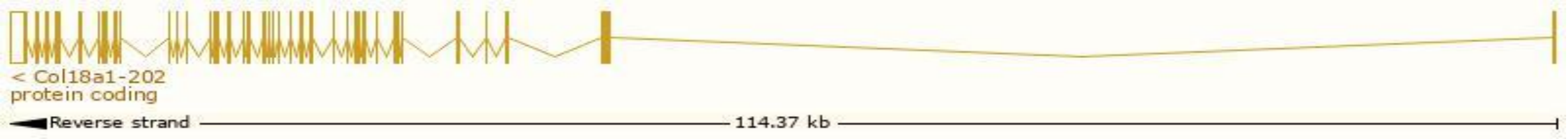
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	10	NC_000076.6 (77052178..77166530, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	10	NC_000076.5 (76514924..76629275, complement)

Transcript information（Ensembl）

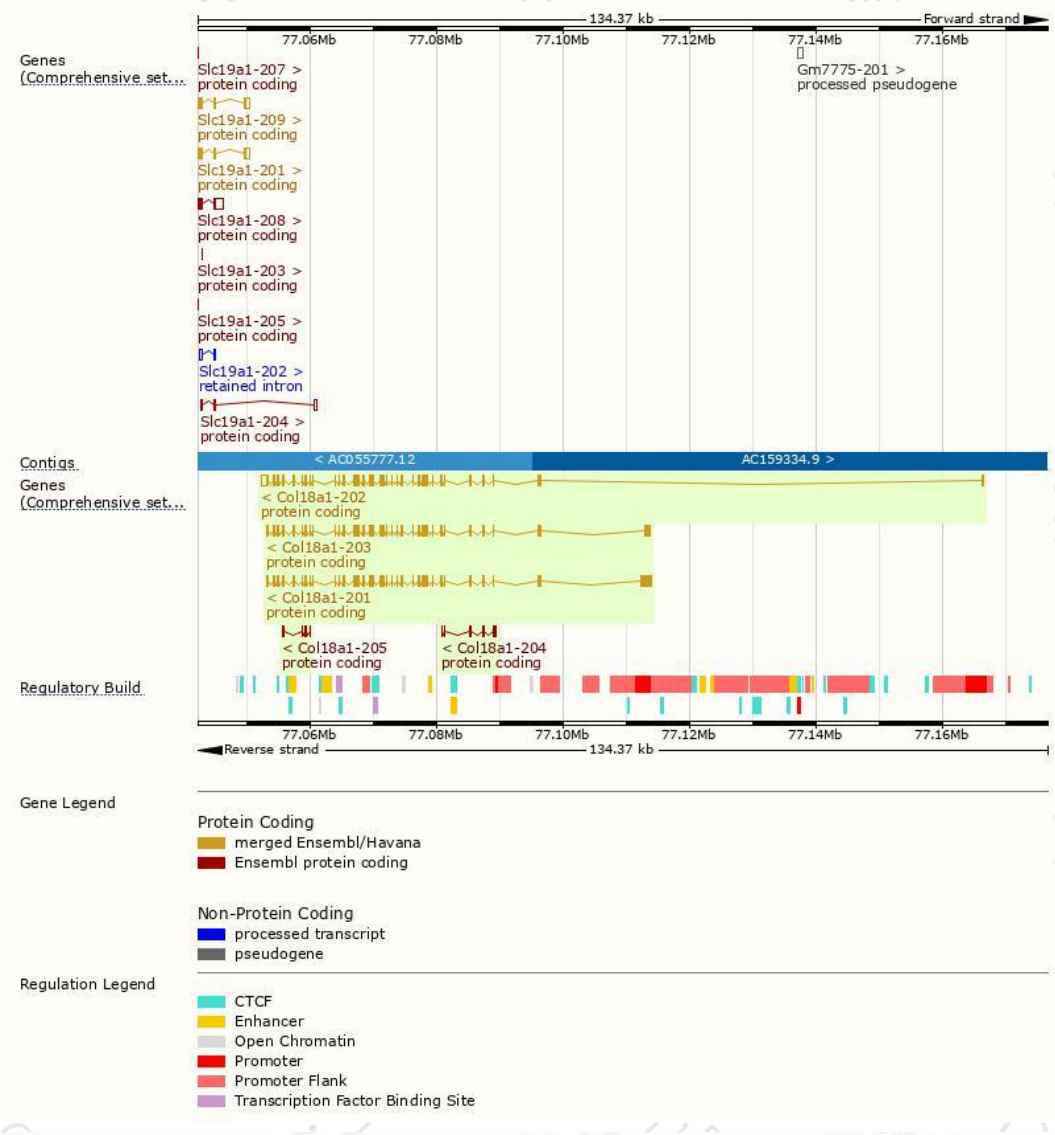
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Col18a1-202	ENSMUST00000081654.12	5063	1315aa	Protein coding	CCDS23953	P39061	TSL:1 GENCODE basic
Col18a1-203	ENSMUST00000105409.7	4791	1527aa	Protein coding	CCDS48604	P39061	TSL:1 GENCODE basic APPRIS P2
Col18a1-201	ENSMUST00000072755.11	5595	1774aa	Protein coding	-	E9QPX1	TSL:5 GENCODE basic APPRIS ALT2
Col18a1-204	ENSMUST00000156009.1	717	161aa	Protein coding	-	D3Z556	CDS 3' incomplete TSL:3
Col18a1-205	ENSMUST00000218407.1	675	225aa	Protein coding	-	A0A1W2P6N8	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3

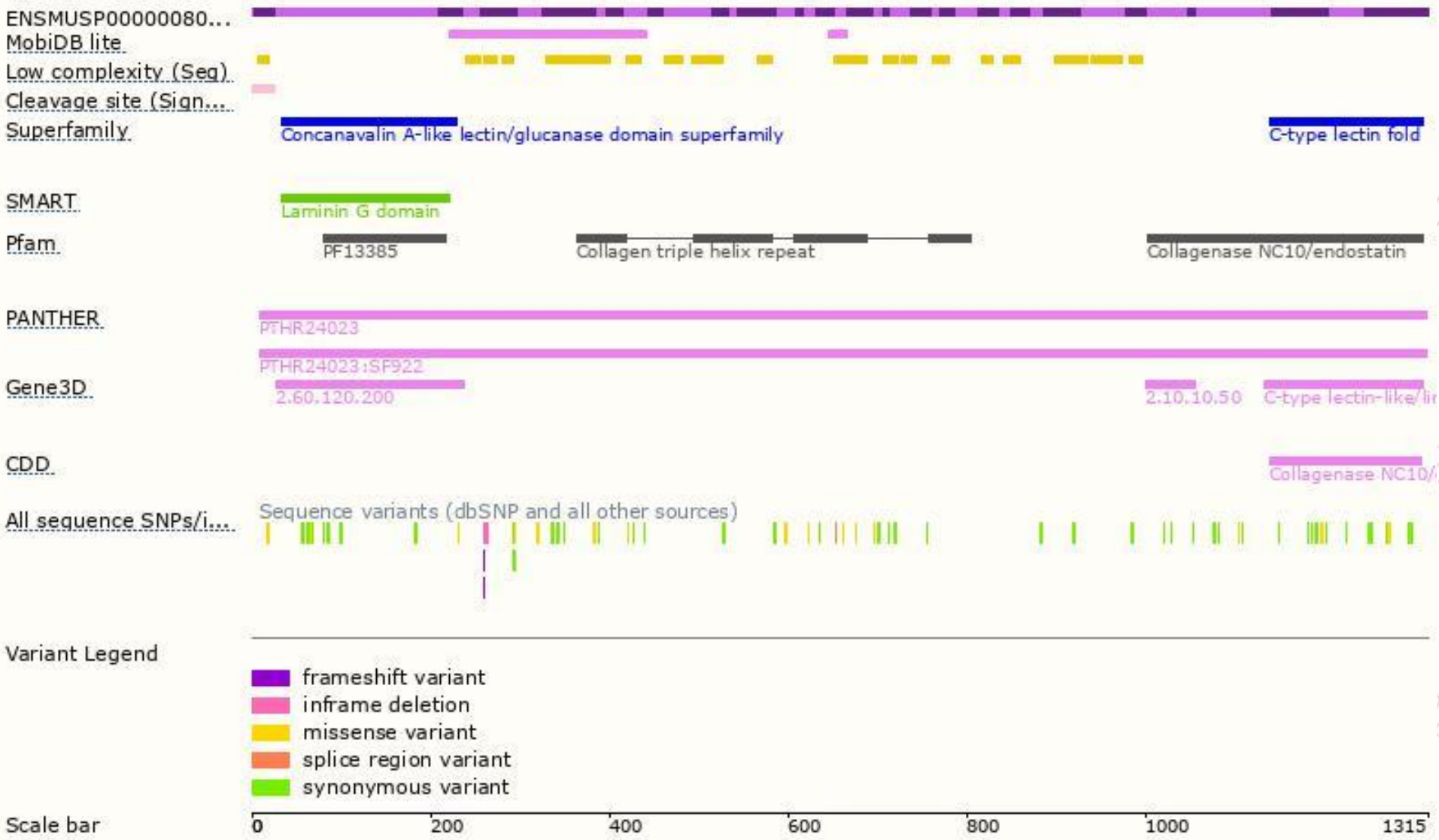
The strategy is based on the design of *Col18a1-202* transcript,The transcription is shown below



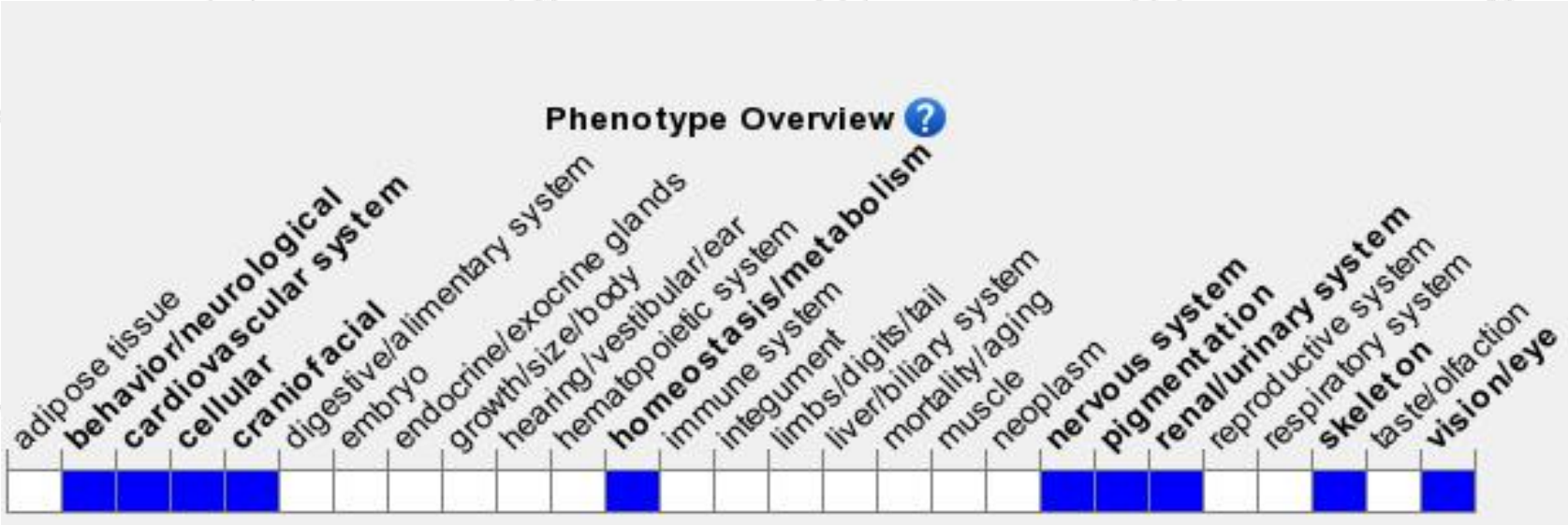
Genomic location distribution



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 defects in hyaloid vessel regression, attenuated visual function, abnormal electroretinograms, broad proximal tubule basement membrane, podocyte effacement, and softened glomeruli.

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