Rnf7 Cas9-CKO Strategy

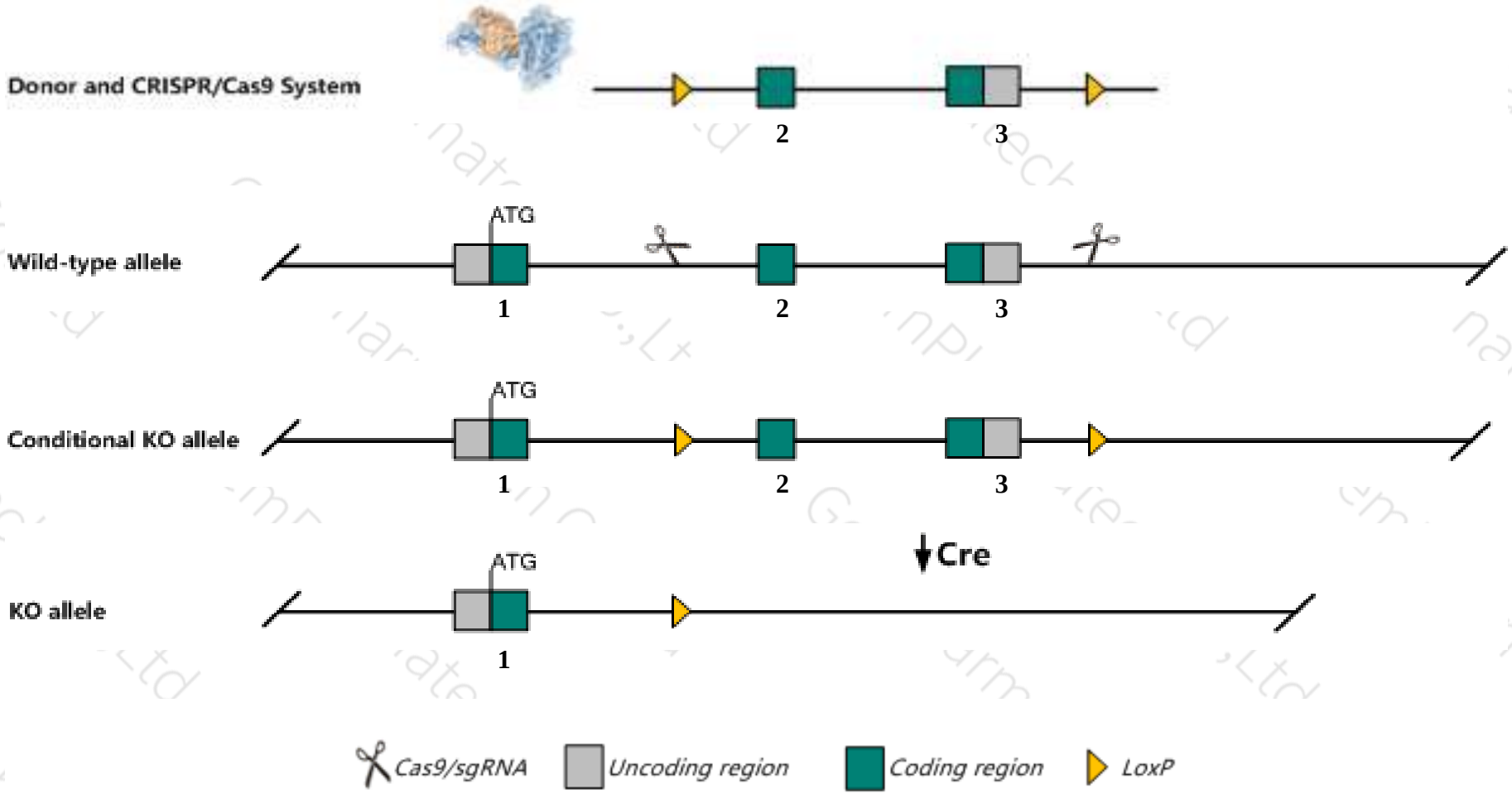
Designer: Yanhua Shen
Design Date: 2019-08-05

Project Overview

Project Name	***Rnf7***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rnf7* gene. The schematic diagram is as follows:



Technical routes

- The *Rnf7* gene has 3 transcripts. According to the structure of *Rnf7* gene, exon2-exon3 of *Rnf7-201* (ENSMUST00000057500.5) transcript is recommended as the knockout region.The region contains 167bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Rnf7* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector 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 null mutation display complete embryonic lethality during organogenesis with defects in angiogenesis, widespread apoptosis, impaired cell cycle progression of neuronal precursors and embryonic growth retardation.

➢ The *Rnf7* gene is located on the Chr9. 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

Rnf7 ring finger protein 7 [Mus musculus (house mouse)]

Gene ID: 19823, updated on 31-Jan-2019

Summary

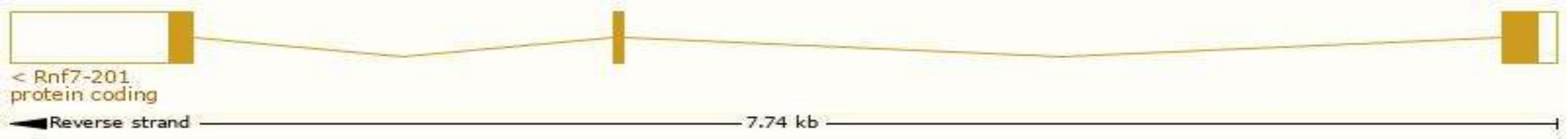
Official Symbol	Rnf7 provided by MGI
Official Full Name	ring finger protein 7 provided by MGI
Primary source	MGI:MGI:1337096
See related	Ensembl:ENSMUSG00000051234
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Rbx2, SAG
Expression	Ubiquitous expression in CNS E11.5 (RPKM 73.0), CNS E14 (RPKM 56.6) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

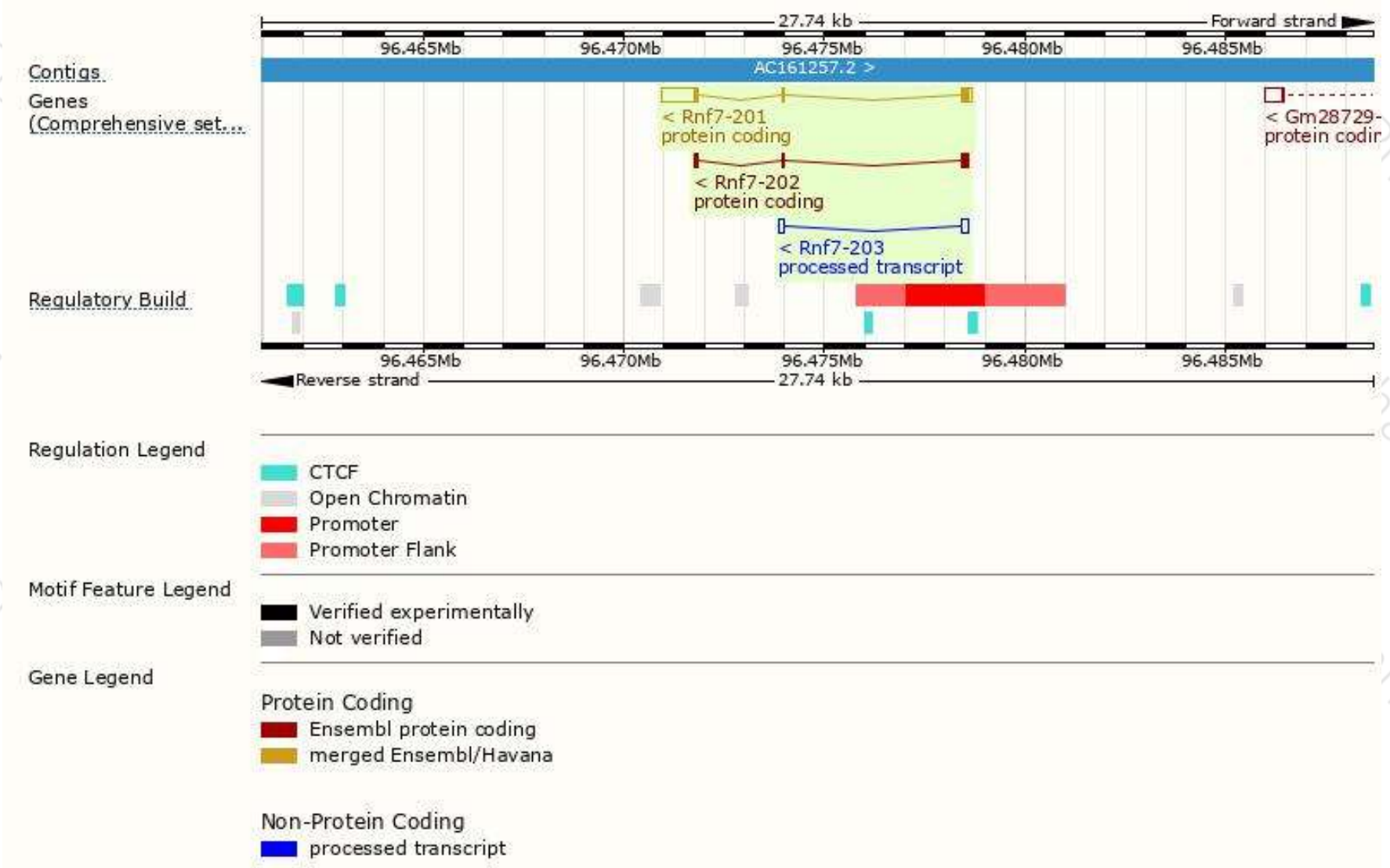
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rnf7-201	ENSMUST00000057500.5	1232	113aa	Protein coding	CCDS40730	Q9WTZ1	TSL:1 GENCODE basic APPRIS P1
Rnf7-202	ENSMUST00000071301.4	334	90aa	Protein coding	CCDS81056	D3Z392	TSL:2 GENCODE basic
Rnf7-203	ENSMUST00000128955.1	325	No protein	Processed transcript	-	-	TSL:2

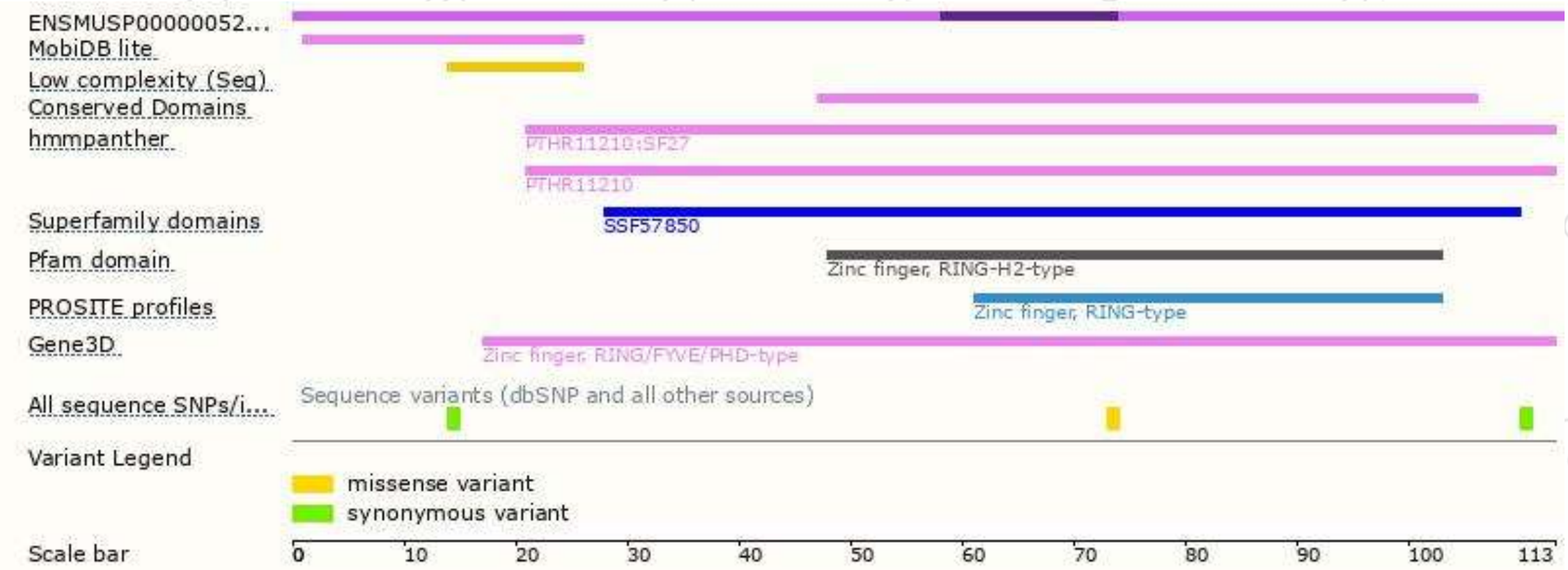
The strategy is based on the design of *Rnf7-201* transcript,The transcription is shown below



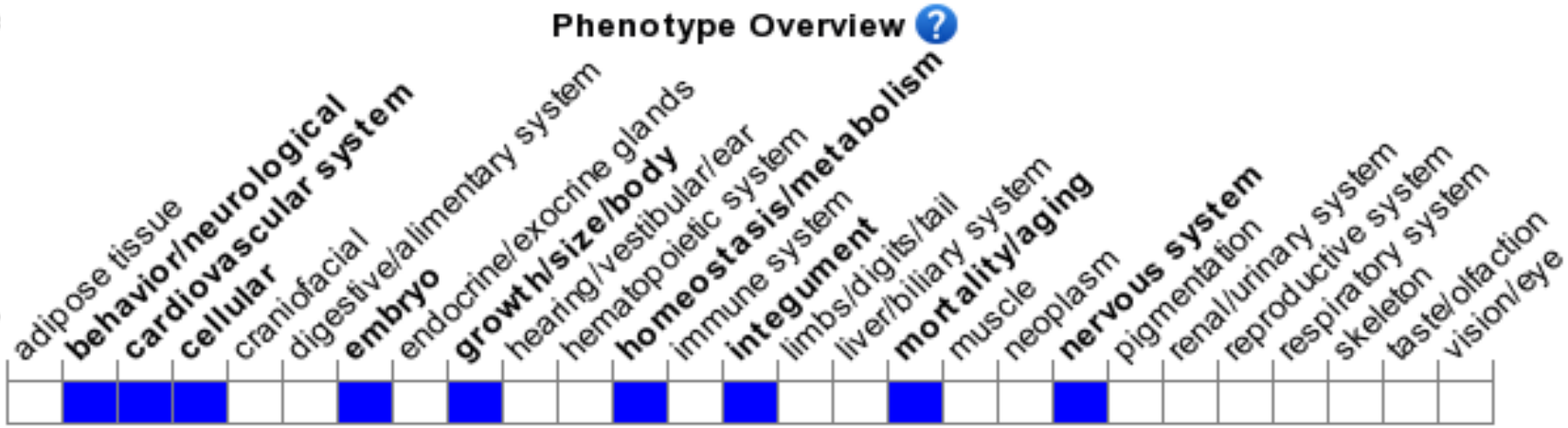
Genomic location distribution



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 null mutation display complete embryonic lethality during organogenesis with defects in angiogenesis, widespread apoptosis, impaired cell cycle progression of neuronal precursors and embryonic growth retardation.

集萃药康
GemPharmatech

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