Procr Cas9-CKO Strategy

Designer: Shilei Zhu

Reviewer: Linyan Wu

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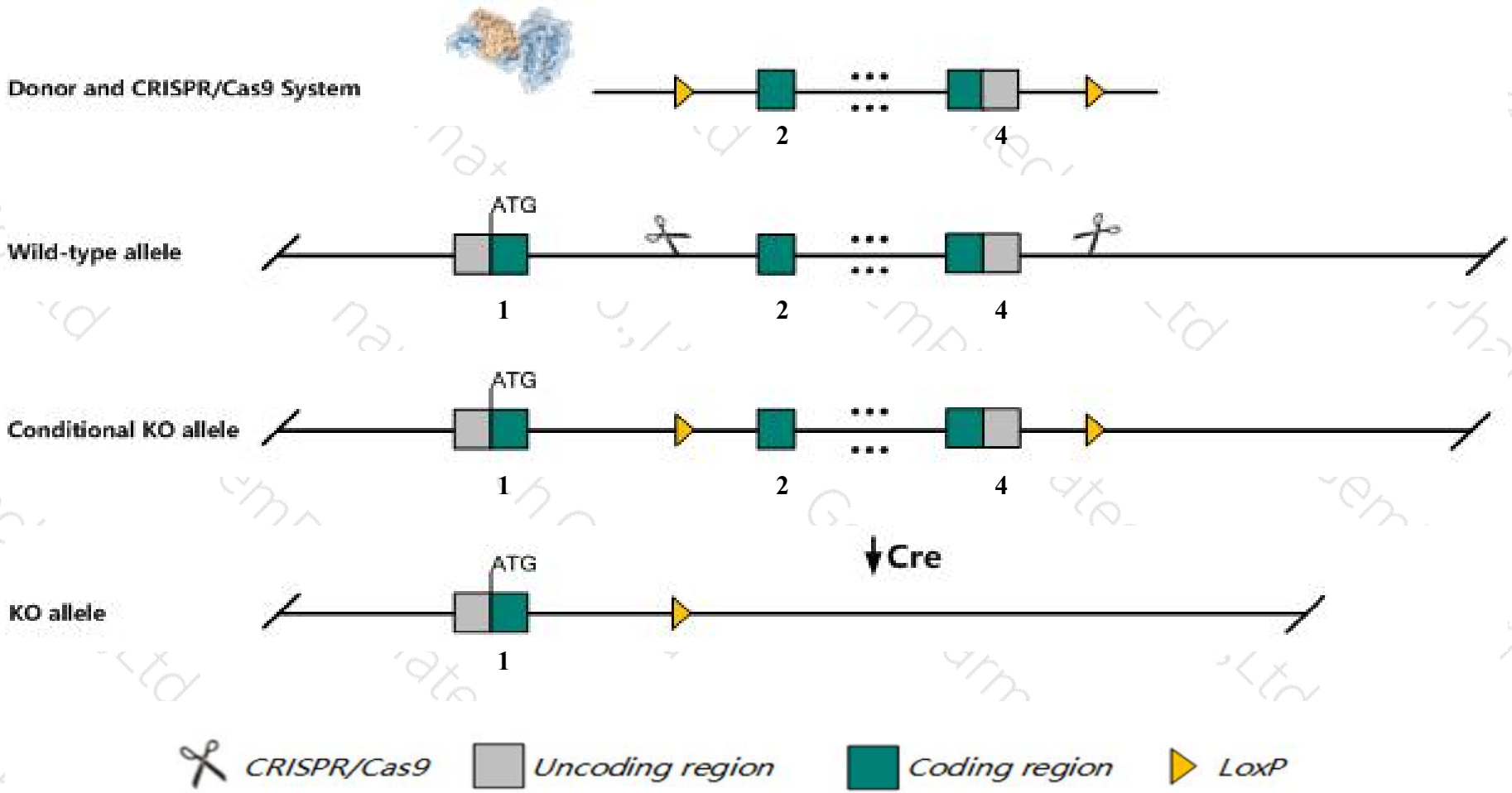
Project Overview

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

Conditional Knockout strategy

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



Technical routes

➢ The *Procr* gene has 4 transcripts. According to the structure of *Procr* gene, exon2-exon4 of *Procr-201*(ENSMUST00000029140.11) transcript is recommended as the knockout region.The region contains 662bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Procr* 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,nullizygous embryos die by E10.5 showing placental thrombosis, small size, and incomplete turning. Mice with a severe deficiency survive and reproduce normally. Homozygotes for the R84A variant show increased thrombin formation after thrombotic and LPS challenge, splenomegaly, and bone marrow failure.

➢ The *Procr* 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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Procr protein C receptor, endothelial [Mus musculus (house mouse)]

Gene ID: 19124, updated on 13-Mar-2020

Summary

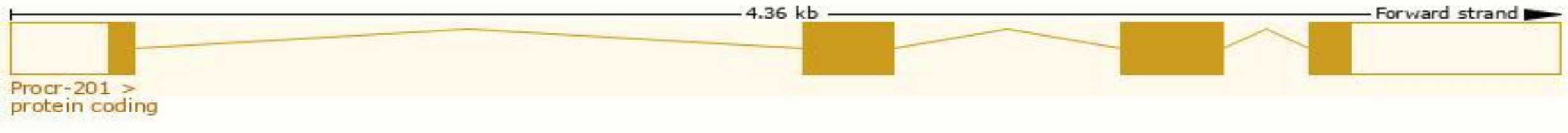
Official Symbol	Procr provided by MGI
Official Full Name	protein C receptor, endothelial provided byMGI
Primary source	MGI:MGI:104596
See related	Ensembl:ENSMUSG00000027611
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI325044, Ccca, Ccd41, Epcr
Expression	Biased expression in placenta adult (RPKM 44.4), bladder adult (RPKM 5.1) and 8 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

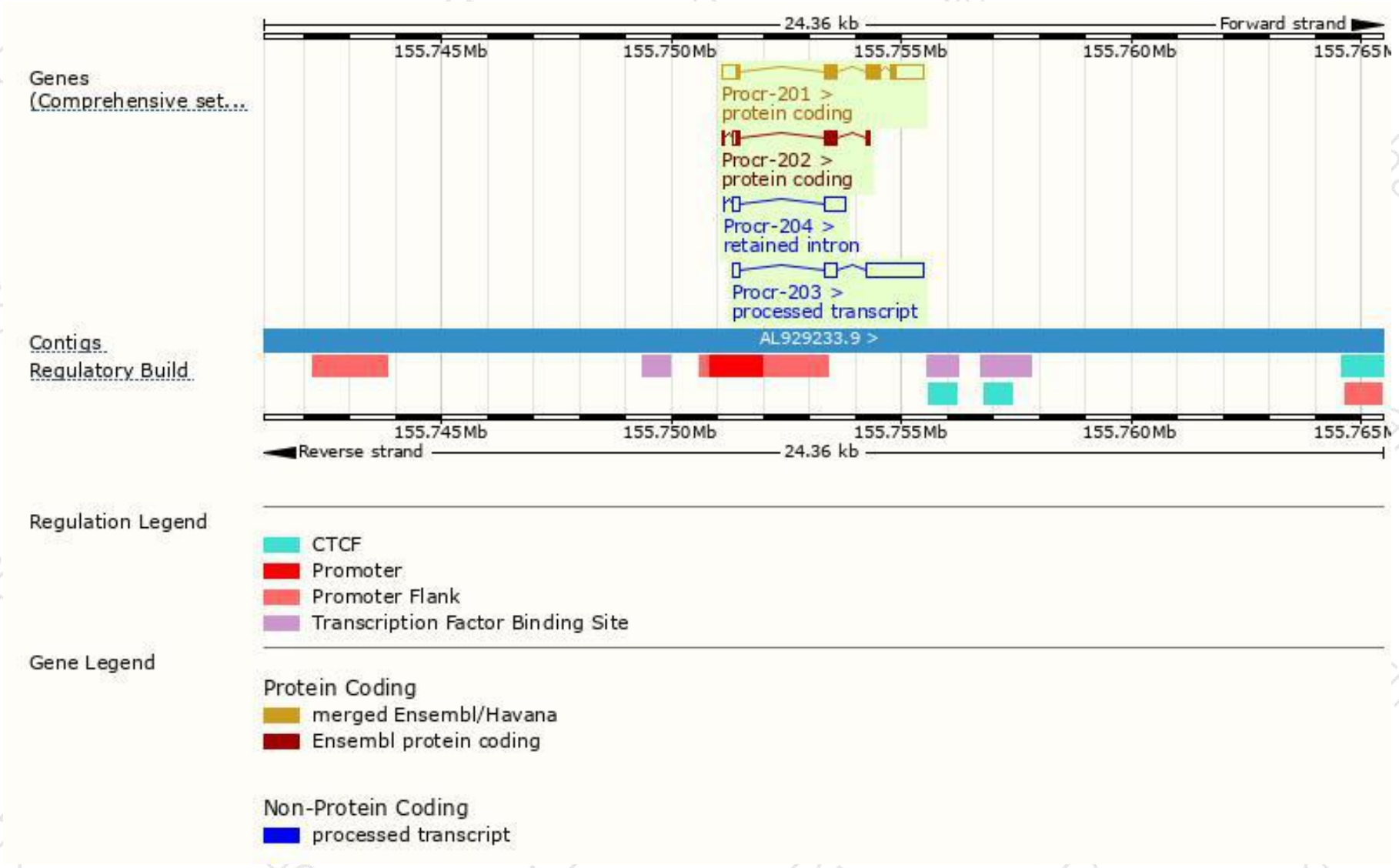
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Procr-201	ENSMUST00000029140.11	1597	242aa	Protein coding	CCDS16954	Q64695	TSL:1 GENCODE basic APPRIS P1
Procr-202	ENSMUST00000132608.1	504	138aa	Protein coding	-	A2AUV5	CDS 3' incomplete TSL:3
Procr-203	ENSMUST00000143493.1	1608	No protein	Processed transcript	-	-	TSL:2
Procr-204	ENSMUST00000155095.1	578	No protein	Retained intron	-	-	TSL:3

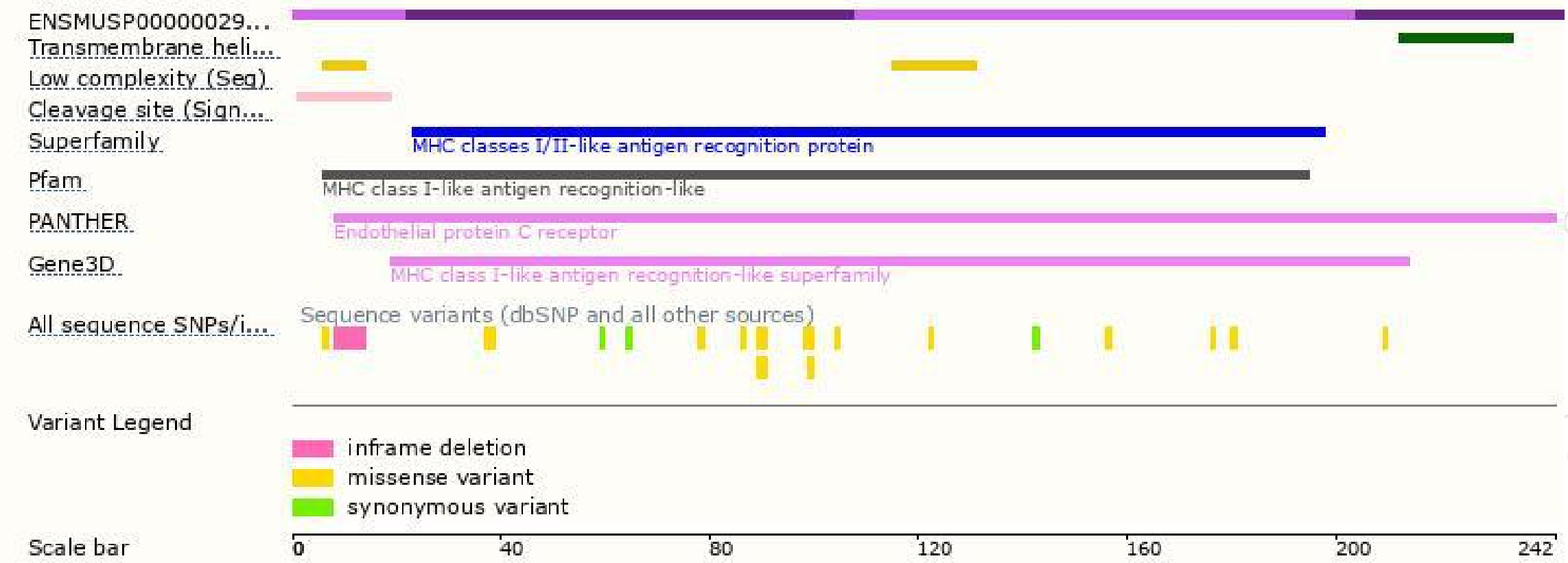
The strategy is based on the design of *Procr-201* transcript,the transcription is shown below:



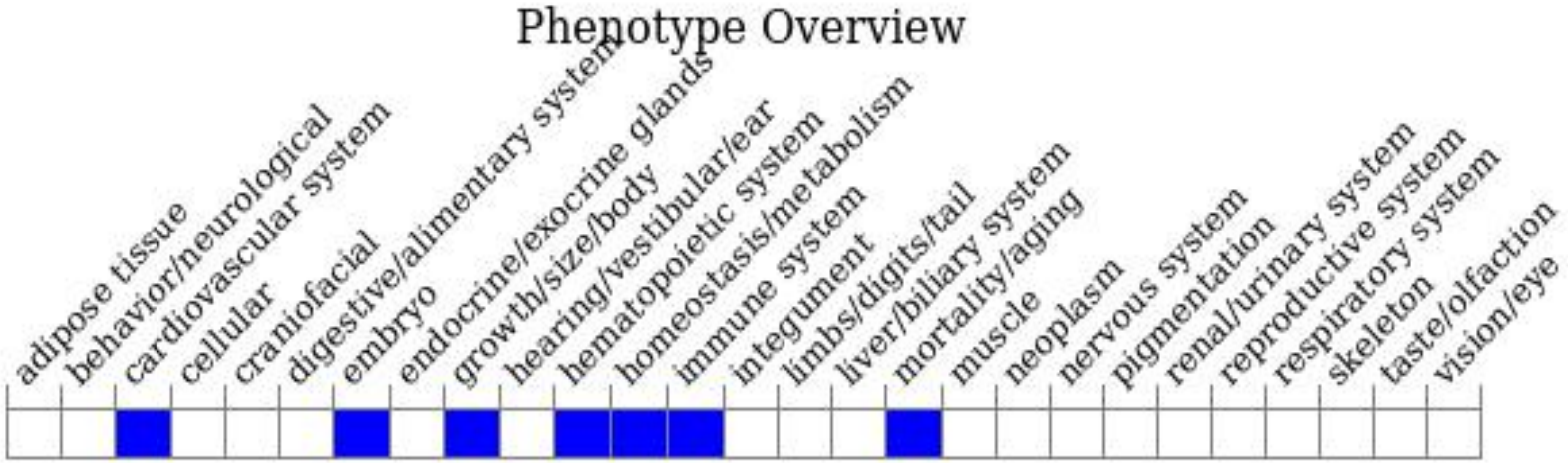
Genomic location distribution



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/).

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd