P2ry6 Cas9-CKO Strategy

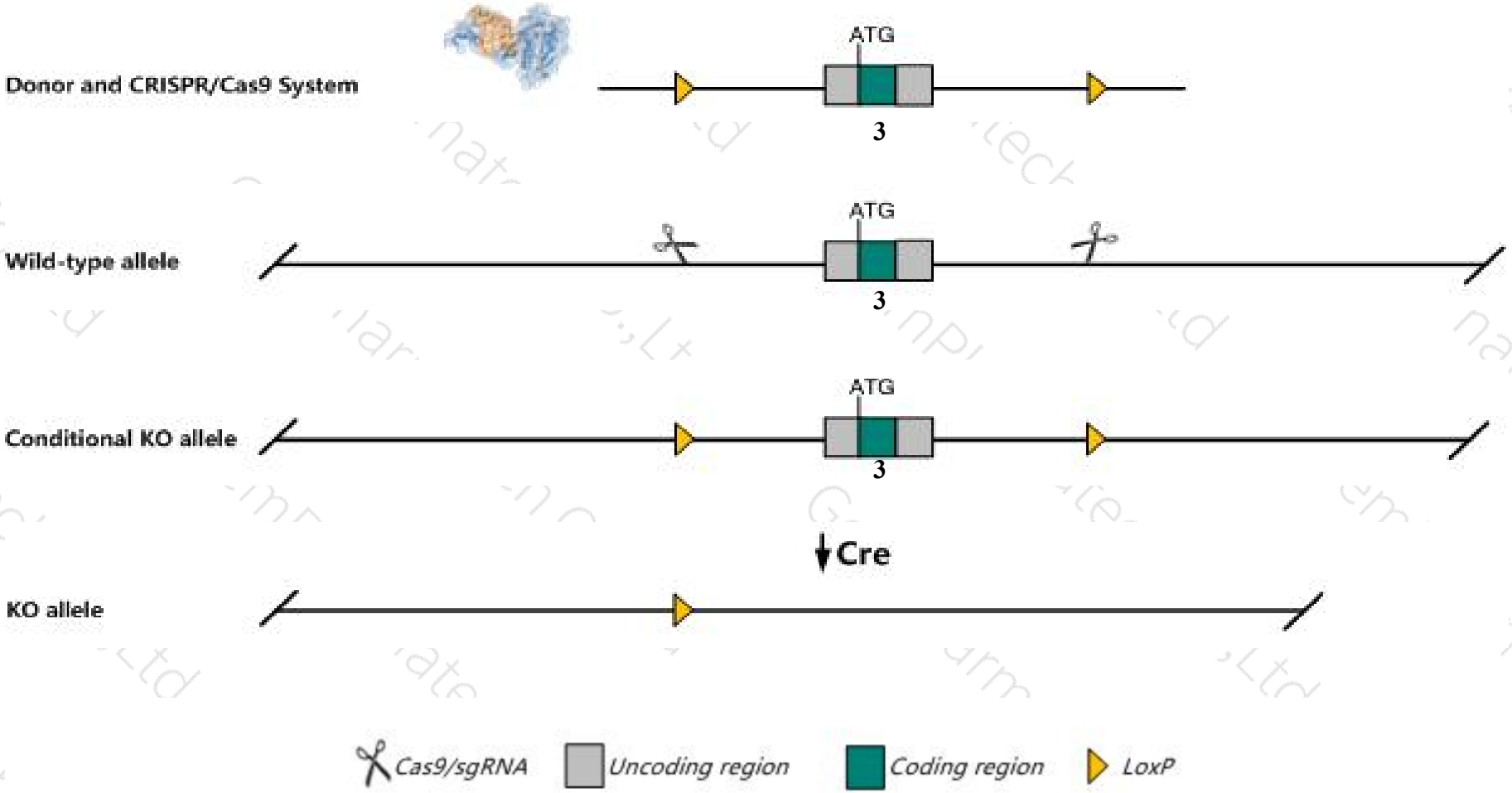
Designer: Lingyan Wu
Reviewer: Jiayuan Yao
Design Date: 2019-7-25

Project Overview

Project Name	***P2ry6***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *P2ry6* gene. The schematic diagram is as follows:



Technical routes

- The *P2ry6* gene has 3 transcripts. According to the structure of *P2ry6* gene, exon3 of *P2ry6-201* (ENSMUST00000060174.5) transcript is recommended as the knockout region.The region contains all of the coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *P2ry6* 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 knock-out allele exhibit abnormal macrophage, endothelial, and vascular smooth muscle response to UTP and UDP.

➢ The *P2ry6* gene is located on the Chr7. 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

P2ry6 pyrimidinergic receptor P2Y, G-protein coupled, 6 [Mus musculus (house mouse)]

Gene ID: 233571, updated on 31-Jan-2019

Summary

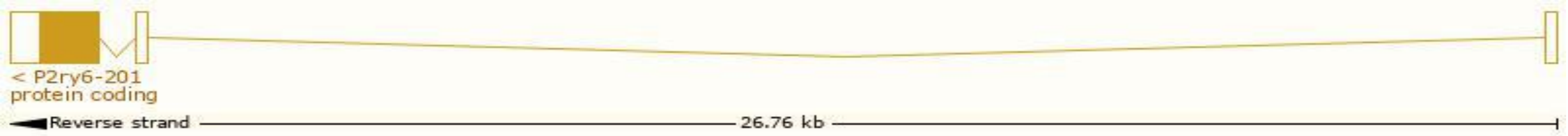
Official Symbol	P2ry6 provided by MGI
Official Full Name	pyrimidinergic receptor P2Y, G-protein coupled, 6 provided by MGI
Primary source	MGI:MGI:2673874
See related	Ensembl:ENSMUSG00000048779
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2010204J23Rik, P2Y6
Expression	Broad expression in colon adult (RPKM 37.0), lung adult (RPKM 23.5) and 16 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

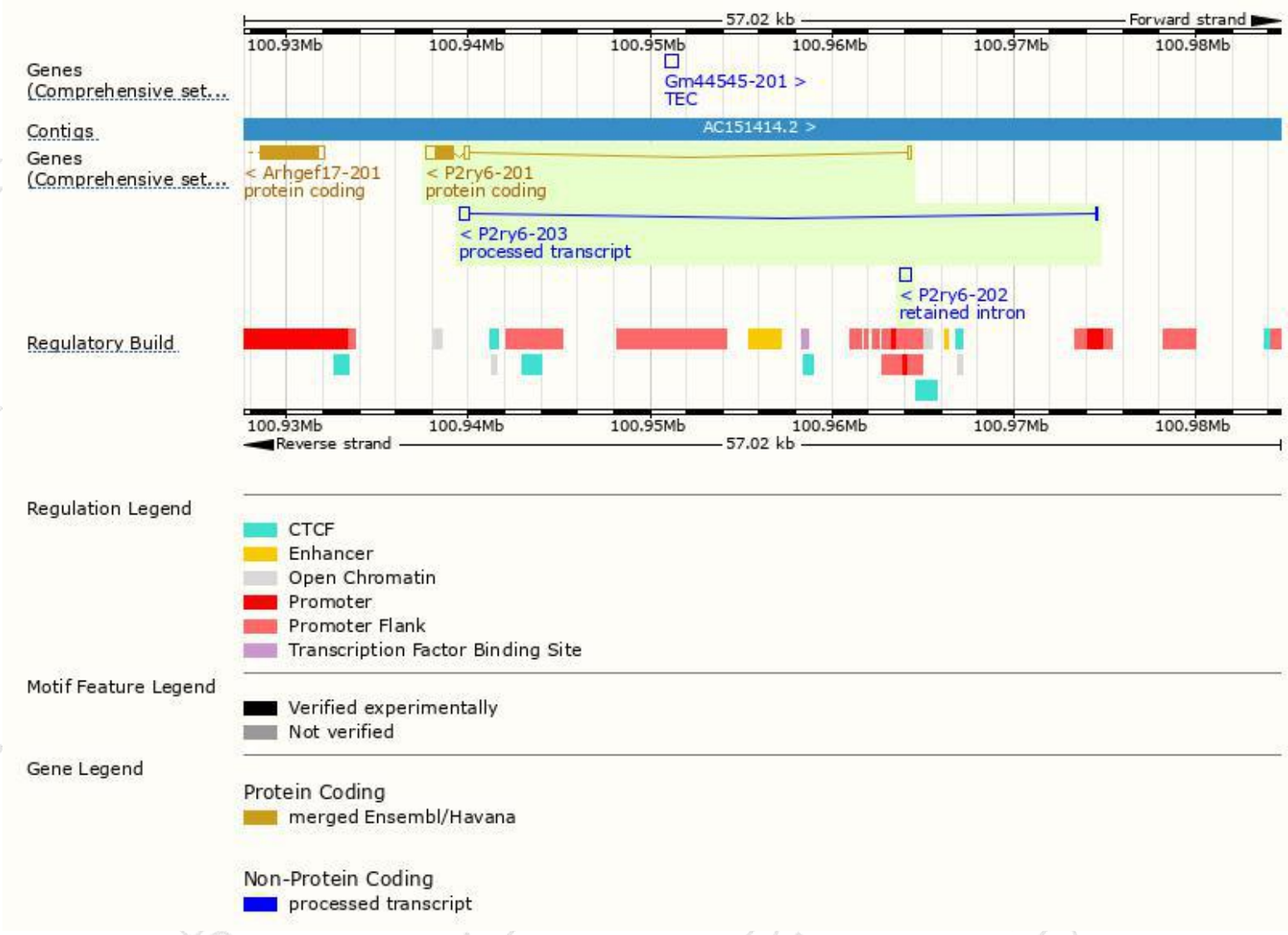
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
P2ry6-201	ENSMUST00000060174.5	1946	328aa	Protein coding	CCDS21507	Q3UQ86 Q9ERK9	TSL:1 GENCODE basic APPRIS P1
P2ry6-203	ENSMUST00000209196.1	676	No protein	Processed transcript	-	-	TSL:3
P2ry6-202	ENSMUST00000208653.1	631	No protein	Retained intron	-	-	TSL:NA

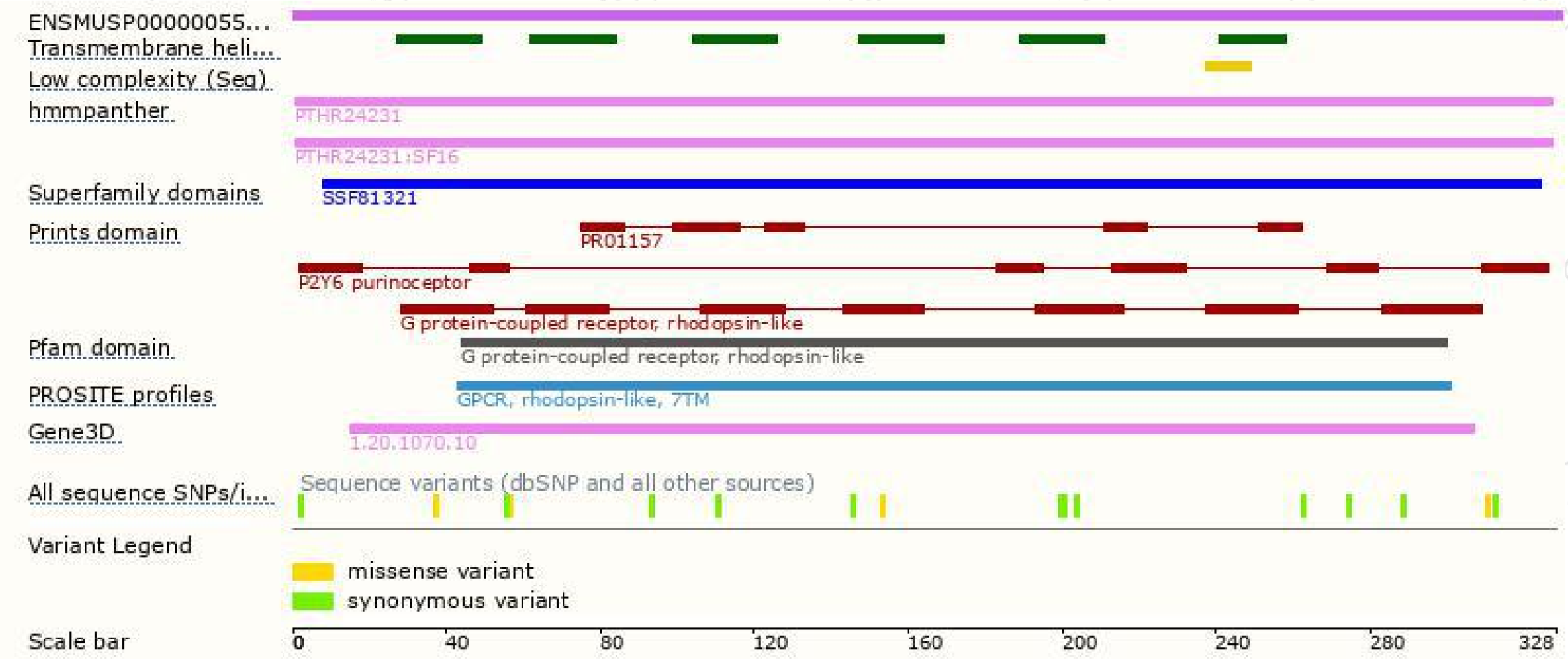
The strategy is based on the design of *P2ry6-201* transcript,The transcription is shown below



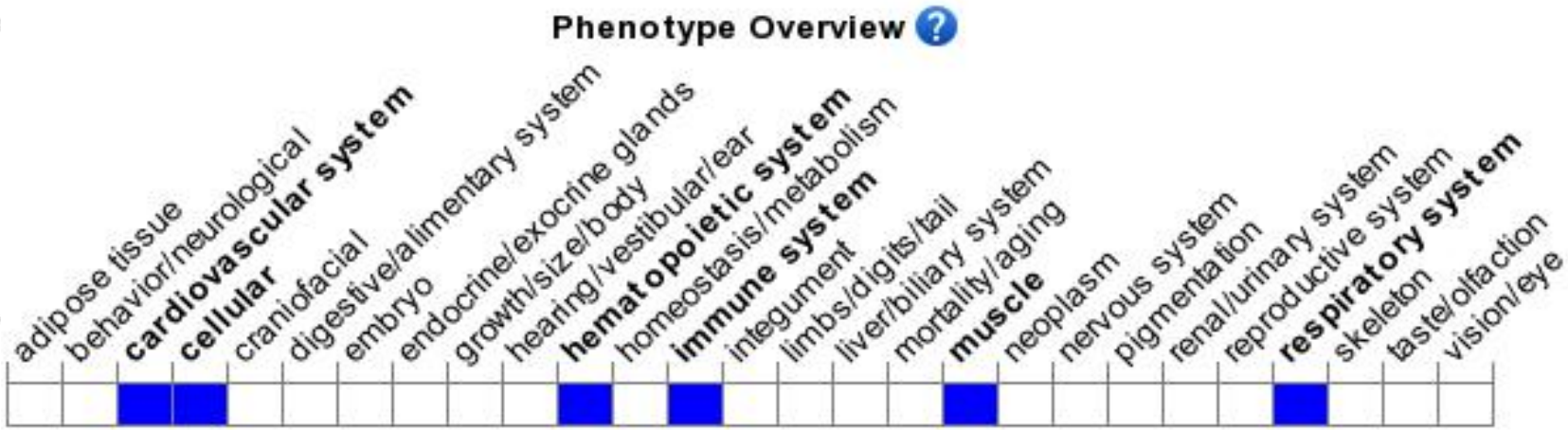
Genomic location distribution



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 abnormal macrophage, endothelial, and vascular smooth muscle response to UTP and UDP.

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