Rassf2 Cas9-KO Strategy

Designer: Xiaojing Li

Reviewer: JiaYu

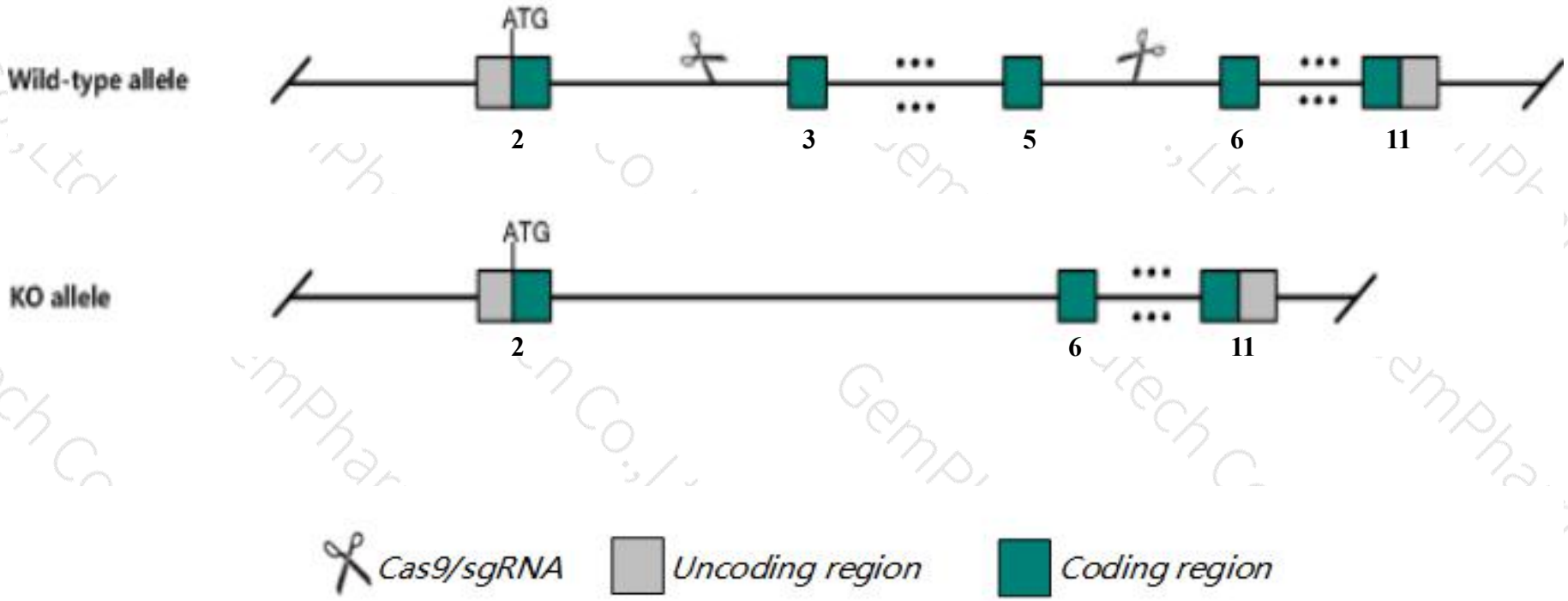
Design Date: 2020-8-27

Project Overview

Project Name	***Rassf2***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rassf2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Rassf2* gene has 5 transcripts. According to the structure of *Rassf2* gene, exon3-exon5 of *Rassf2-201*(ENSMUST00000028814.14) transcript is recommended as the knockout region.The region contains 317bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Rassf2* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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.

Notice

➢ According to the existing MGI data,mice homozygous for a knock-out allele exhibit bone defects and hematopoeitic abnormalities.

➢ The *Rassf2* 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

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Rassf2 Ras association (RalGDS/AF-6) domain family member 2 [Mus musculus (house mouse)]

Gene ID: 215653, updated on 13-Mar-2020

Summary

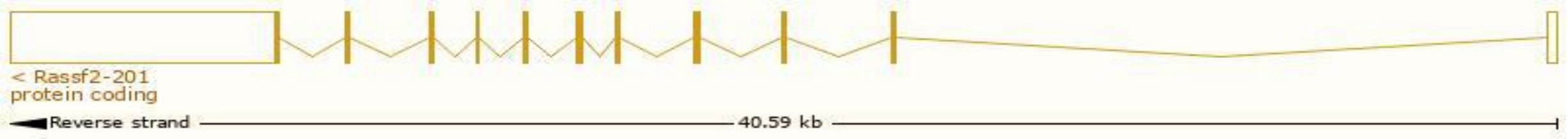
Official Symbol	Rassf2 provided by MGI
Official Full Name	Ras association (RalGDS/AF-6) domain family member 2 provided byMGI
Primary source	MGI:MGI:2442060
See related	Ensembl:ENSMUSG00000027339
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	3830431H01Rik, 9030412M04Rik, AI852669, AW495050
Expression	Broad expression in thymus adult (RPKM 18.3), spleen adult (RPKM 16.9) and 25 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

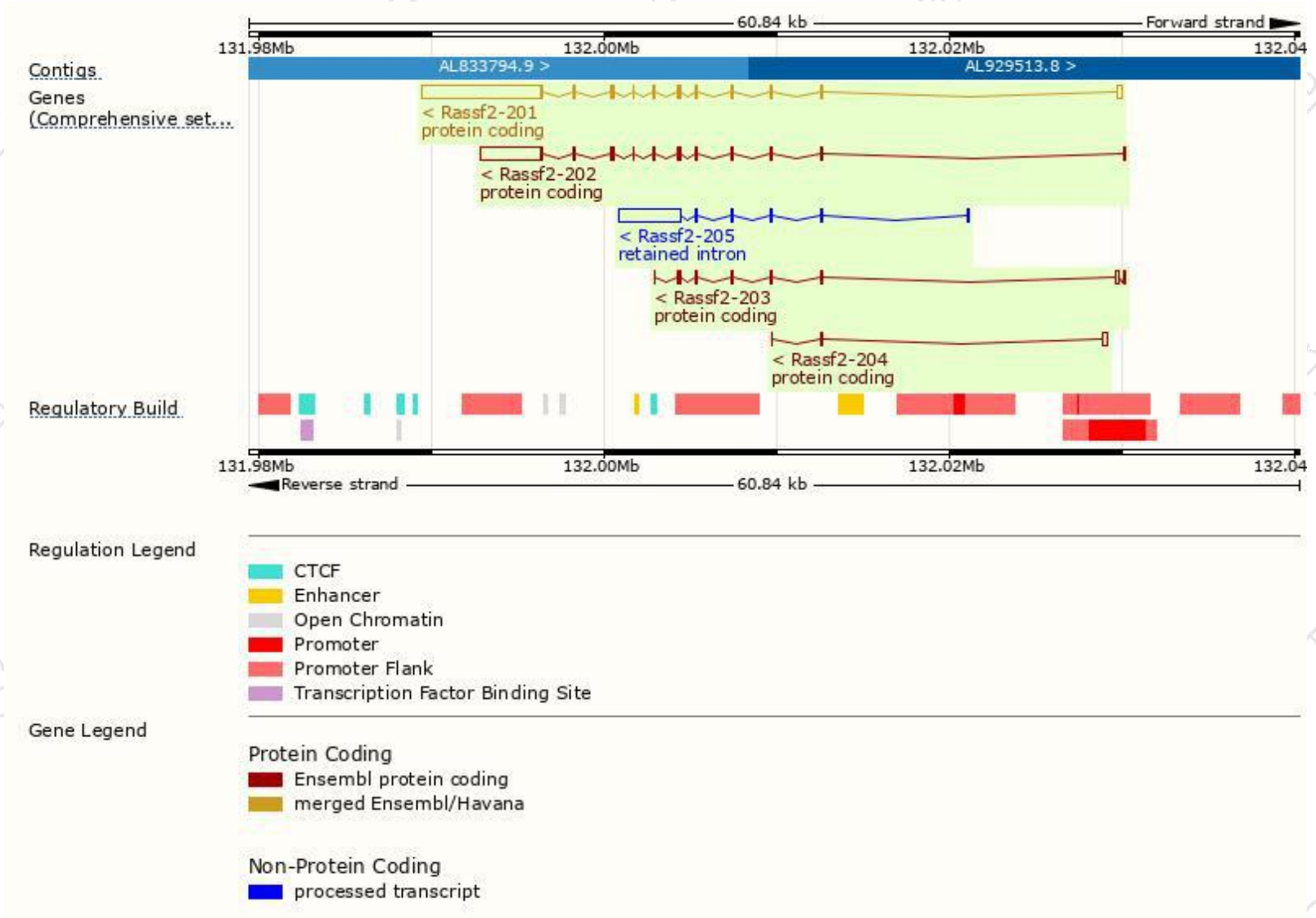
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rassf2-201	ENSMUST00000028814.14	8233	326aa	Protein coding	CCDS16768	Q8BMS9	TSL:1 GENCODE basic APPRIS P1
Rassf2-202	ENSMUST00000103182.7	4589	326aa	Protein coding	CCDS16768	Q8BMS9	TSL:5 GENCODE basic APPRIS P1
Rassf2-203	ENSMUST00000139047.7	924	202aa	Protein coding	-	A2APB1	CDS 3' incomplete TSL:5
Rassf2-204	ENSMUST00000140791.1	405	29aa	Protein coding	-	A2AVI0	CDS 3' incomplete TSL:5
Rassf2-205	ENSMUST00000155829.1	4040	No protein	Retained intron	-	-	TSL:1

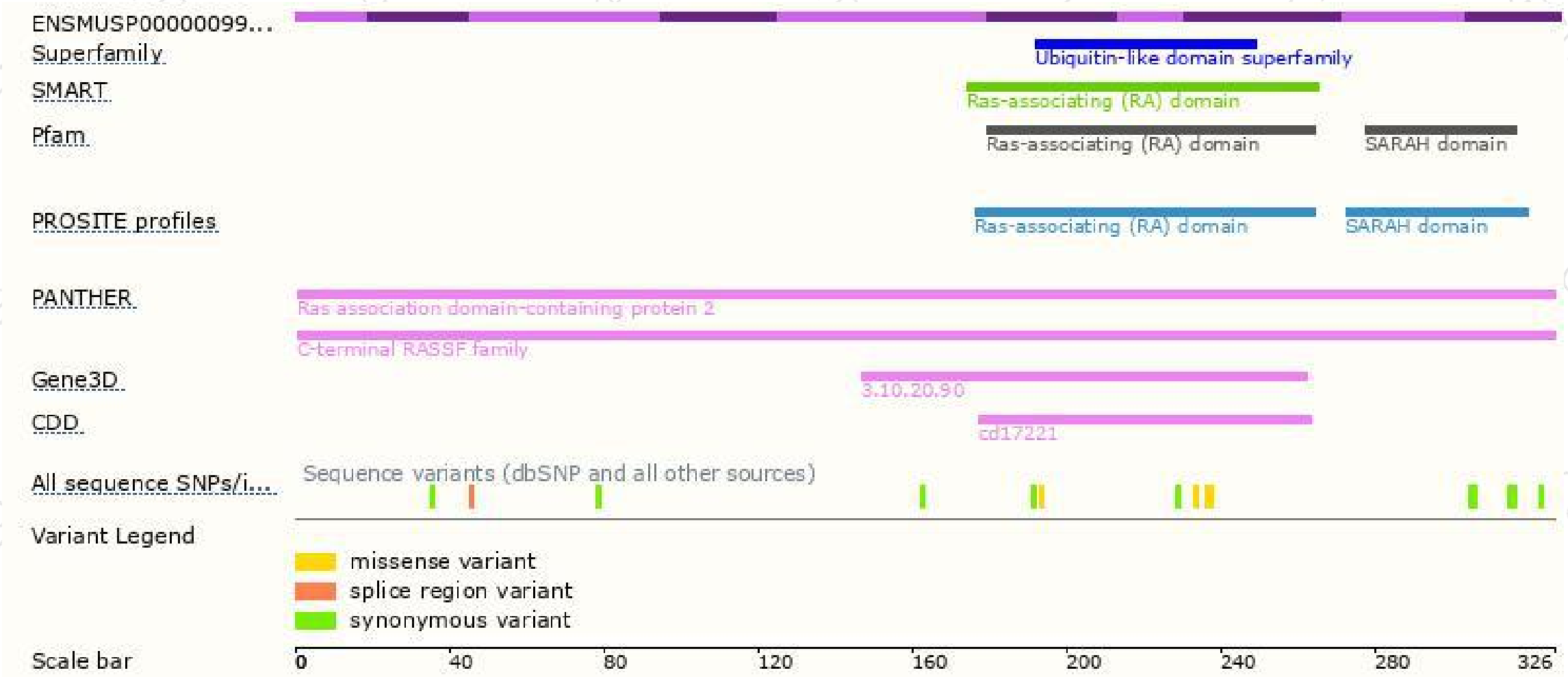
The strategy is based on the design of *Rassf2-201* transcript,the transcription is shown below:



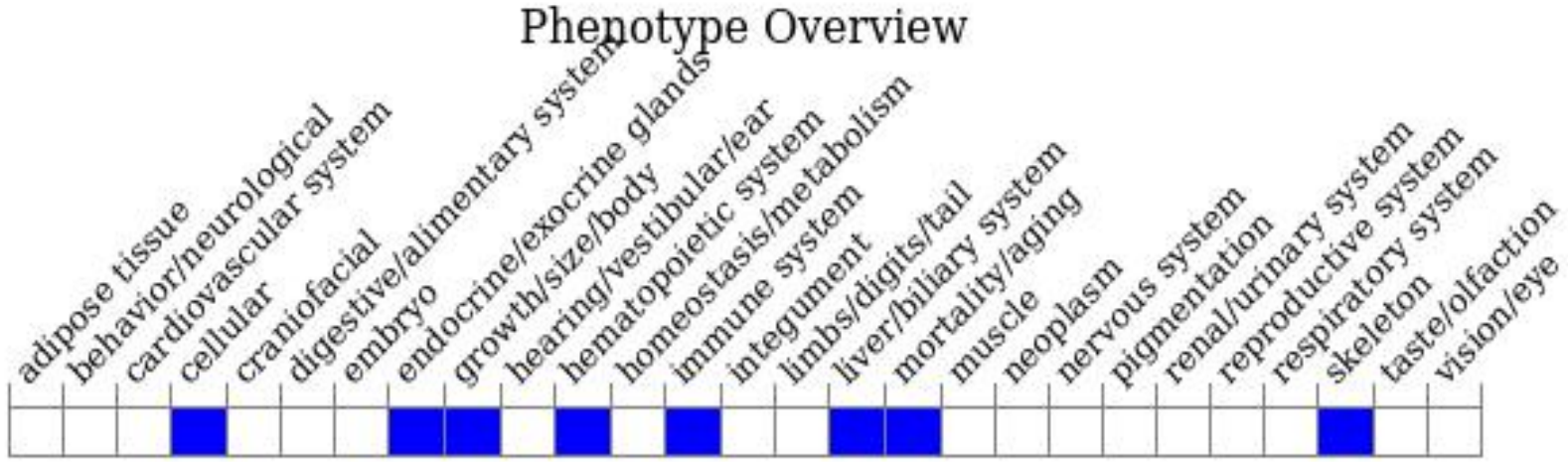
Genomic location distribution



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: 025-5864 1534

集萃药康生物科技
GemPharmatech Co.,Ltd