Rhbdf1 Cas9-CKO Strategy

Designer: Yun Li

Reviewer: Shuang Zhang

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Project Overview

Project Name	***Rhbdf1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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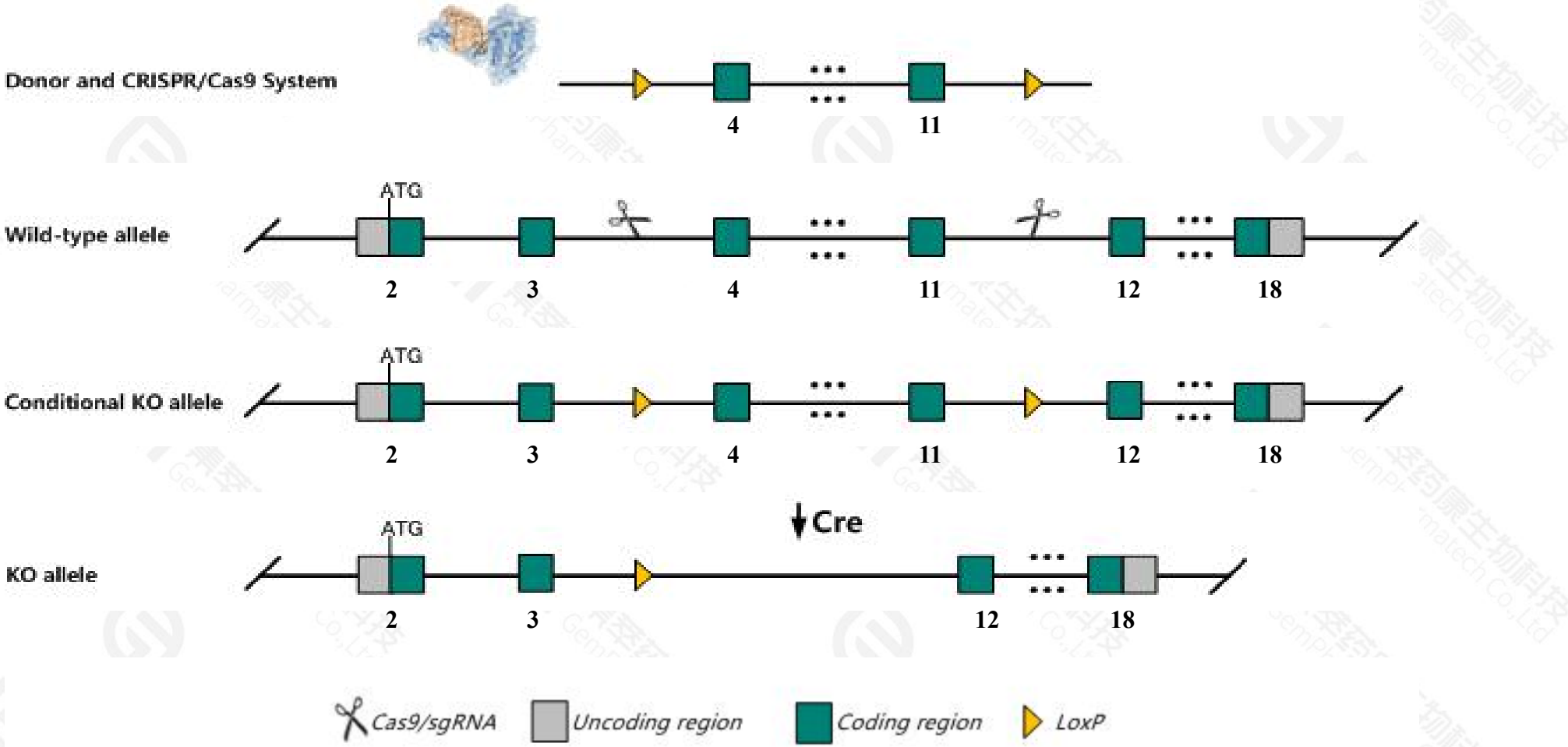
This model will use CRISPR/Cas9 technology to edit the *Rhbdf1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Rhbdf1* gene has 9 transcripts. According to the structure of *Rhbdf1* gene, exon4-exon11 of *Rhbdf1-201*(ENSMUST00000020524.15) transcript is recommended as the knockout region.The region contains 1312bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Rhbdf1* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor 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,homozygotes for a null allele show pleiotropic phenotypes and postnatal lethality largely dependent on the genetic background. Observed defects range from small size, reduced fat mass, and brain haemorrhages to small lymph organs, thrombosis, abnormal pancreatic acini, and behavioral deficits.

➢ The *Rhbdf1* gene is located on the Chr11. 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.

➢Transcripts 206 and 207 will not be disrupted.

➢ 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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Rhbdf1 rhomboid 5 homolog 1 [Mus musculus (house mouse)]

Gene ID: 13650, updated on 7-Mar-2021

Summary

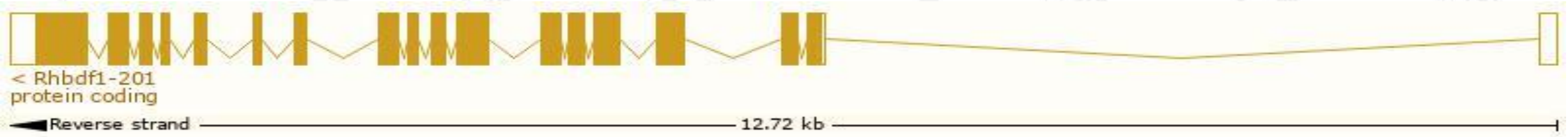
Official Symbol	Rhbdf1 provided by MGI
Official Full Name	rhomboid 5 homolog 1 provided byMGI
Primary source	MGI:MGI:104328
See related	Ensembl:ENSMUSG00000020282
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	C16ORF8, Di, Dis, Dist, Dist1, Egfr, Egfr-rs, mKIAA4242
Expression	Ubiquitous expression in ovary adult (RPKM 45.8), lung adult (RPKM 41.4) and 26 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

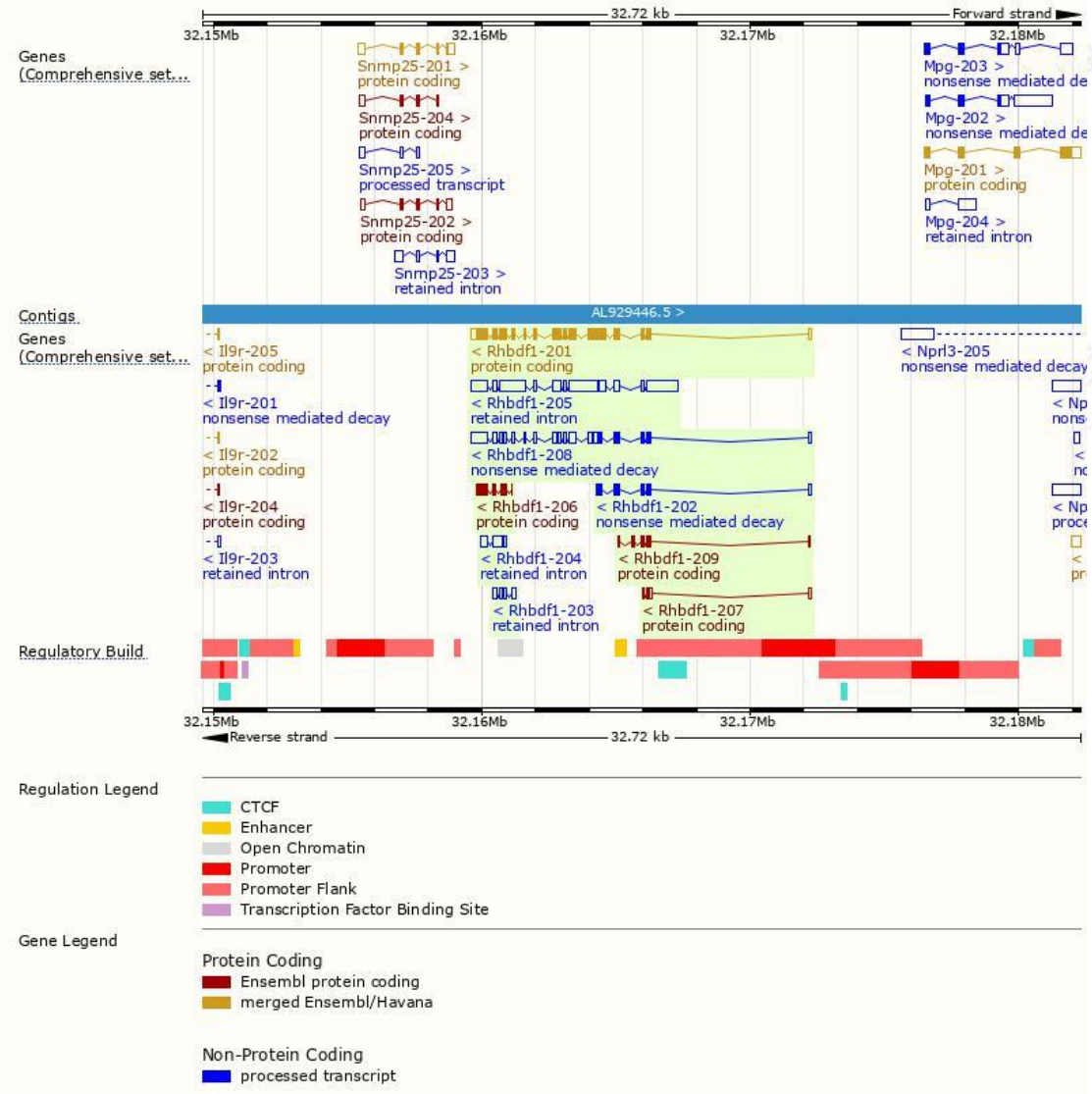
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rhbdf1-201	ENSMUST00000020524.15	2946	856aa	Protein coding	CCDS24519		TSL:1 , GENCODE basic , APPRIS P1 ,
Rhbdf1-206	ENSMUST00000143988.2	696	231aa	Protein coding	-		CDS 5' incomplete , TSL:5 ,
Rhbdf1-209	ENSMUST00000150381.2	509	136aa	Protein coding	-		CDS 3' incomplete , TSL:3 ,
Rhbdf1-207	ENSMUST00000144902.2	353	57aa	Protein coding	-		CDS 3' incomplete , TSL:3 ,
Rhbdf1-208	ENSMUST00000146179.8	2805	168aa	Nonsense mediated decay	-		TSL:1 ,
Rhbdf1-202	ENSMUST00000132578.8	708	168aa	Nonsense mediated decay	-		TSL:5 ,
Rhbdf1-205	ENSMUST00000143036.8	5089	No protein	Retained intron	-		TSL:2 ,
Rhbdf1-204	ENSMUST00000142274.2	709	No protein	Retained intron	-		TSL:3 ,
Rhbdf1-203	ENSMUST00000137125.2	490	No protein	Retained intron	-		TSL:5 ,

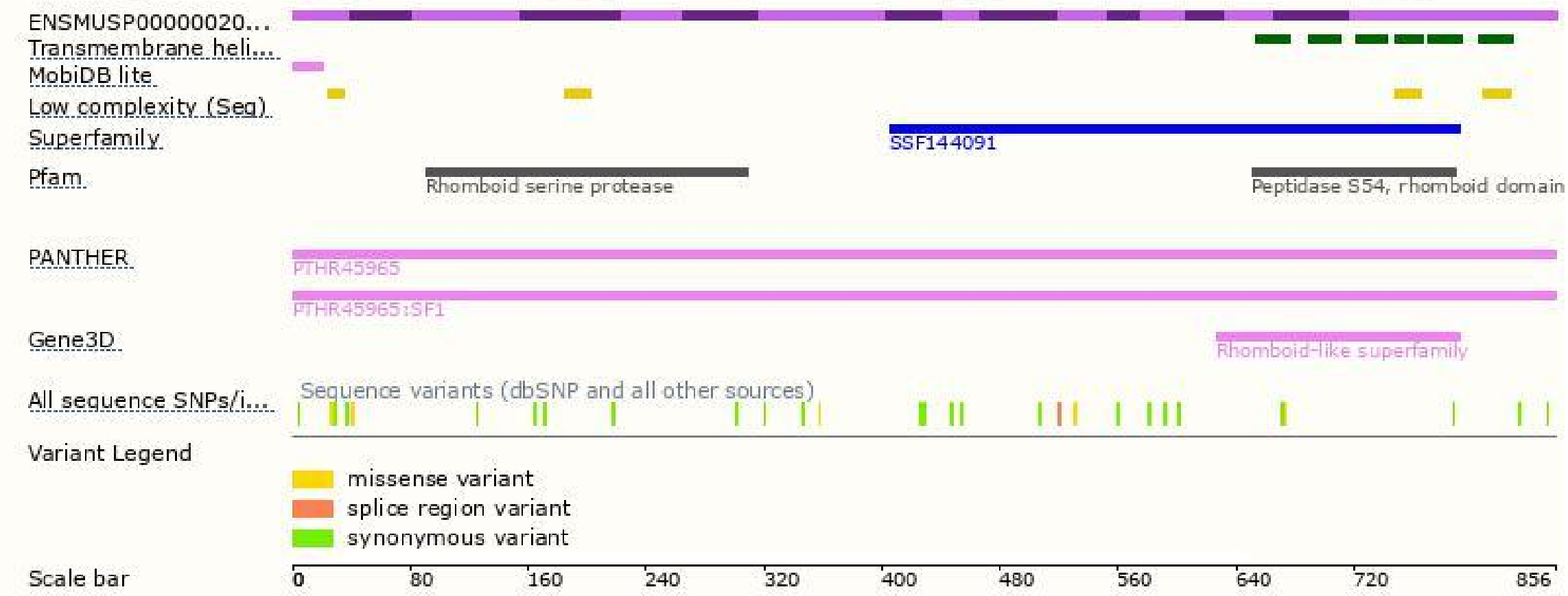
The strategy is based on the design of *Rhbdf1-201* transcript,the transcription is shown below:



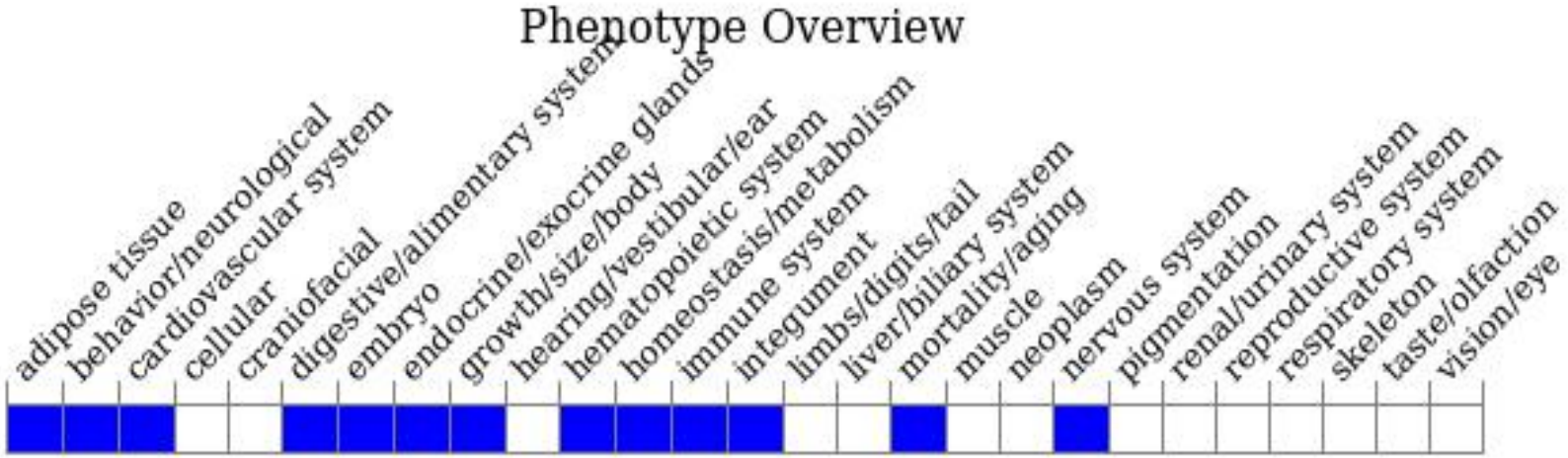
Genomic location distribution



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

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GemPharmatech Co.,Ltd