Ctsd Cas9-CKO Strategy

Designer: Jinling Wang

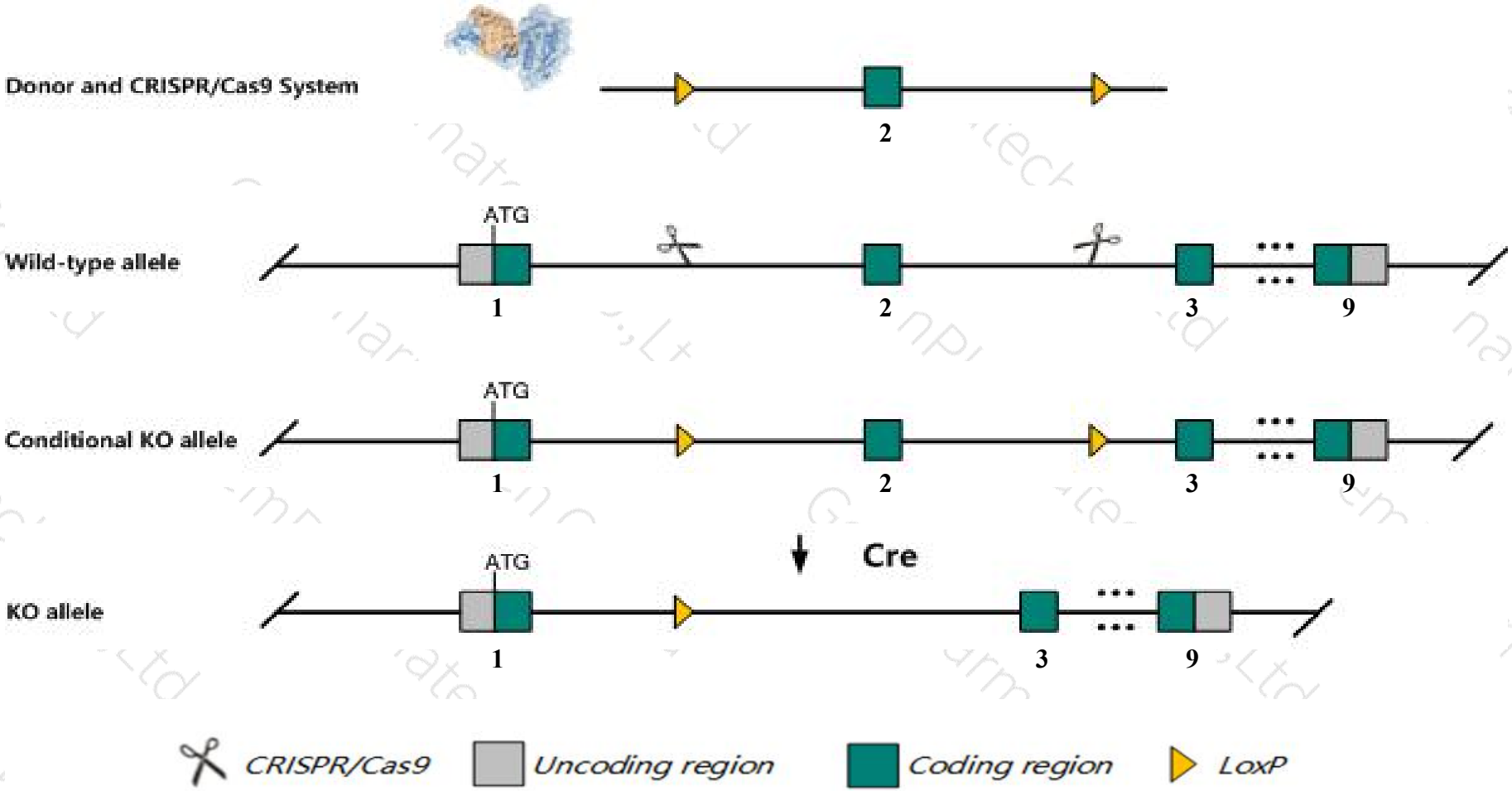
Design Date: 2019-9-30

Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Ctsd* gene has 3 transcripts. According to the structure of *Ctsd* gene, exon2 of *Ctsd-202* (ENSMUST00000151120.8) transcript is recommended as the knockout region.The region contains 160bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Ctsd* 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,Mice homozygous for a null mutation die in a state of anorexia at ~P26, displaying severe atrophy of the intestinal mucosa, and massive destruction of the thymus and spleen with loss of T and B cells; near the terminal stage, affected mice have seizures,display retinal atrophy, and become blind.

- The *Ctsd* 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

Ctsd cathepsin D [Mus musculus (house mouse)]

Gene ID: 13033, updated on 19-Mar-2019

Summary

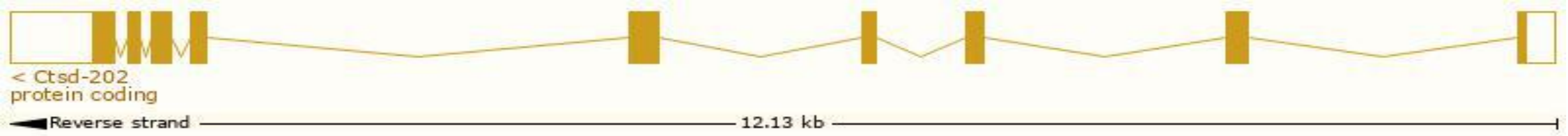
Official Symbol	Ctsd provided by MGI
Official Full Name	cathepsin D provided byMGI
Primary source	MGI:MGI:88562
See related	Ensembl:ENSMUSG00000007891
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	CD, CatD
Expression	Broad expression in subcutaneous fat pad adult (RPKM 1266.1), mammary gland adult (RPKM 682.4) and 23 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

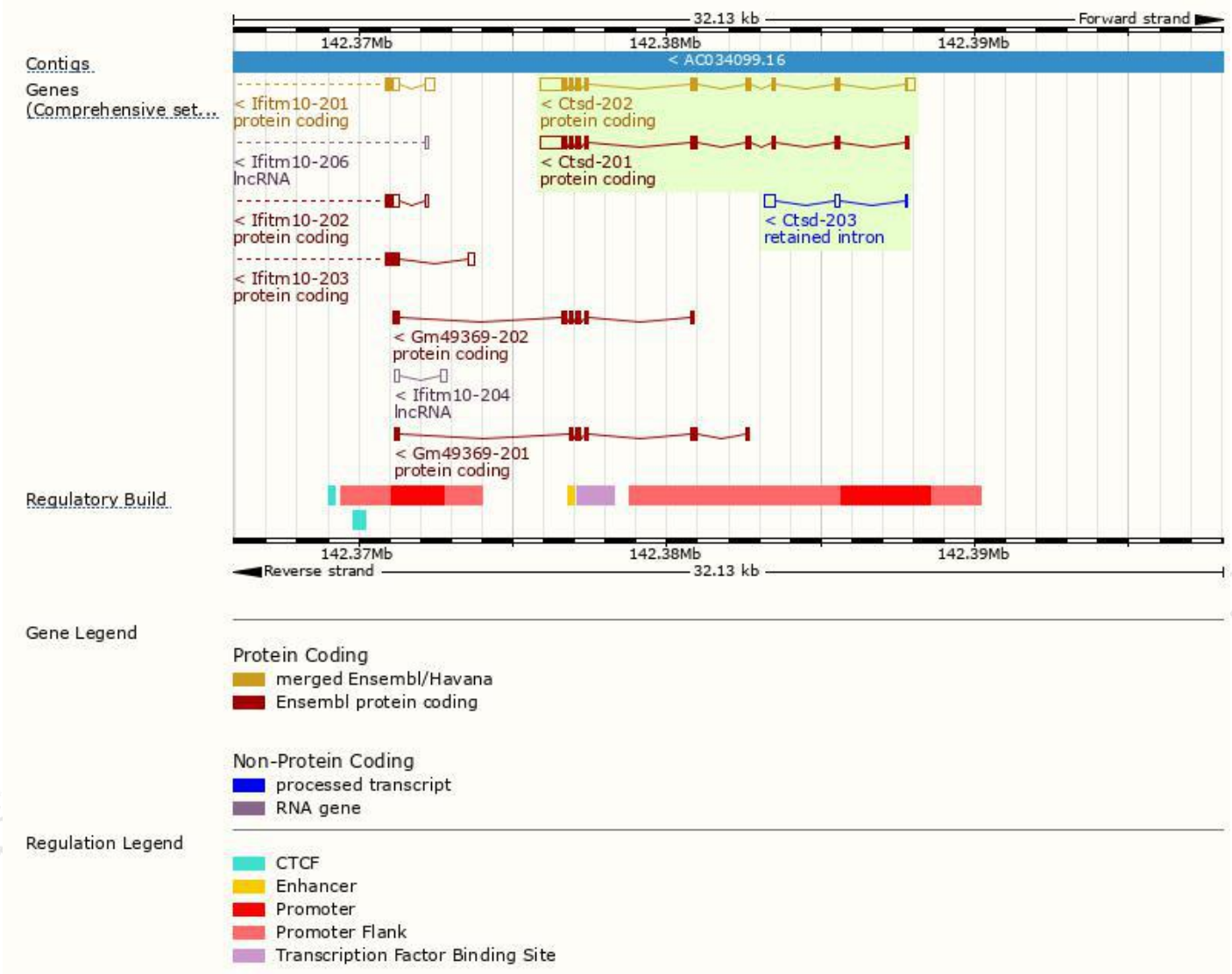
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ctsd-202	ENSMUST00000151120.8	2127	410aa	Protein coding	CCDS22029	P18242 Q3UCD9	TSL:1 GENCODE basic APPRIS P2
Ctsd-201	ENSMUST00000066401.6	1922	404aa	Protein coding	-	F8WIR1	TSL:1 GENCODE basic APPRIS ALT2
Ctsd-203	ENSMUST00000153679.1	591	No protein	Retained intron	-	-	TSL:2

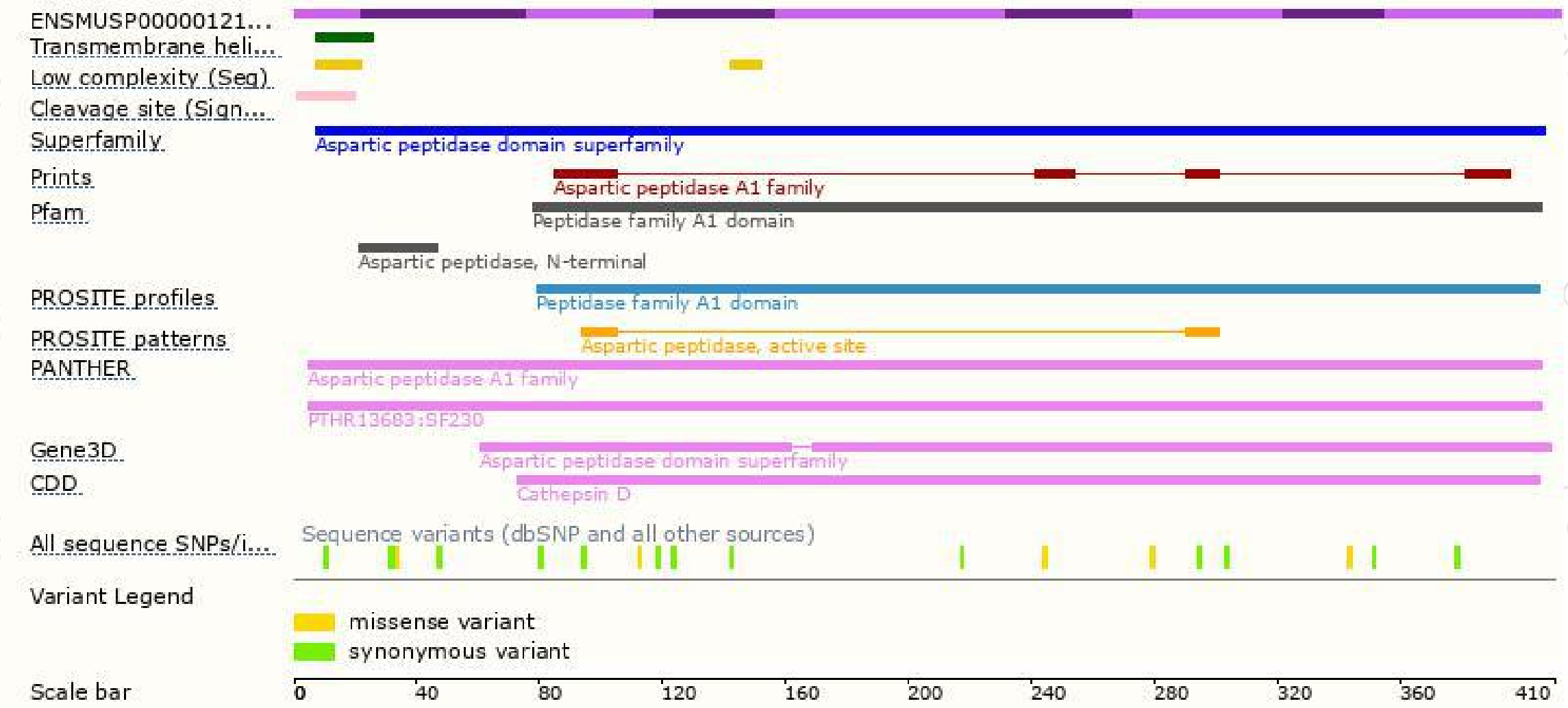
The strategy is based on the design of *Ctsd-202* transcript,The transcription is shown below



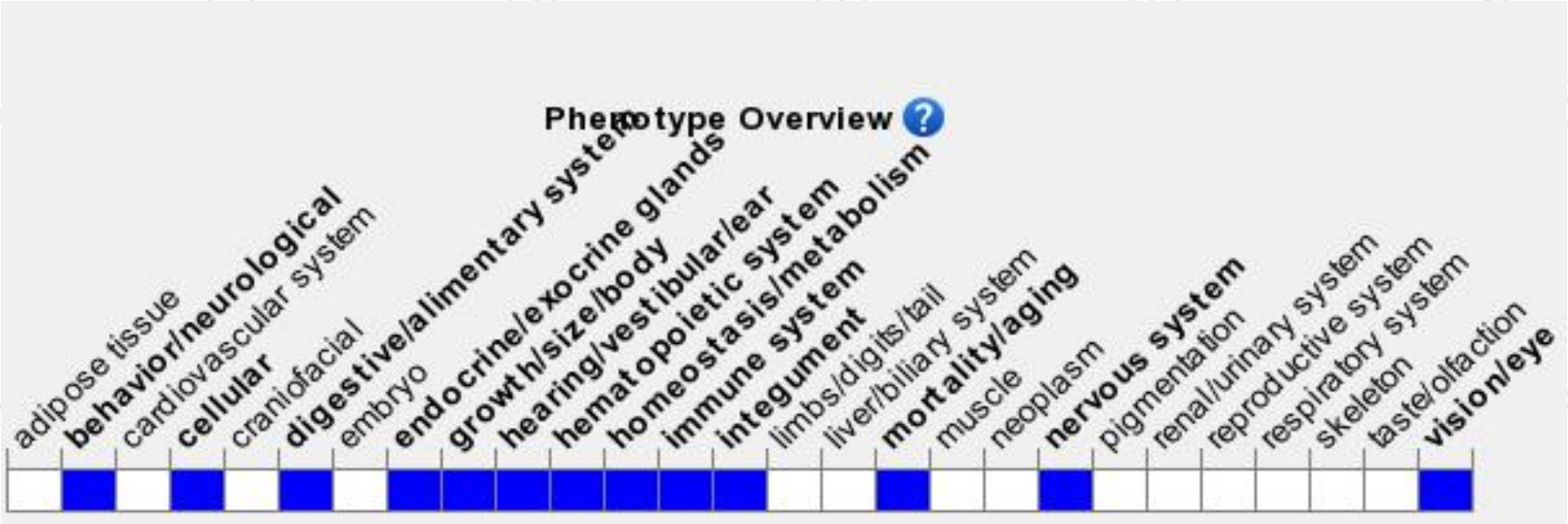
Genomic location distribution



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 die in a state of anorexia at ~P26, displaying severe atrophy of the intestinal mucosa, and massive destruction of the thymus and spleen with loss of T and B cells; near the terminal stage, affected mice have seizures,display retinal atrophy, and become blind.

集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd