

Anpep Cas9-CKO Strategy

Designer:

Daohua Xu

Reviewer:

Huimin Su

Design Date:

2019-10-18

Project Overview

Project Name

Anpep

Project type

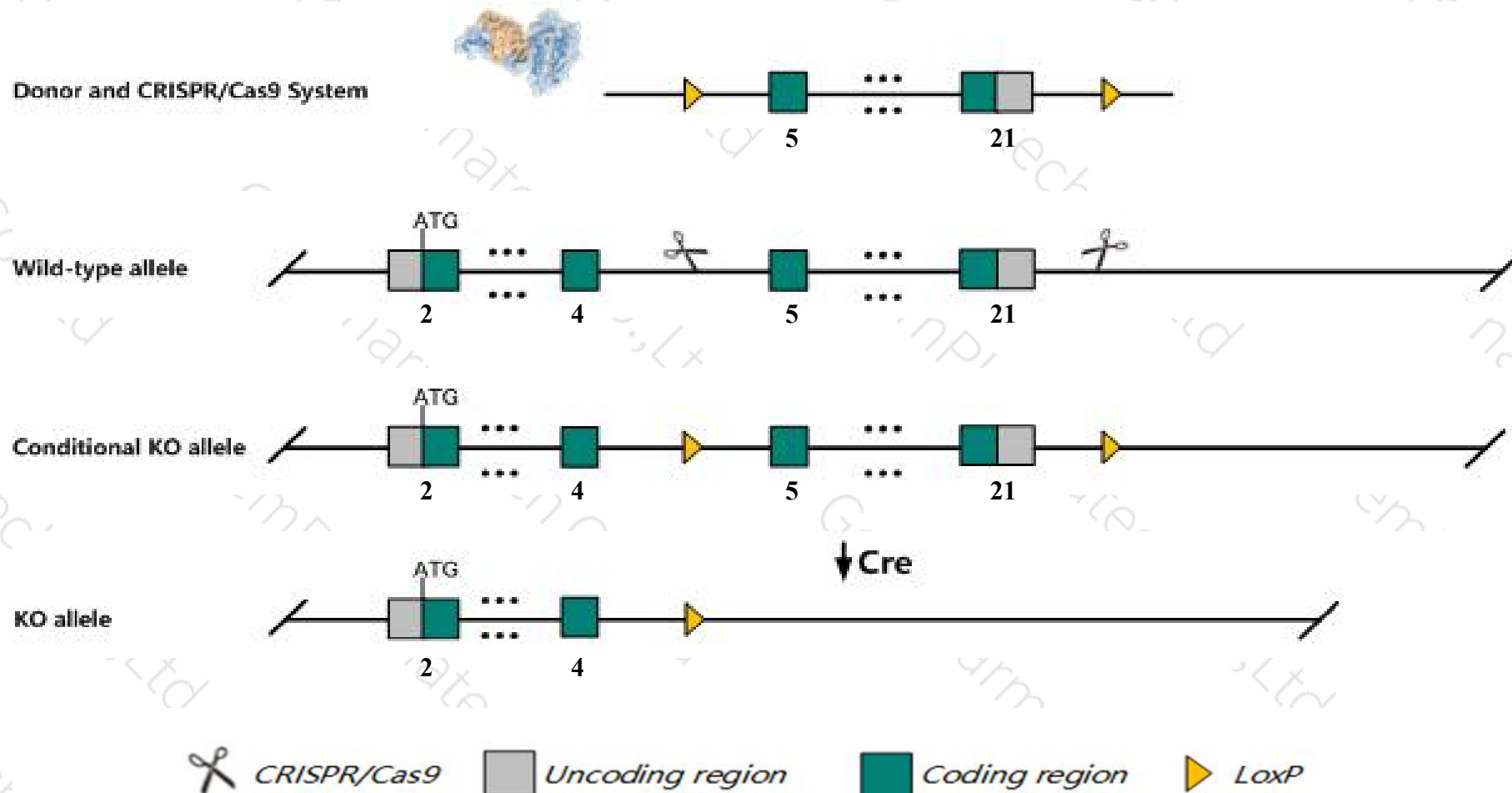
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- The *Anpep* gene has 6 transcripts. According to the structure of *Anpep* gene, exon5-exon21 of *Anpep*-202 (ENSMUST00000107392.7) transcript is recommended as the knockout region. The region contains most 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 *Anpep* 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.

- According to the existing MGI data, Mice homozygous for different knock-out alleles exhibit an increase in CD4+ thymocytes, altered macrophage adhesion, pathological neovascularization and/or altered mammary gland morphology during gestation.
- The *Anpep* 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)

Anpep alanyl (membrane) aminopeptidase [Mus musculus (house mouse)]

Gene ID: 16790, updated on 31-Jan-2019

Summary



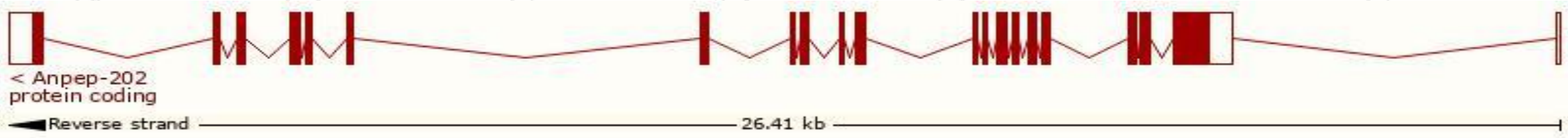
Official Symbol	Anpep provided by MGI
Official Full Name	alanyl (membrane) aminopeptidase provided by MGI
Primary source	MGI:MGI:5000466
See related	Ensembl:ENSMUSG00000039062
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AP-M, AP-N, Apn, Cd13, P150
Expression	Biased expression in large intestine adult (RPKM 1188.9), duodenum adult (RPKM 786.8) and 2 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

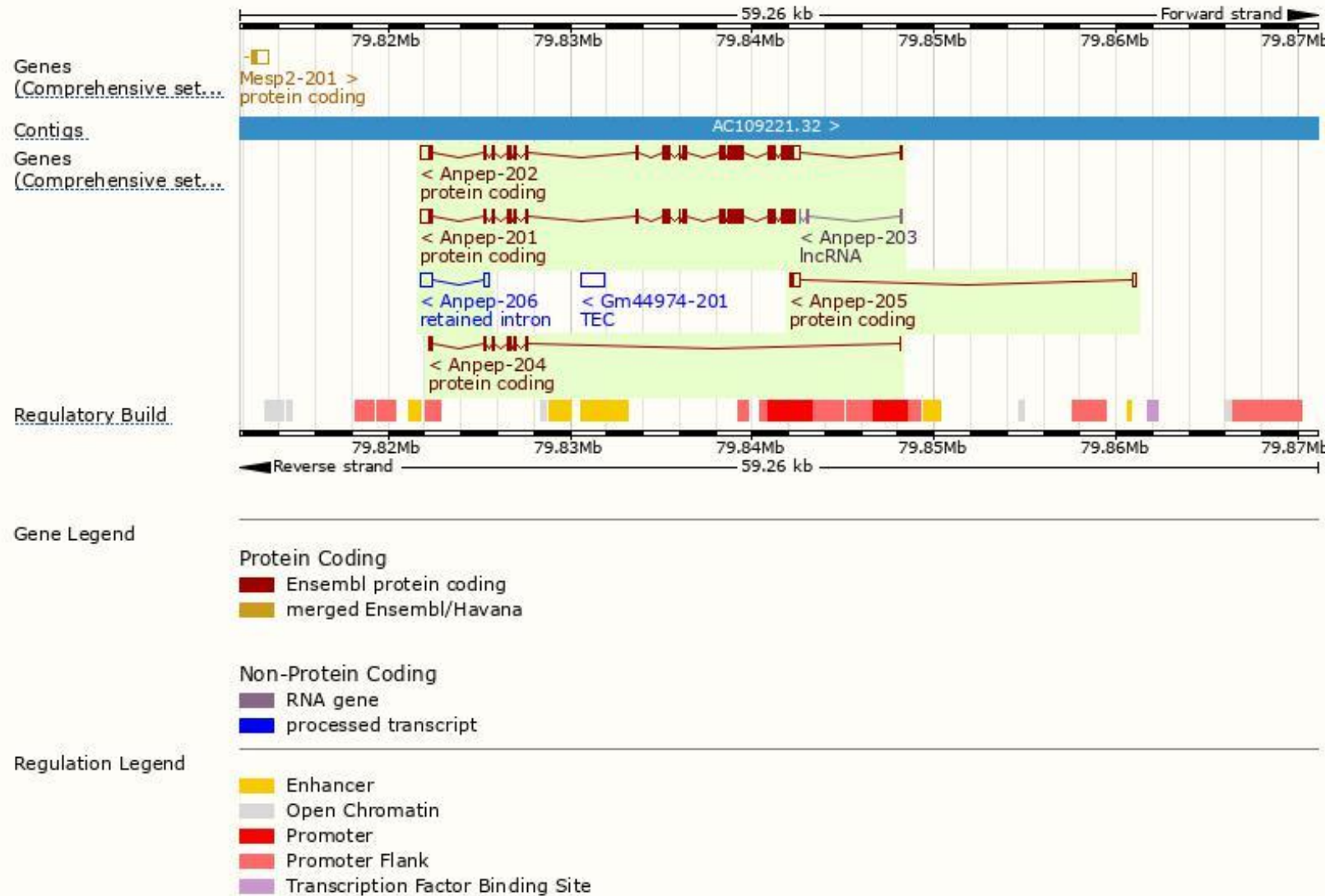
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Anpep-202	ENSMUST00000107392.7	3797	966aa	Protein coding	CCDS21388	P97449	TSL:1 GENCODE basic APPRIS P1
Anpep-201	ENSMUST00000049004.7	3422	966aa	Protein coding	CCDS21388	P97449	TSL:1 GENCODE basic APPRIS P1
Anpep-204	ENSMUST00000205502.1	746	207aa	Protein coding	-	A0A0U1RNS3	CDS 3' incomplete TSL:3
Anpep-205	ENSMUST00000206235.1	746	65aa	Protein coding	-	A0A0U1RPR2	CDS 3' incomplete TSL:3
Anpep-206	ENSMUST00000206682.1	770	No protein	Retained intron	-	-	TSL:2
Anpep-203	ENSMUST00000149164.1	178	No protein	lncRNA	-	-	TSL:3

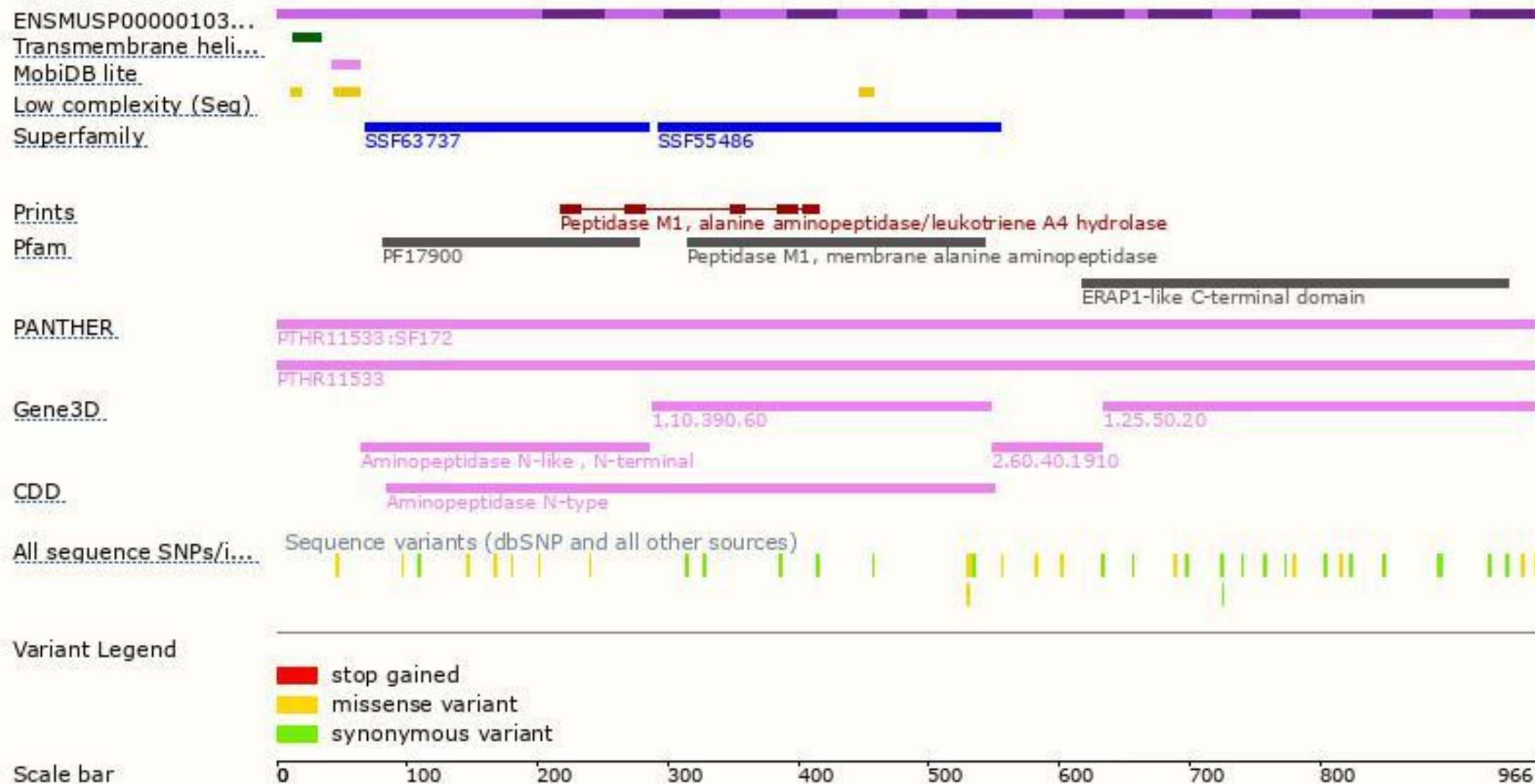
The strategy is based on the design of *Anpep-202* transcript,The transcription is shown below



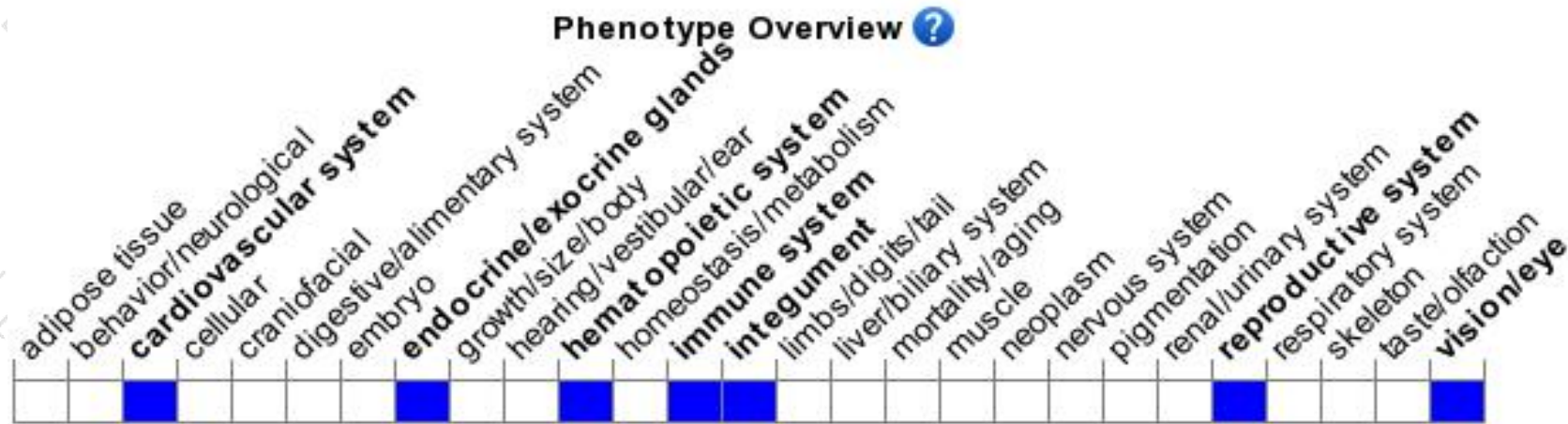
Genomic location distribution



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 different knock-out alleles exhibit an increase in CD4⁺ thymocytes, altered macrophage adhesion, pathological neovascularization and/or altered mammary gland morphology during gestation.

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

Tel: 400-9660890

