Xpnpep1 Cas9-CKO Strategy

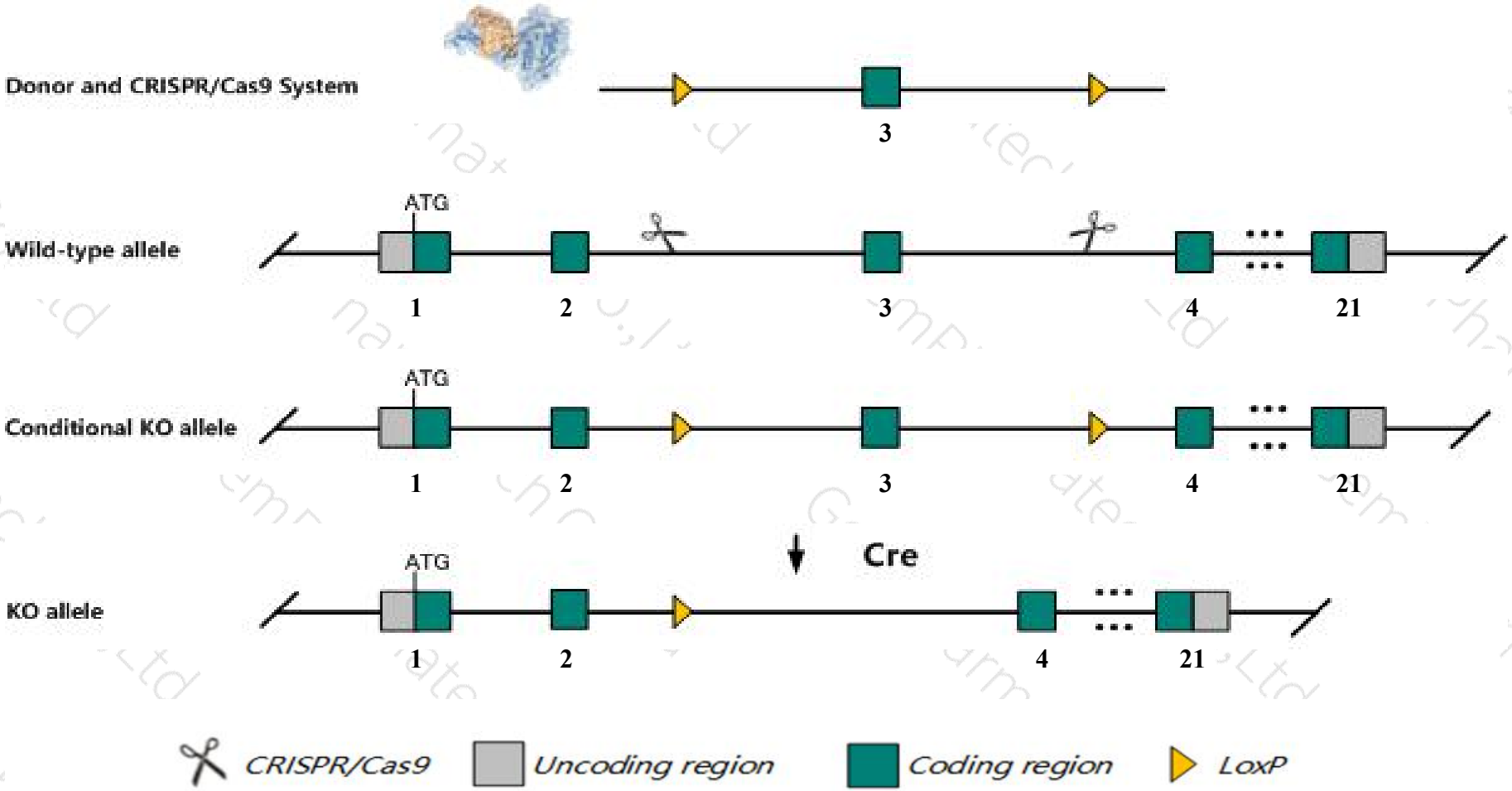
Designer: Huan Wang
Reviewer: Shanhong Tao
Design Date: 2020-8-4

Project Overview

Project Name	***Xpnpep1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Xpnpep1* gene. The schematic diagram is as follows:



Technical routes

- The *Xpnpep1* gene has 17 transcripts. According to the structure of *Xpnpep1* gene, exon3 of *Xpnpep1-209* (ENSMUST00000183108.7) transcript is recommended as the knockout region.The region contains 125bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Xpnpep1* 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 gene trap allele exhibit pre and postnatal lethality, reduced male survival, growth retardation with decreased body weight, size and length, microcephaly and peptiduria.
- The *Xpnpep1* gene is located on the Chr19. 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

Xpnpep1 X-prolyl aminopeptidase (aminopeptidase P) 1, soluble [Mus musculus (house mouse)]

Gene ID: 170750, updated on 20-Mar-2020

Summary

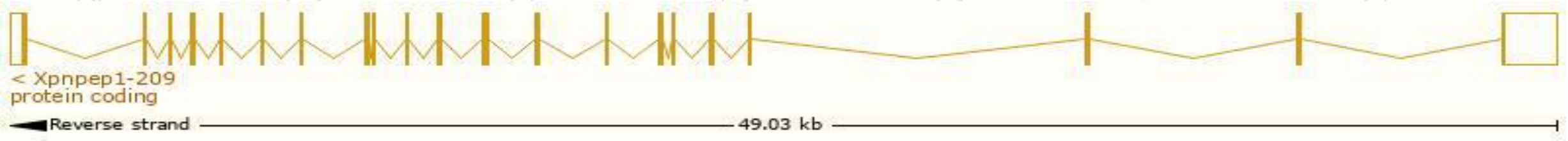
Official Symbol	Xpnpep1 provided by MGI
Official Full Name	X-prolyl aminopeptidase (aminopeptidase P) 1, soluble provided byMGI
Primary source	MGI:MGI:2180003
See related	Ensembl:ENSMUSG00000025027
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	D230045I08Rik, sAMP
Expression	Ubiquitous expression in large intestine adult (RPKM 157.6), small intestine adult (RPKM 86.1) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

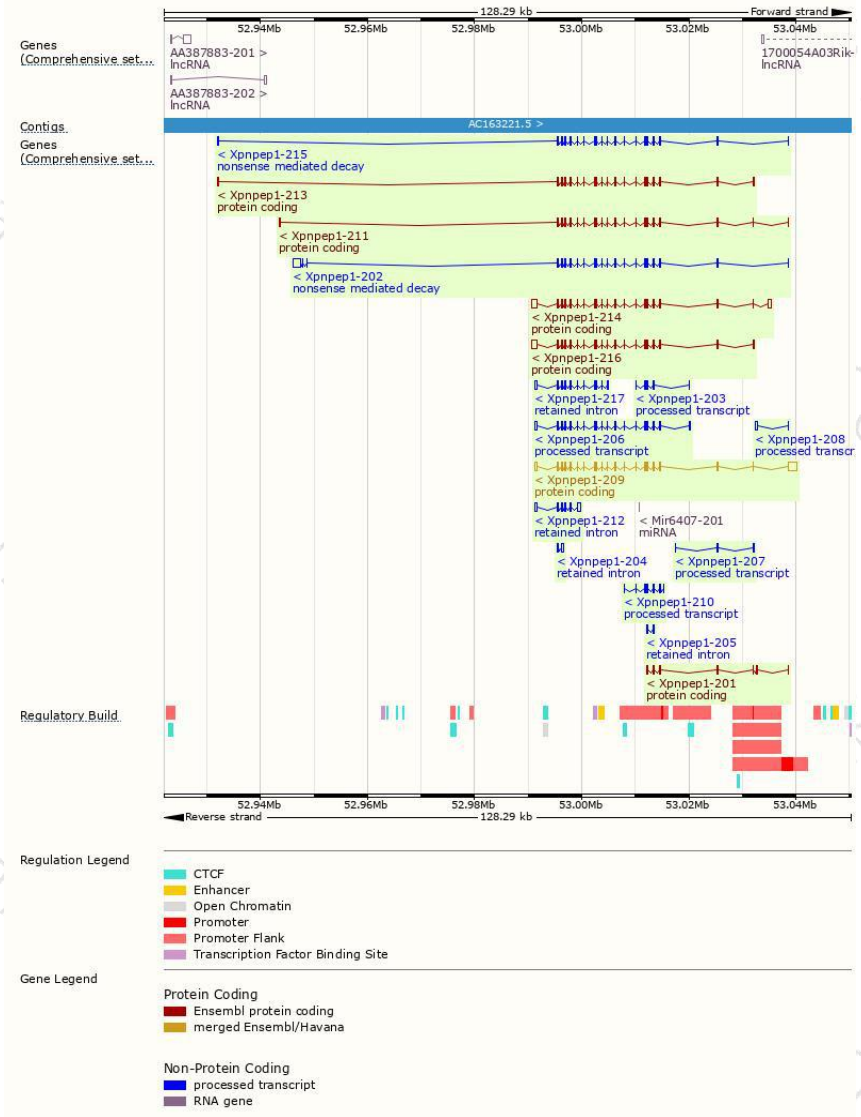
The gene has 17 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Xpnpep1-209	ENSMUST00000183108.7	4078	666aa	Protein coding	CCDS29899	Q3UE92	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P2
Xpnpep1-214	ENSMUST00000236058.1	3442	623aa	Protein coding	-	Q3UKF5 Q6P1B1	GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT1
Xpnpep1-216	ENSMUST00000237294.1	2956	623aa	Protein coding	-	Q3UKF5 Q6P1B1	GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT1
Xpnpep1-213	ENSMUST00000236008.1	2221	587aa	Protein coding	-	A0A494BBG8	GENCODE basic
Xpnpep1-211	ENSMUST00000183274.7	2120	635aa	Protein coding	-	S4R1I3	TSL:1 GENCODE basic
Xpnpep1-201	ENSMUST00000182097.1	698	119aa	Protein coding	-	S4R228	CDS 3' incomplete TSL:3
Xpnpep1-202	ENSMUST00000182500.7	3307	59aa	Nonsense mediated decay	-	S4R167	TSL:1
Xpnpep1-215	ENSMUST00000236456.1	2216	59aa	Nonsense mediated decay	-	S4R167	
Xpnpep1-206	ENSMUST00000182877.7	2475	No protein	Processed transcript	-	-	TSL:1
Xpnpep1-210	ENSMUST00000183188.7	689	No protein	Processed transcript	-	-	TSL:5
Xpnpep1-203	ENSMUST00000182534.1	585	No protein	Processed transcript	-	-	TSL:3
Xpnpep1-208	ENSMUST00000182939.1	378	No protein	Processed transcript	-	-	TSL:3
Xpnpep1-207	ENSMUST00000182887.1	350	No protein	Processed transcript	-	-	TSL:5
Xpnpep1-212	ENSMUST00000235453.1	1679	No protein	Retained intron	-	-	
Xpnpep1-217	ENSMUST00000238001.1	1422	No protein	Retained intron	-	-	
Xpnpep1-204	ENSMUST00000182728.1	541	No protein	Retained intron	-	-	TSL:3
Xpnpep1-205	ENSMUST00000182844.1	306	No protein	Retained intron	-	-	TSL:5

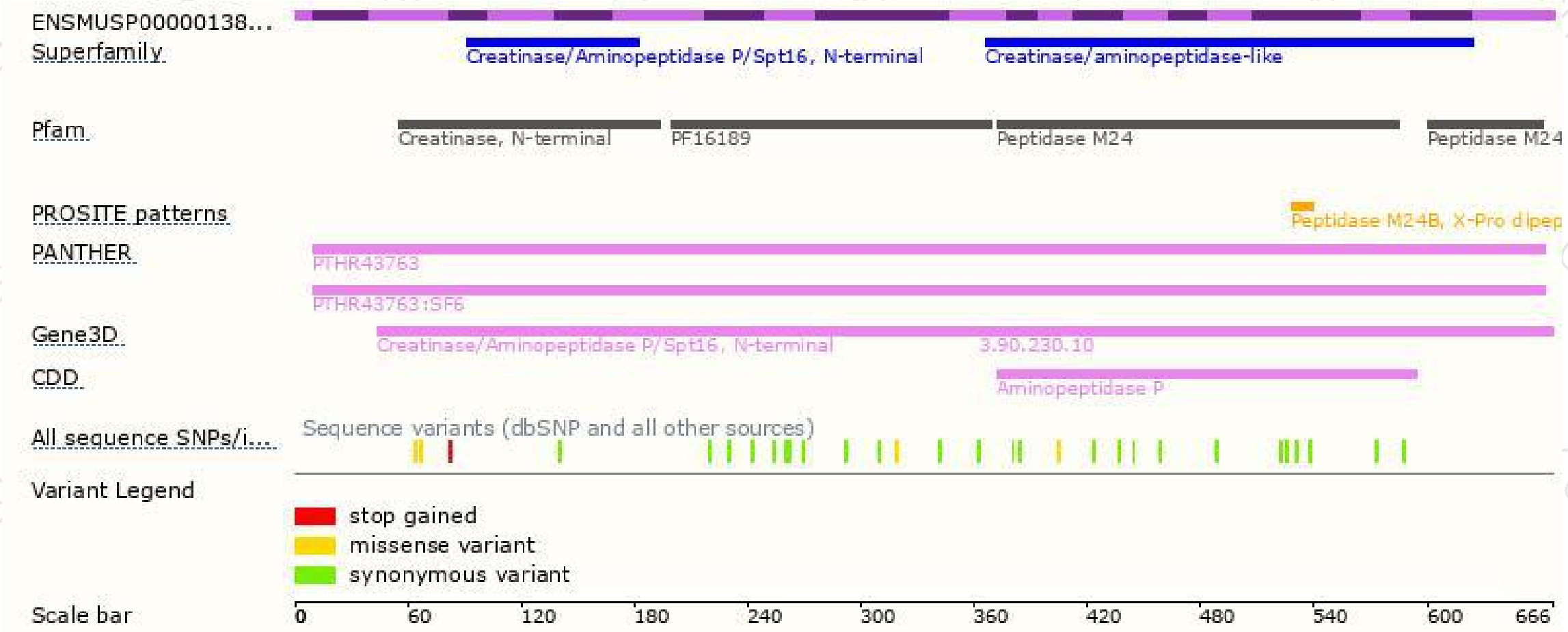
The strategy is based on the design of *Xpnpep1-209* transcript,the transcription is shown below



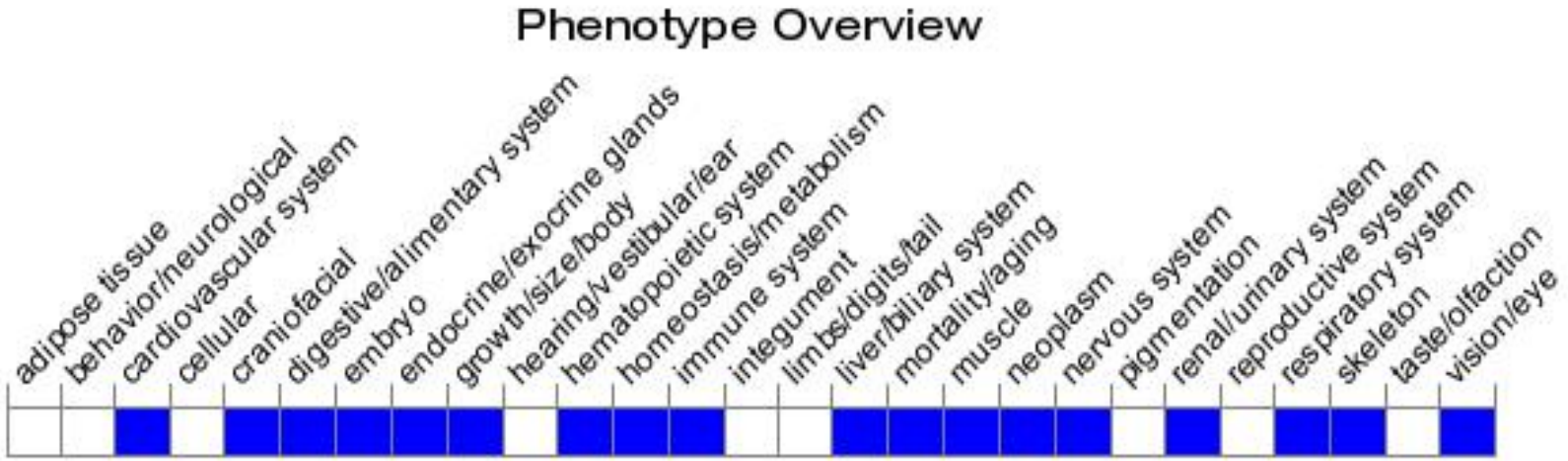
Genomic location distribution



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 gene trap allele exhibit pre and postnatal lethality, reduced male survival, growth retardation with decreased body weight, size and length, microcephaly and peptiduria.

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