

Enpep Cas9-CKO Strategy

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Project Overview

Project Name

Enpep

Project type

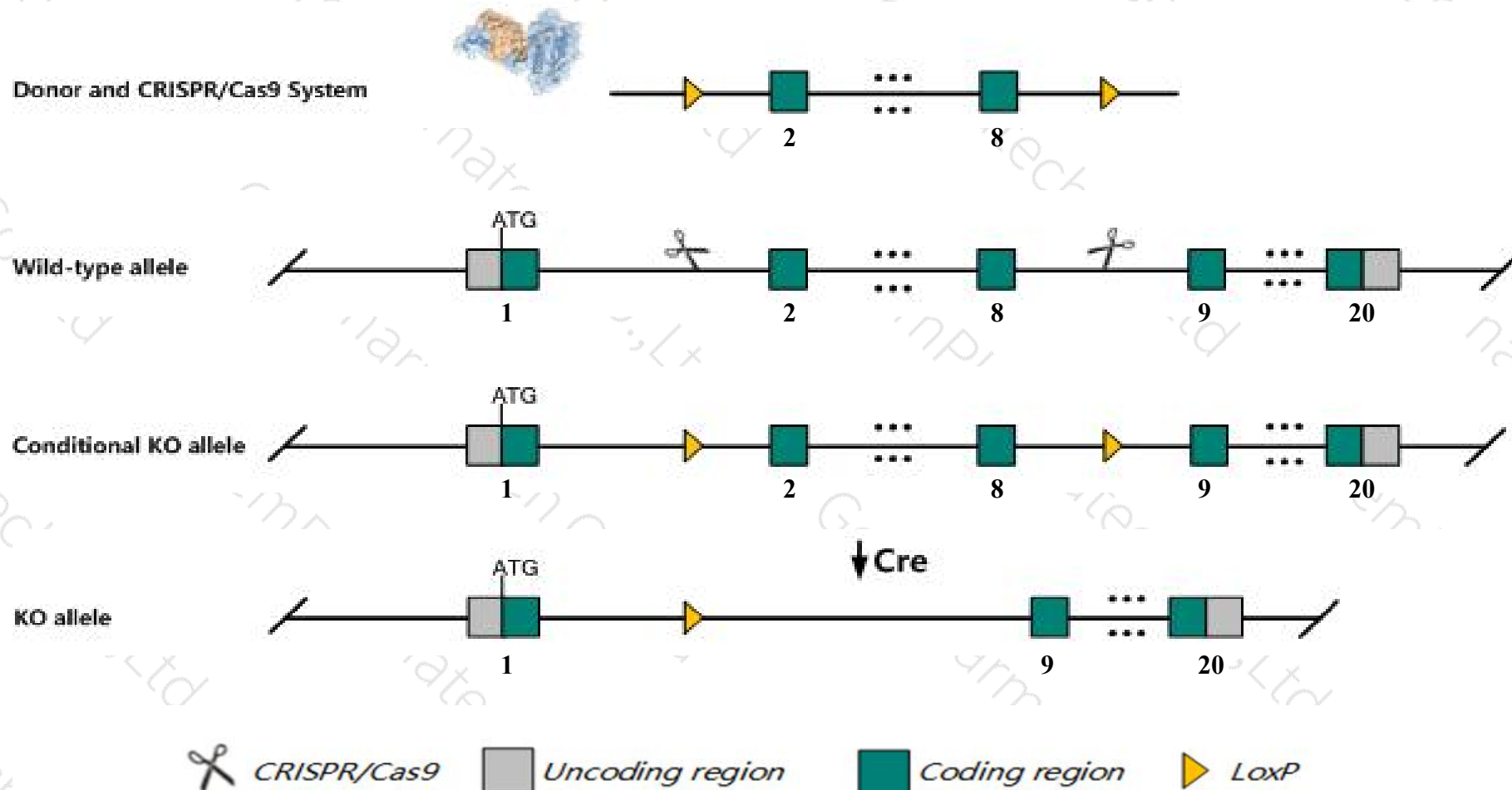
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



Technical routes

- The *Enpep* gene has 5 transcripts. According to the structure of *Enpep* gene, exon2-exon8 of *Enpep*-201 (ENSMUST00000029658.13) transcript is recommended as the knockout region. The region contains 865bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Enpep* 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 a targeted null mutation are viable, fertile and morphologically unaffected with normal B and T cell development.
- Transcript *Enpep*-202 may not be affected.
- The *Enpep* gene is located on the Chr3. 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)

Enpep glutamyl aminopeptidase [*Mus musculus* (house mouse)]

Gene ID: 13809, updated on 17-Dec-2019

Summary

- Official Symbol** Enpep provided by MGI
- Official Full Name** glutamyl aminopeptidase provided by MGI
- Primary source** MGI:MGI:106645
- See related** Ensembl:ENSMUSG00000028024
- 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** APA; Ly51; Ly-51; Bp-1/6C3; 6030431M22Rik
- Expression** Biased expression in large intestine adult (RPKM 77.9), subcutaneous fat pad adult (RPKM 16.1) and 9 other tissues [See more](#)
- Orthologs** [human](#) [all](#)

Genomic context

Location: 3 G3; 3 57.92 cM See Enpep in [Genome Data Viewer](#)

Exon count: 20

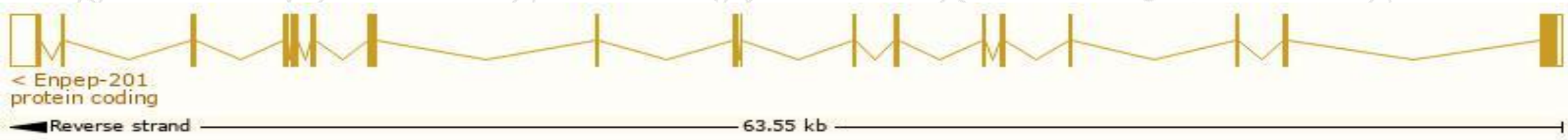
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_0000001635.26)	3	NC_000069.6 (129269177..129332749, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_0000001635.18)	3	NC_000069.5 (128972095..129035667, complement)

Transcript information (Ensembl)

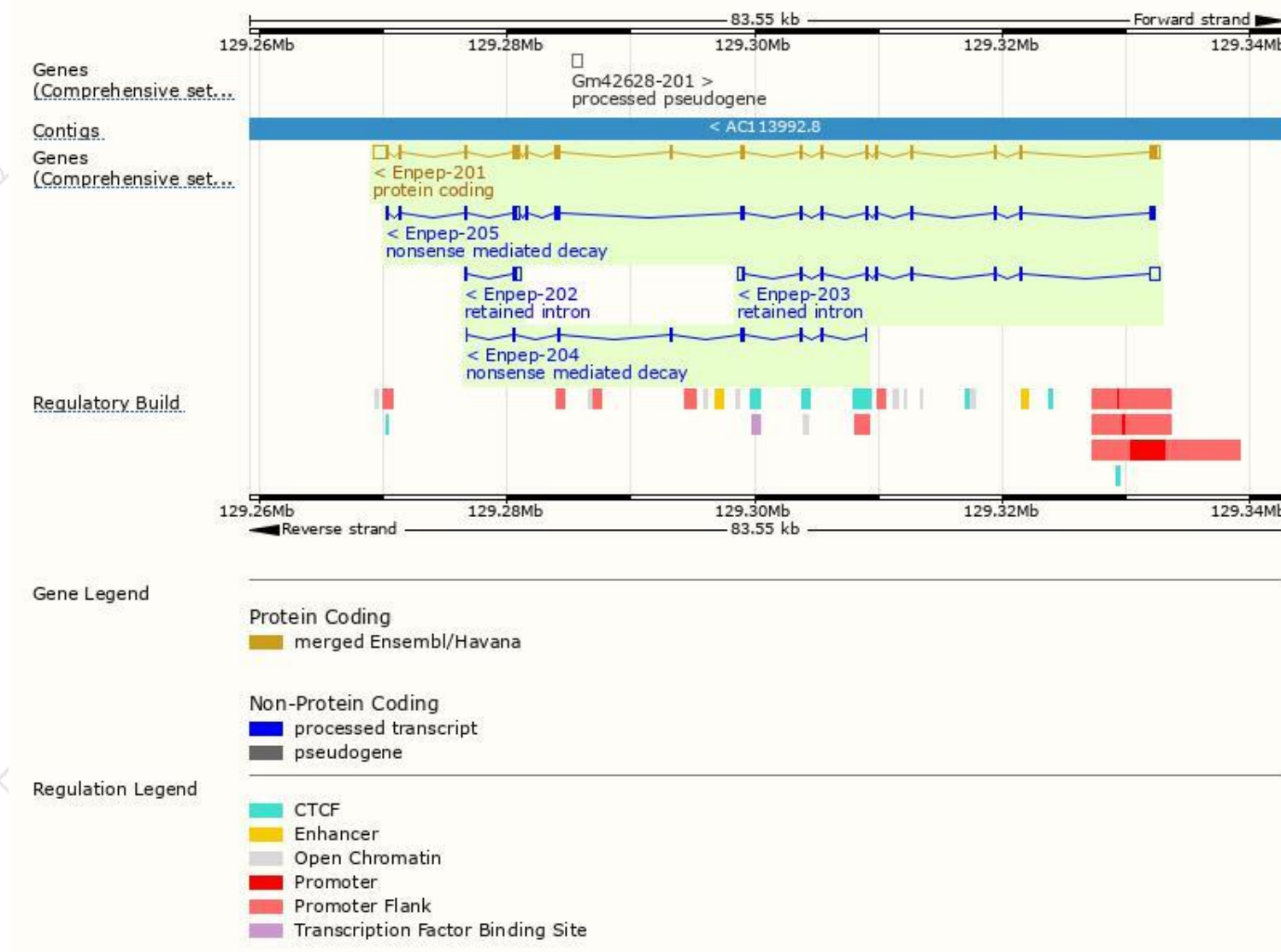
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Enpep-201	ENSMUST00000029658.13	4181	945aa	Protein coding	CCDS17831	P16406 Q52JJ6	TSL:1 GENCODE basic APPRIS P1
Enpep-205	ENSMUST00000170918.7	2655	501aa	Nonsense mediated decay	-	F7B9G4	CDS 5' incomplete TSL:5
Enpep-204	ENSMUST00000169240.1	836	235aa	Nonsense mediated decay	-	F6YHW2	CDS 5' incomplete TSL:5
Enpep-203	ENSMUST00000165217.1	2178	No protein	Retained intron	-	-	TSL:1
Enpep-202	ENSMUST00000164443.1	780	No protein	Retained intron	-	-	TSL:3

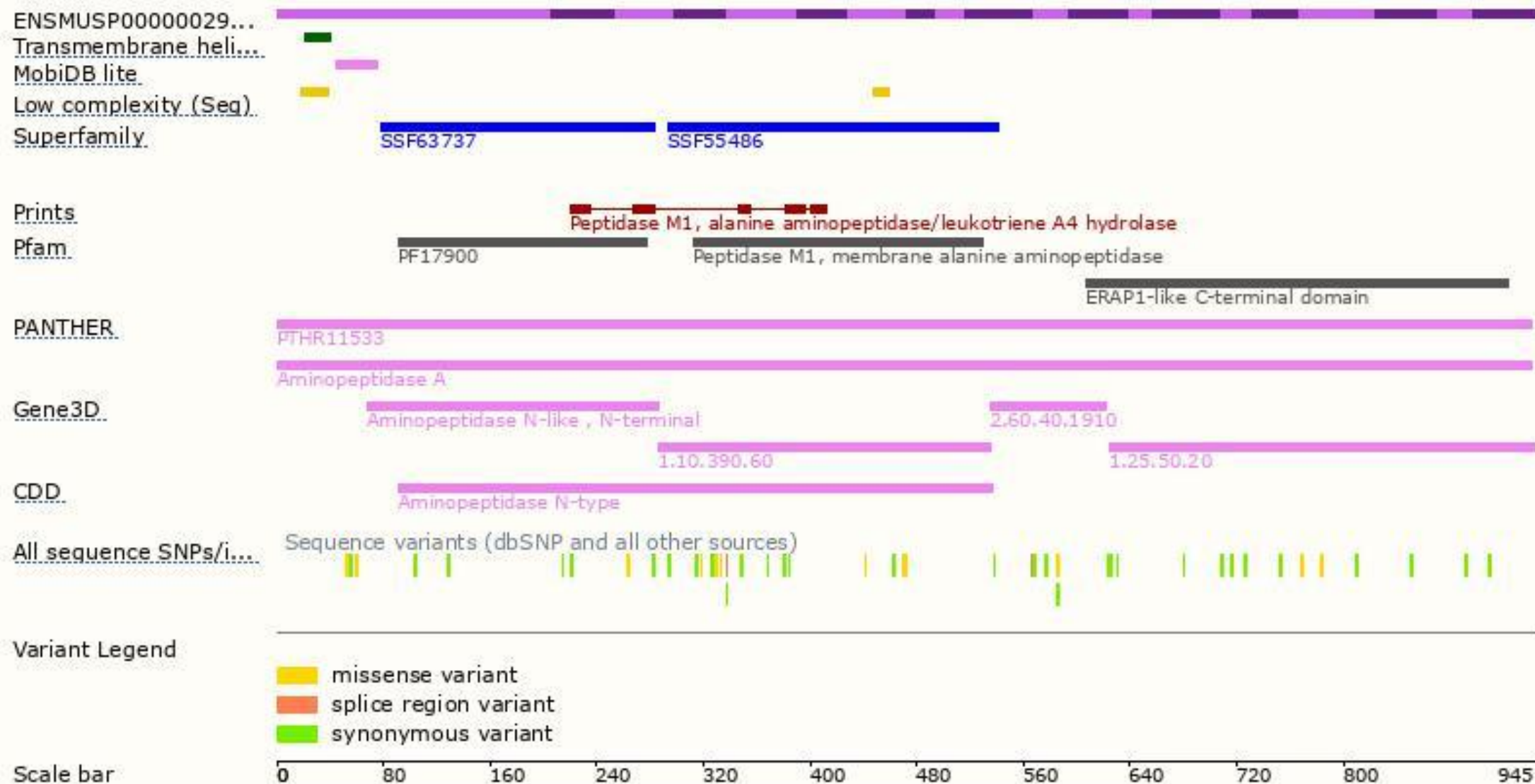
The strategy is based on the design of *Enpep-201* transcript,The transcription is shown below



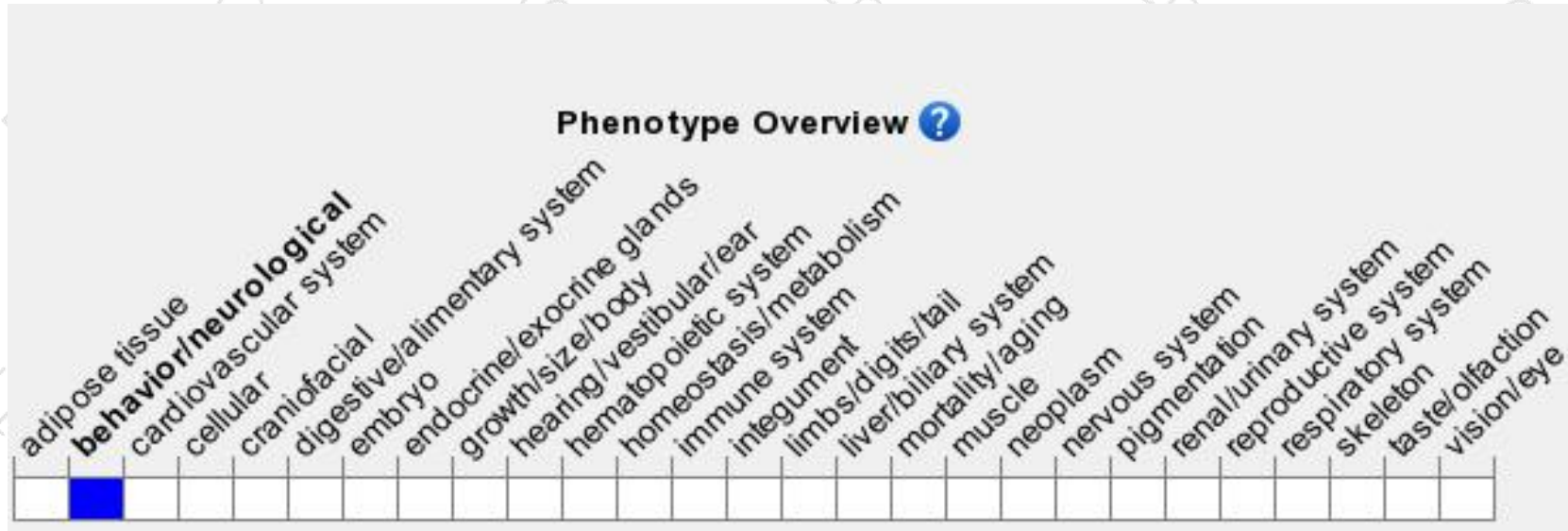
Genomic location distribution



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/>).

According to the existing MGI data, Mice homozygous for a targeted null mutation are viable, fertile and morphologically unaffected with normal B and T cell development.

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

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