

***Klk4* Cas9-CKO Strategy**

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Reviewer:

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Project Overview

Project Name

Klk4

Project type

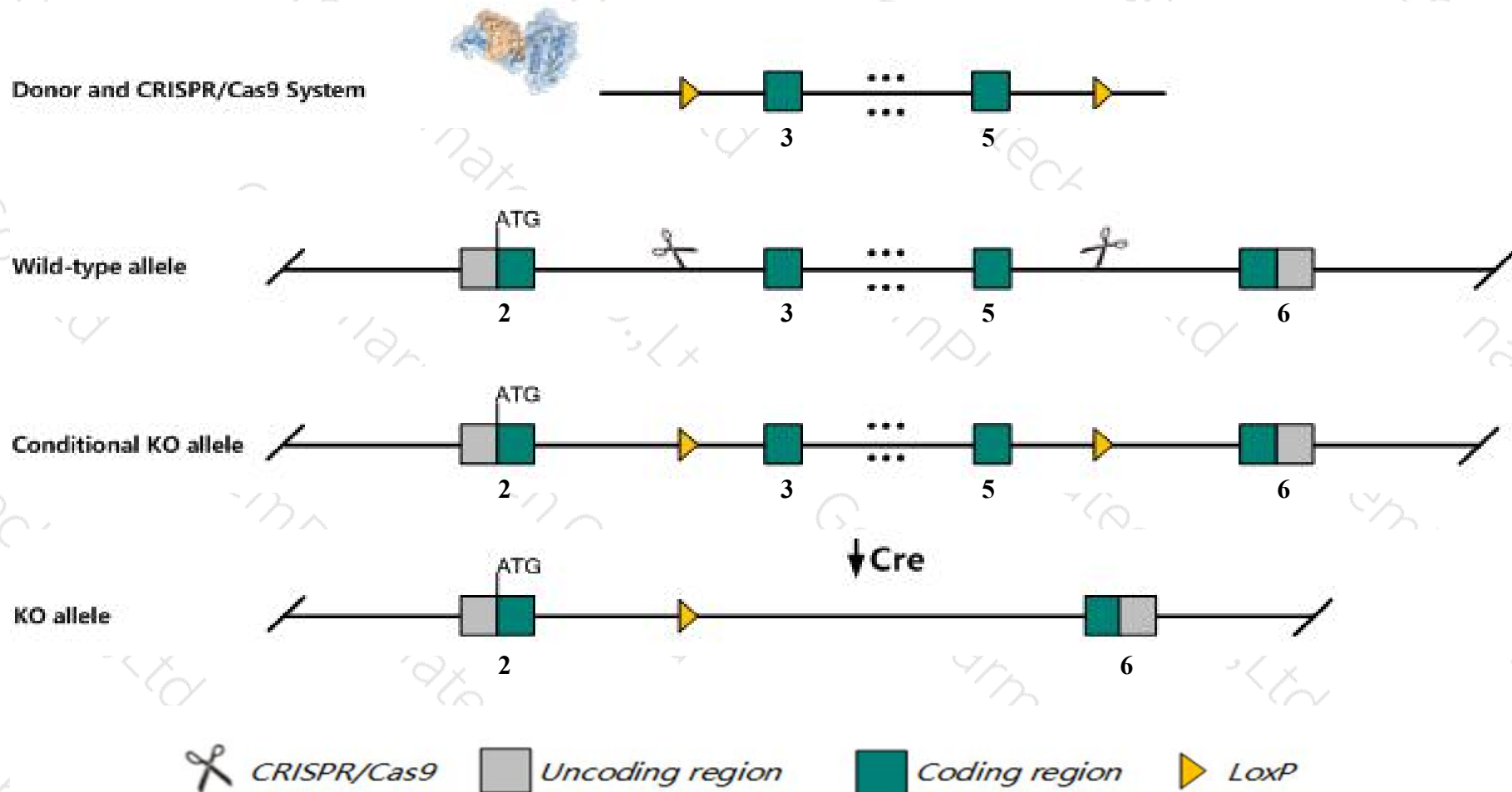
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Klk4* gene. The schematic diagram is as follows:



Technical routes

- The *Klk4* gene has 1 transcript. According to the structure of *Klk4* gene, exon3-exon5 of *Klk4-201* (ENSMUST00000007161.7) transcript is recommended as the knockout region. The region contains 551bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Klk4* 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 null allele exhibit reduced enamel strength that leads to enamel fracturing, delayed postnatal growth, and decreased survival to maturity.
- The *Klk4* 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)

Klk4 kallikrein related-peptidase 4 (prostase, enamel matrix, prostate) [Mus musculus (house mouse)]

Gene ID: 56640, updated on 31-Jan-2019

Summary



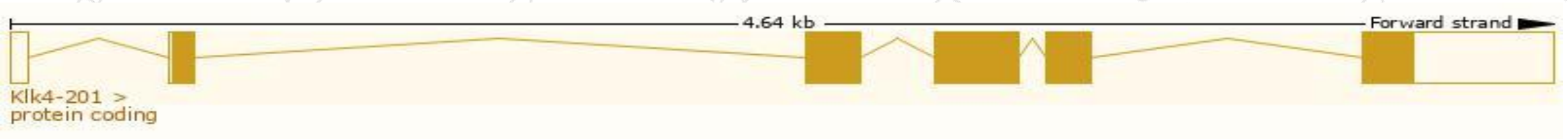
Official Symbol	Klk4 provided by MGI
Official Full Name	kallikrein related-peptidase 4 (prostase, enamel matrix, prostate) provided by MGI
Primary source	MGI:MGI:1861379
See related	Ensembl:ENSMUSG00000006948
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	ESMP1, KLK-L1, PSTS, Prss17
Expression	Low expression observed in reference dataset See more
Orthologs	human all

Transcript information (Ensembl)

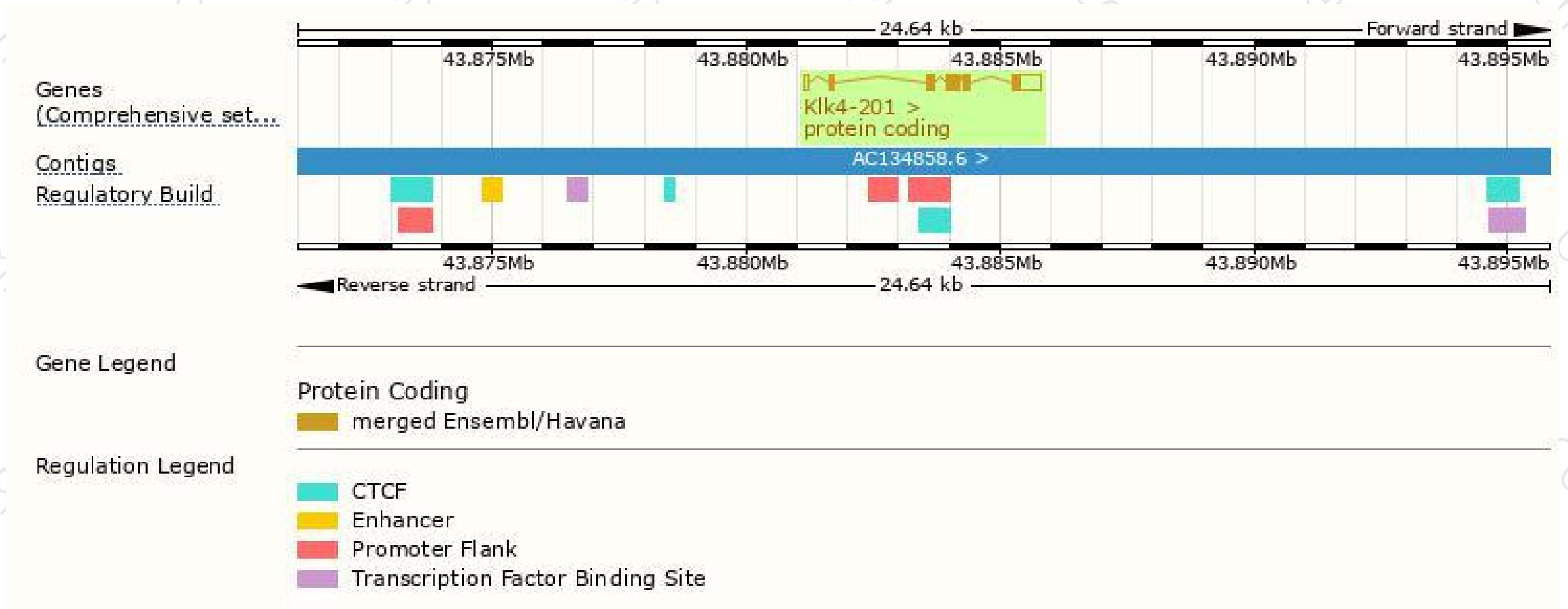
The gene has 1 transcript, and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Klk4-201	ENSMUST00000007161.7	1249	255aa	Protein coding	CCDS21186	A0A1R3UDT0_Q9Z0M1	TSL:1 GENCODE basic APPRIS P1

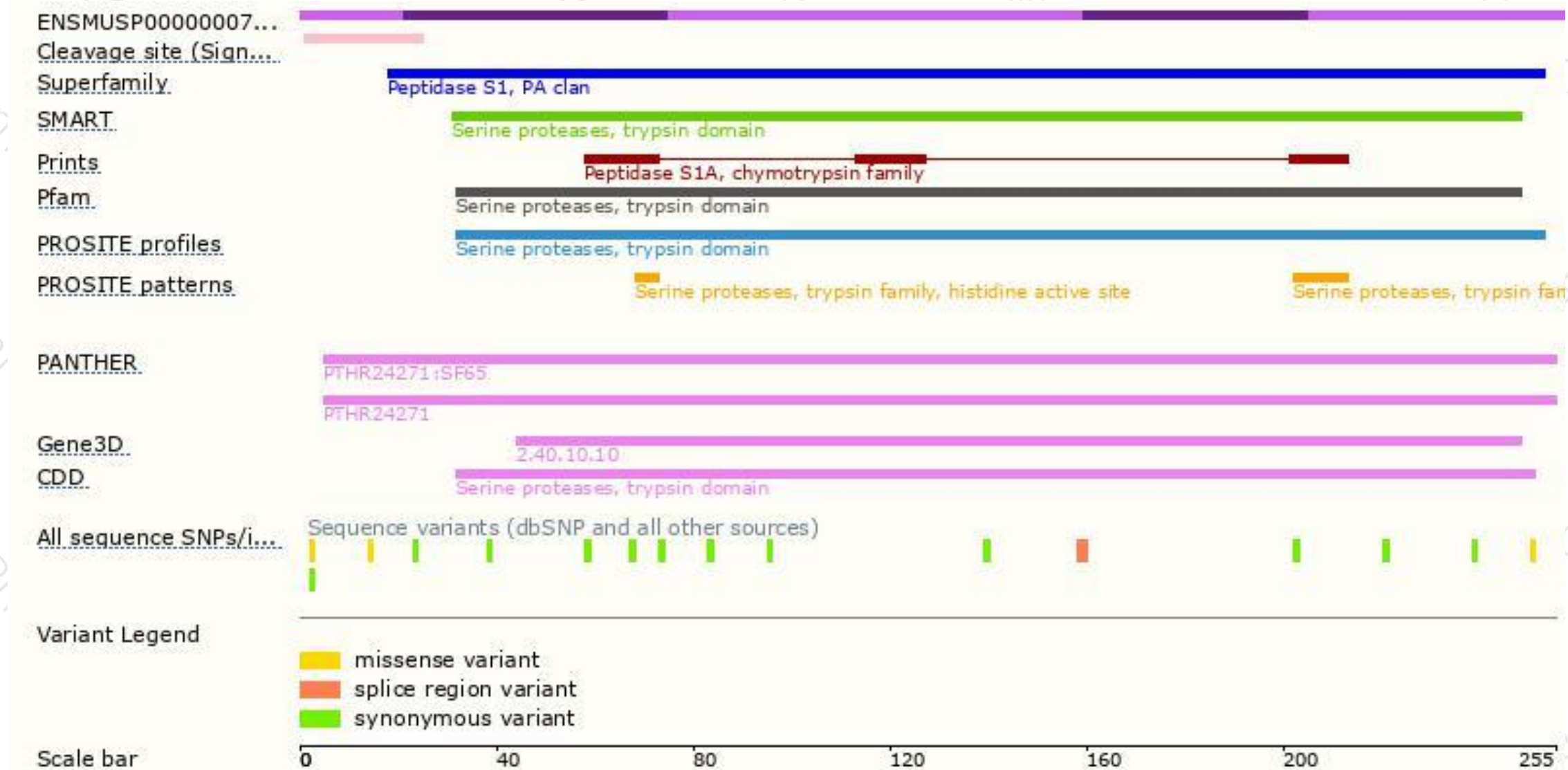
The strategy is based on the design of *Klk4-201* transcript, The transcription is shown below



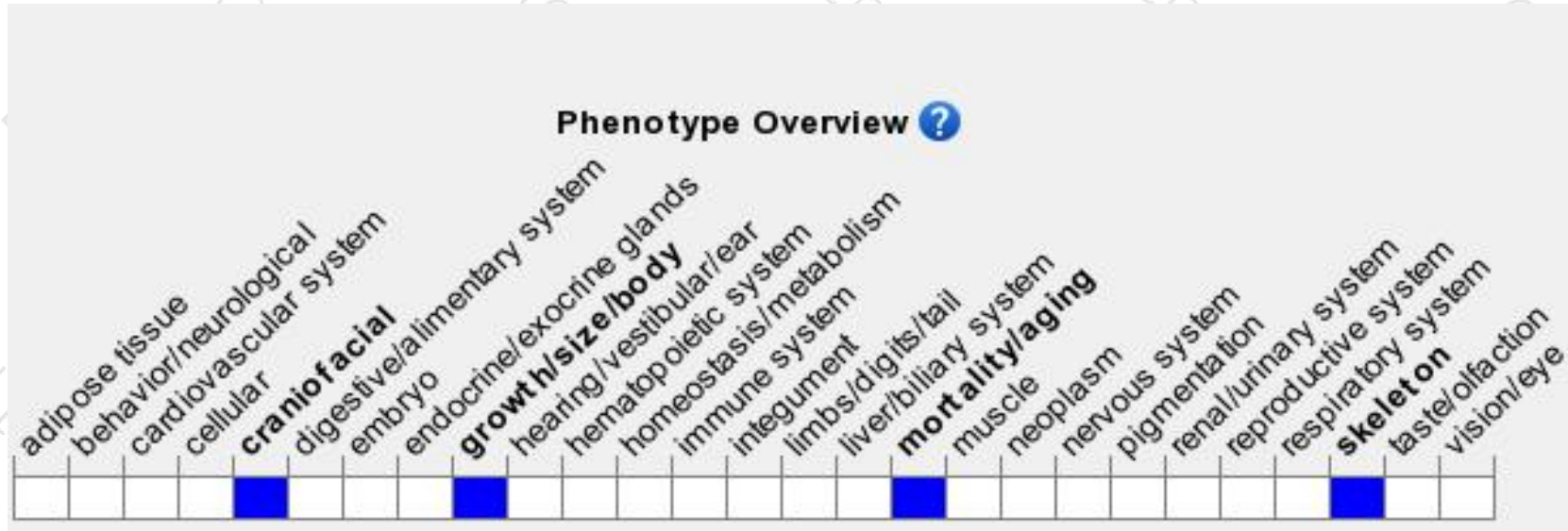
Genomic location distribution



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 null allele exhibit reduced enamel strength that leads to enamel fracturing, delayed postnatal growth, and decreased survival to maturity.

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

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