

***Grk5* Cas9-CKO Strategy**

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Reviewer: Huan Wang

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Project Overview

Project Name

Grk5

Project type

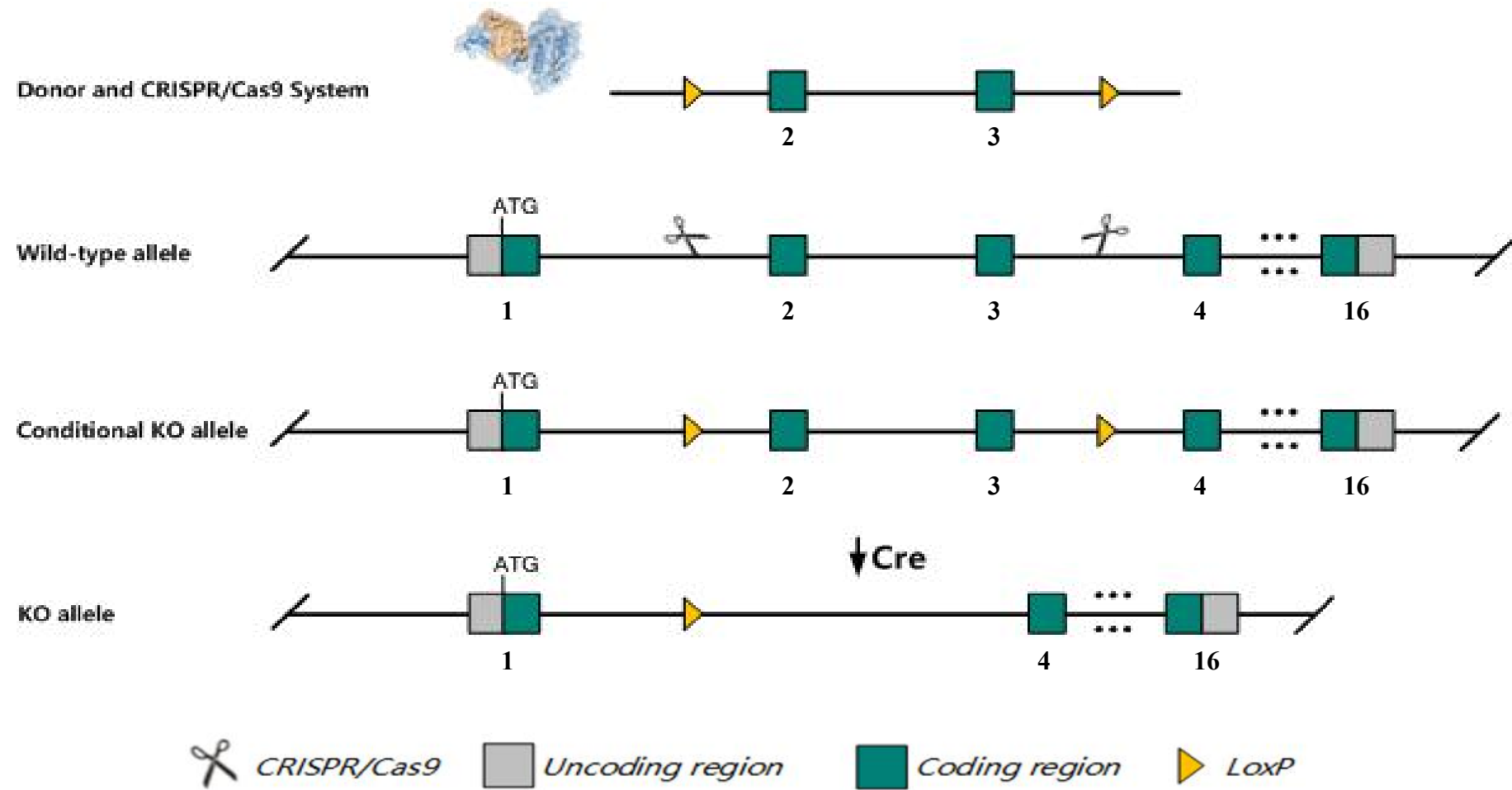
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Grk5* gene. The schematic diagram is as follows:



The *Grk5* gene has 3 transcripts. According to the structure of *Grk5* gene, exon2-exon3 of *Grk5-201* (ENSMUST00000003313.9) transcript is recommended as the knockout region. The region contains 209bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Grk5* 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, homozygous mutation of this gene results in a decrease in thermal pain sensation. mice homozygous for a knock-out allele exhibit decreased response of heart to induced stress. *Gm22520* will be deleted.

The *Grk5* 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.

Grk5 G protein-coupled receptor kinase 5 [Mus musculus (house mouse)]

Gene ID: 14773, updated on 13-Mar-2020

Summary**Official Symbol** Grk5 provided by [MGI](#)**Official Full Name** G protein-coupled receptor kinase 5 provided by [MGI](#)**Primary source** [MGI:MGI:109161](#)**See related** [Ensembl:ENSMUSG00000003228](#)**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** Gprk5**Expression** Ubiquitous expression in lung adult (RPKM 3.7), subcutaneous fat pad adult (RPKM 2.5) and 28 other tissues [See more](#)**Orthologs** [human](#) [all](#)

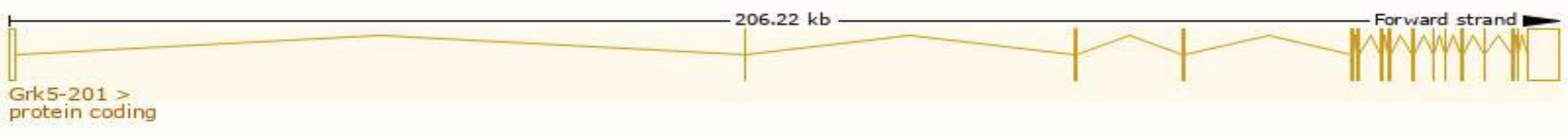
Transcript information Ensembl



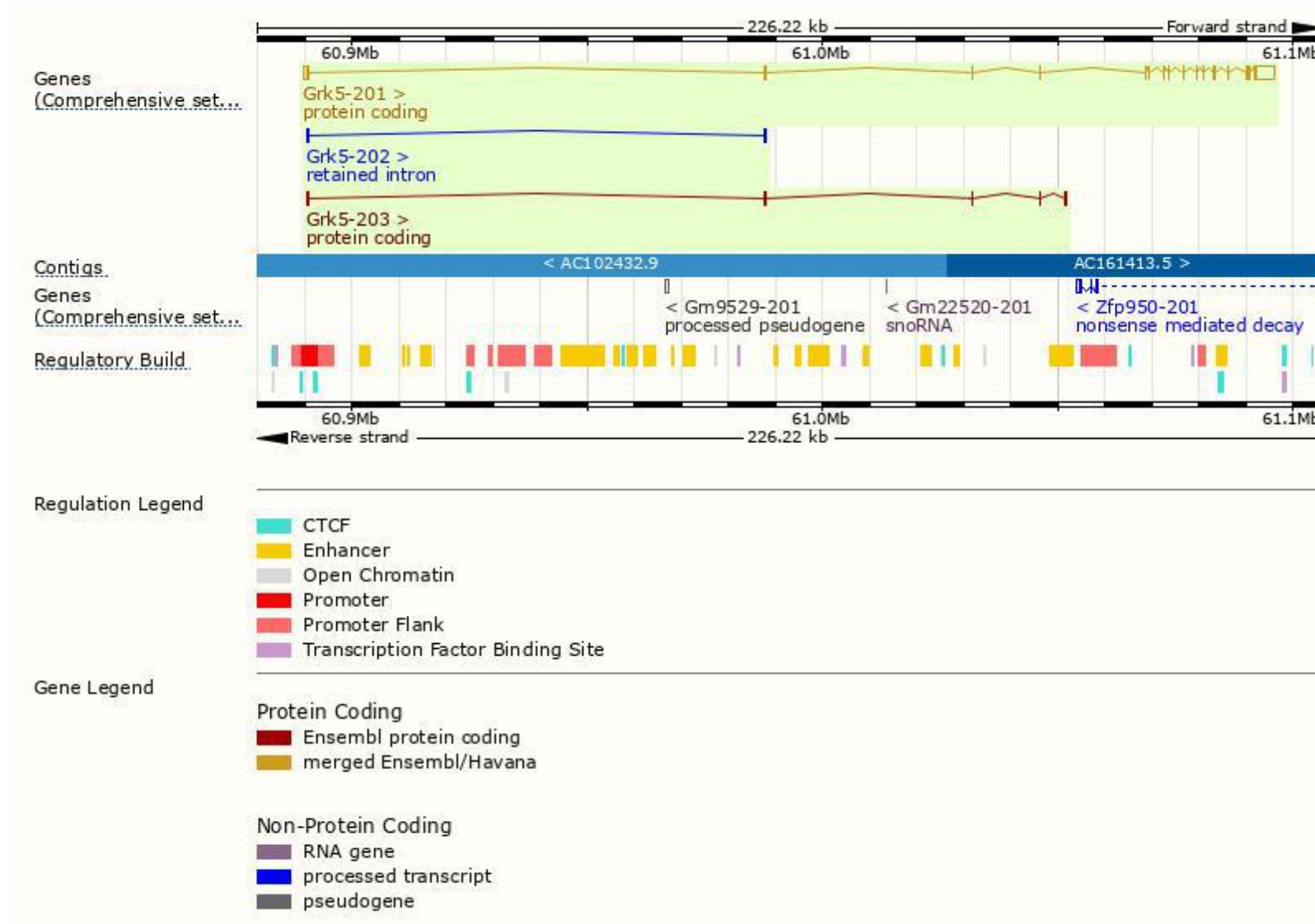
The gene has 3 transcripts,all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|----------|--------------------------------------|------|-----------------------|-----------------|---------------------------|-------------------------------|---|
| Grk5-201 | ENSMUST00000003313.9 | 6580 | 590aa | Protein coding | CCDS29945 | Q3TST4 Q8VEB1 | 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 P1 |
| Grk5-203 | ENSMUST00000236876.1 | 634 | 125aa | Protein coding | - | A0A494B9K8 | GENCODE basic |
| Grk5-202 | ENSMUST00000235623.1 | 568 | No protein | Retained intron | - | - | |

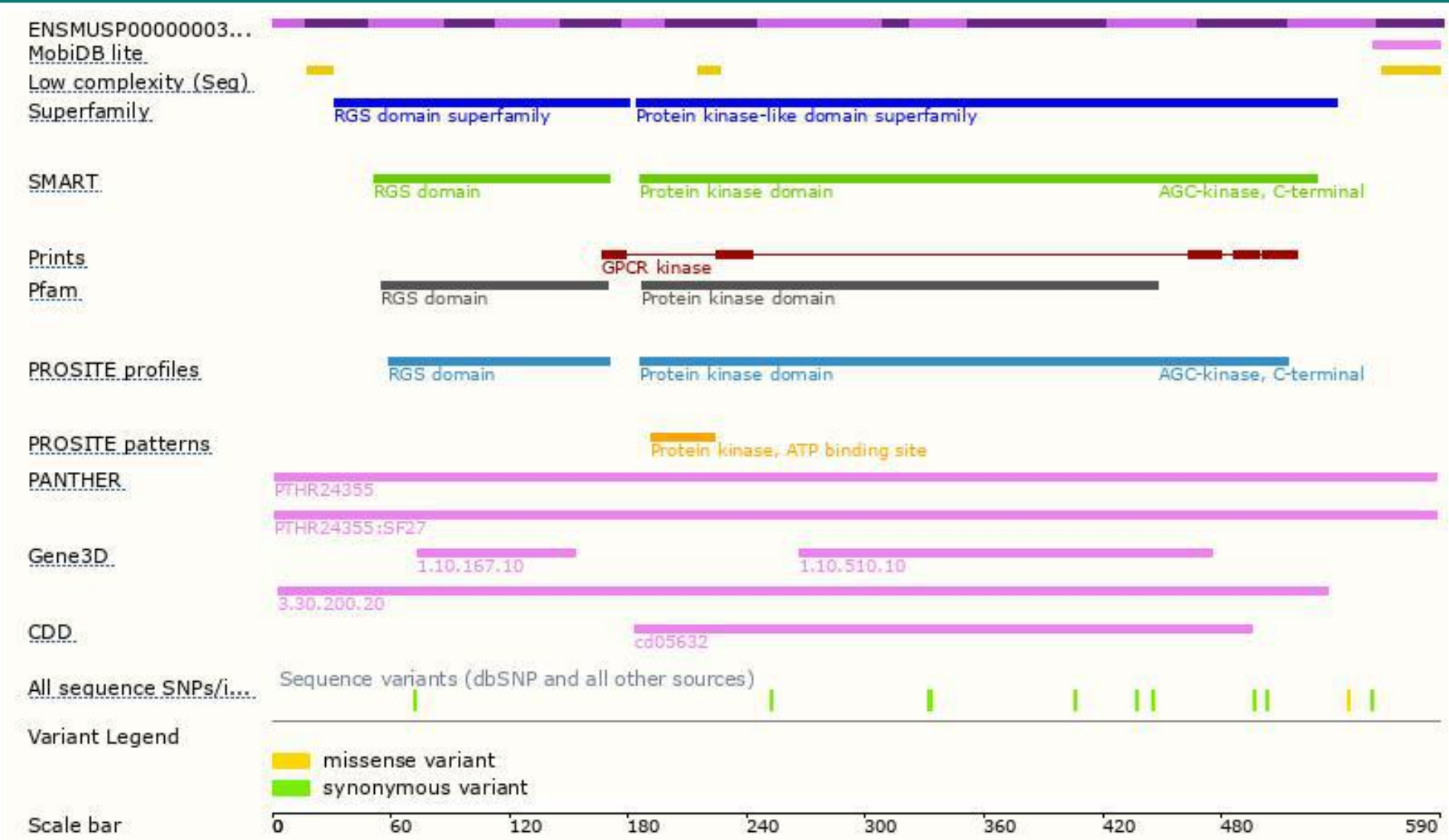
The strategy is based on the design of *Grk5-201* transcript,the transcription is shown below:



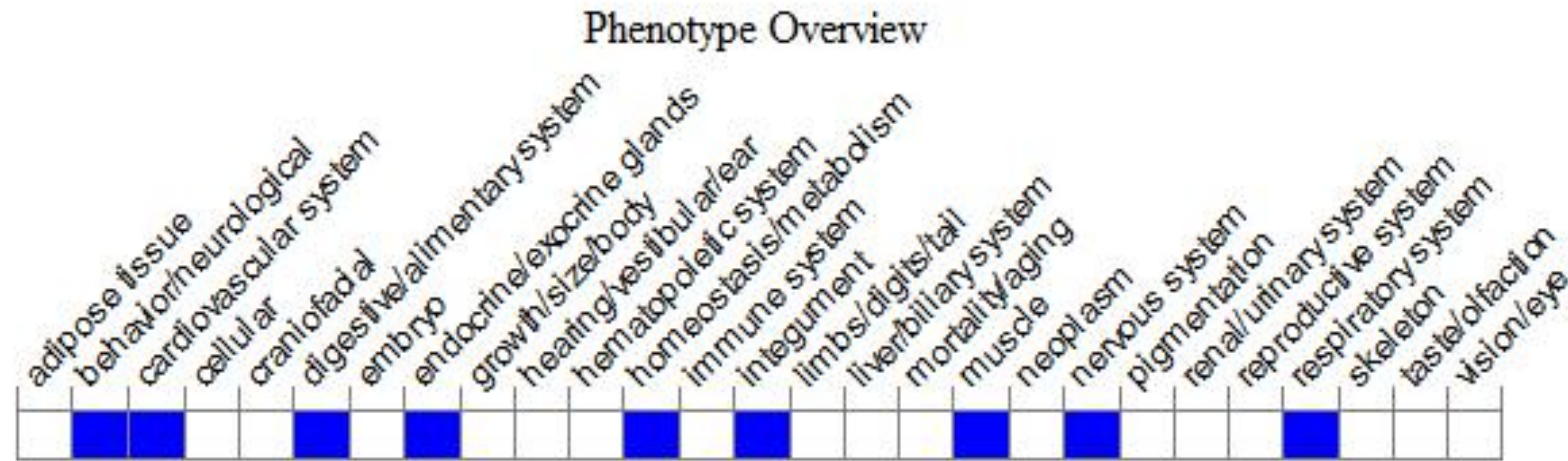
Genomic location distribution



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, homozygous mutation of this gene results in a decrease in thermal pain sensation.

Mice homozygous for a knock-out allele exhibit decreased response of heart to induced stress.

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