

***Pth2-P2A-iCre* cas9-ki Strategy**

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Reviewer

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Design Date:

2021-3-19

Project Overview

Project Name

Pth2-P2A-iCre

Project type

cas9-ki

Strain background

C57BL/6JGpt

- The *Pth2* gene has 2 transcripts. According to the structure of *Pth2* gene, *Pth2-201*(ENSMUST00000042754.7) is selected for presentation of the recommended strategy.
- *Pth2-201* gene has 3 exons, with the ATG start codon in exon2 and TGA stop codon in exon3.
- We make *Pth2-P2A-iCre* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near stop codon(TGA) of *Pth2* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in P2A-iCre before stop coding(TGA) of *Pth2* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

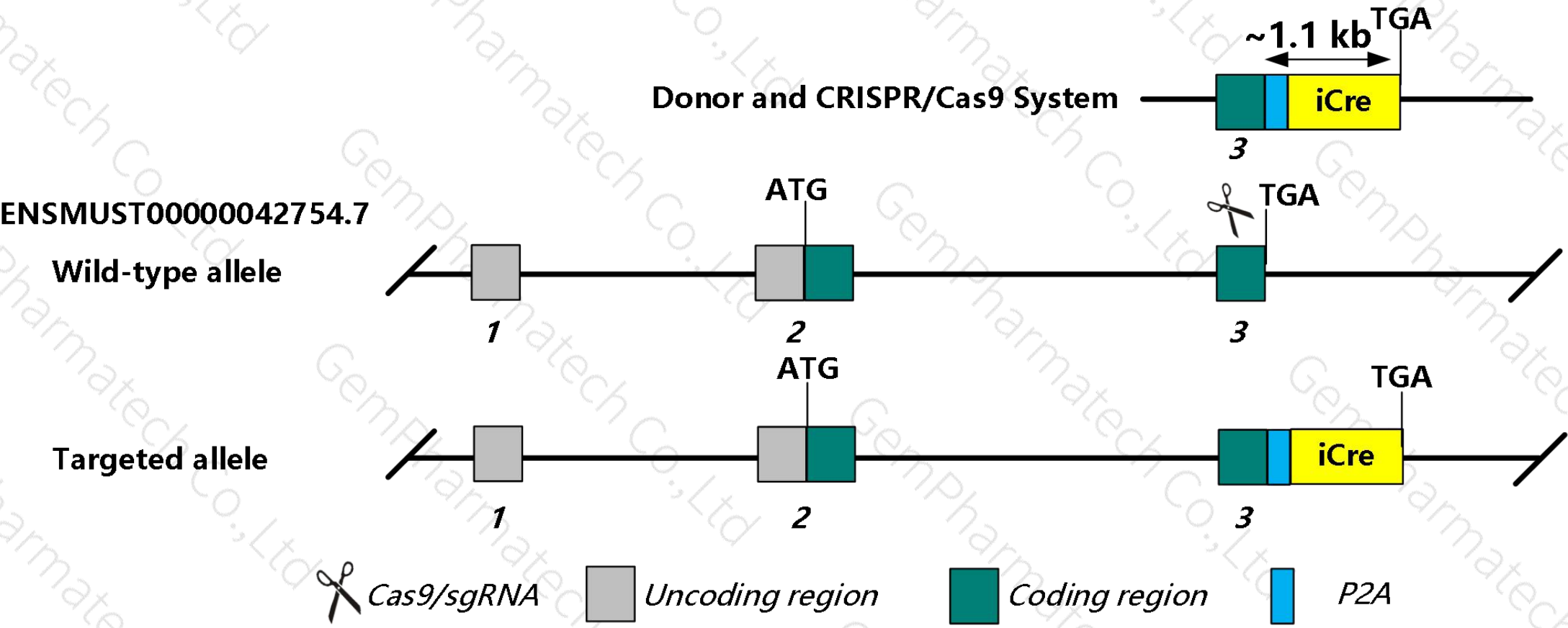
Coding Sequence of Codon-Optimized *Cre* Gene ^[1](*iCre* :1056 bp)

ATGGTGCCCAAGAAGAAGAGGAAAGTCTCCAACCTGCTGACTGTGCACCAAAACCTGCCTGCCCTCCCTGTGGATGC
CACCTCTGATGAAGTCAGGAAGAACCTGATGGACATGTTTCAGGGACAGGCAGGCCTTCTCTGAACACACCTGGAAG
ATGCTCCTGTCTGTGTGCAGATCCTGGGCTGCCTGGTGCAAGCTGAACAACAGGAAATGGTTCCCTGCTGAACCTGA
GGATGTGAGGGACTACCTCCTGTACCTGCAAGCCAGAGGCCTGGCTGTGAAGACCATCCAACAGCACCTGGGGCCAG
CTCAACATGCTGCACAGGAGATCTGGCCTGCCTCGCCCTTCTGACTCCAATGCTGTGTCCCTGGTGATGAGGAGAATC
AGAAAGGAGAATGTGGATGCTGGGGAGAGAGCCAAGCAGGCCCTGGCCTTTGAACGCACTGACTTTGACCAAGTCA
GATCCCTGATGGAGAACTCTGACAGATGCCAGGACATCAGGAACCTGGCCTTCCTGGGCATTGCCTACAACACCCTG
CTGCGCATTGCCGAAATTGCCAGAATCAGAGTGAAGGACATCTCCCGCACCGATGGTGGGAGAATGCTGATCCACAT
TGGCAGGACCAAGACCCTGGTGTCCACAGCTGGTGTGGAGAAGGCCCTGTCCCTGGGGGGTTACCAAGCTGGTGGAG
AGATGGATCTCTGTGTCTGGTGTGGCTGATGACCCCAACAACCTGTTCTGCCGGGTCAGAAAGAATGGTGTGGCT
GCCCCTTCTGCCACCTCCCAACTGTCCACCCGGGGCCCTGGAAGGGATCTTTGAGGCCACCCACCGCCTGATCTATGGT
GCCAAGGATGACTCTGGGCAGAGATACCTGGCCTGGTCTGGCCACTCTGCCAGAGTGGGTGCTGCCAGGGACATGGC
CAGGGCTGGTGTGTCCATCCCTGAAATCATGCAGGCTGGTGGCTGGACCAATGTGAACATTGTGATGAACTACATCAG
AAACCTGGACTCTGAGACTGGGGCCATGGTGAGGCTGCTCGAGGATGGGGACTGA

Shimshek DR, Kim J, Hübner MR, Spergel DJ. Codon-improved Cre recombinase (iCre) expression in the mouse. *Genesis*.2002 Jan.32(1):19-26.

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Pth2* gene. The schematic diagram is as follows:



- According to the existing MGI data, both sexes of mice homozygous for a null allele are sterile. Female homozygotes display very small atretic ovaries without follicles while male homozygotes lack postmeiotic germ cells due to a block during prophase of meiosis I and subsequent death of developing spermatocytes.
- The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide.
- Insertion of P2A-iCre may affect the regulation of the 3' end of the *Pth2* gene.
- There may be 1 to 2 amino acid synonymous mutation in exon3 of *Pth2* gene in this strategy.
- There may be base mutations in the modeling process because of the repetitive sequences(PolyG) downstream of the insertion site.
- The site of insertion of P2A-iCre is nearly to the C-terminal of *Kash5* gene(~1.8kb) and N-terminal of *Gfy* gene(~2.2kb), this strategy may influence the regulatory function of the C-terminal of *Kash5* gene and N-terminal of *Gfy* gene.
- The *Pth2* gene is located on the Chr7. If the knockin mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- The scheme is designed according to the genetic information in the existing database. Inserting a foreign gene after the gene coding region may affect the expression of endogenous and foreign genes. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Gene information (NCBI)

Pth2 parathyroid hormone 2 [*Mus musculus* (house mouse)]

Gene ID: 114640, updated on 10-Oct-2020

Download Datasets

Summary

- Official Symbol

Pth2 provided by MGI
- Official Full Name

parathyroid hormone 2 provided by MGI
- Primary source

MGI:MGI:2152297
- See related

Ensembl:ENSMUSG00000038300
- Gene type

protein coding
- RefSeq status

REVIEWED
- Organism

Mus musculus
- Lineage

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
- Also known as

TIP; Tfp; Tip39; Tfp39
- Summary

This gene encodes the precursor of a peptide hormone that shares sequence similarity with the parathyroid hormone. This gene is expressed in various regions of the brain where it plays a role in the release of pituitary hormones, anxiety and nociception. The encoded precursor protein is proteolytically processed to generate the biologically active neuropeptide. Mice lacking the encoded protein display increased fear and anxiety after exposure to stressful events, and decreased sensitivity to pain. [provided by RefSeq, Aug 2015]
- Expression

Restricted expression toward testis adult (RPKM 12.4) See more
- Orthologs

human all
- NEW

Try the new Gene table
- Try the new Transcript table

Genomic context

Location: 7; 7 B3

See Pth2 in Genome Data Viewer

Exon count: 5

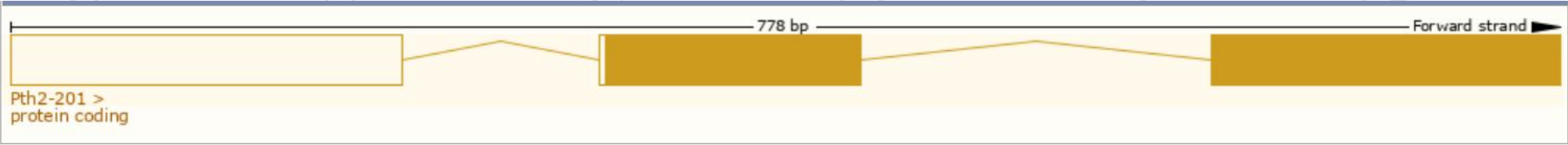
Annotation release	Status	Assembly	Chr	Location
109	current	GRCm39 (GCF_000001635.27)	7	NC_000073.7 (44829447..44831262)
108.20200622	previous assembly	GRCm38.p6 (GCF_000001635.26)	7	NC_000073.6 (45179701..45181838)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	7	NC_000073.5 (52436365..52437208)

Transcript information (Ensembl)

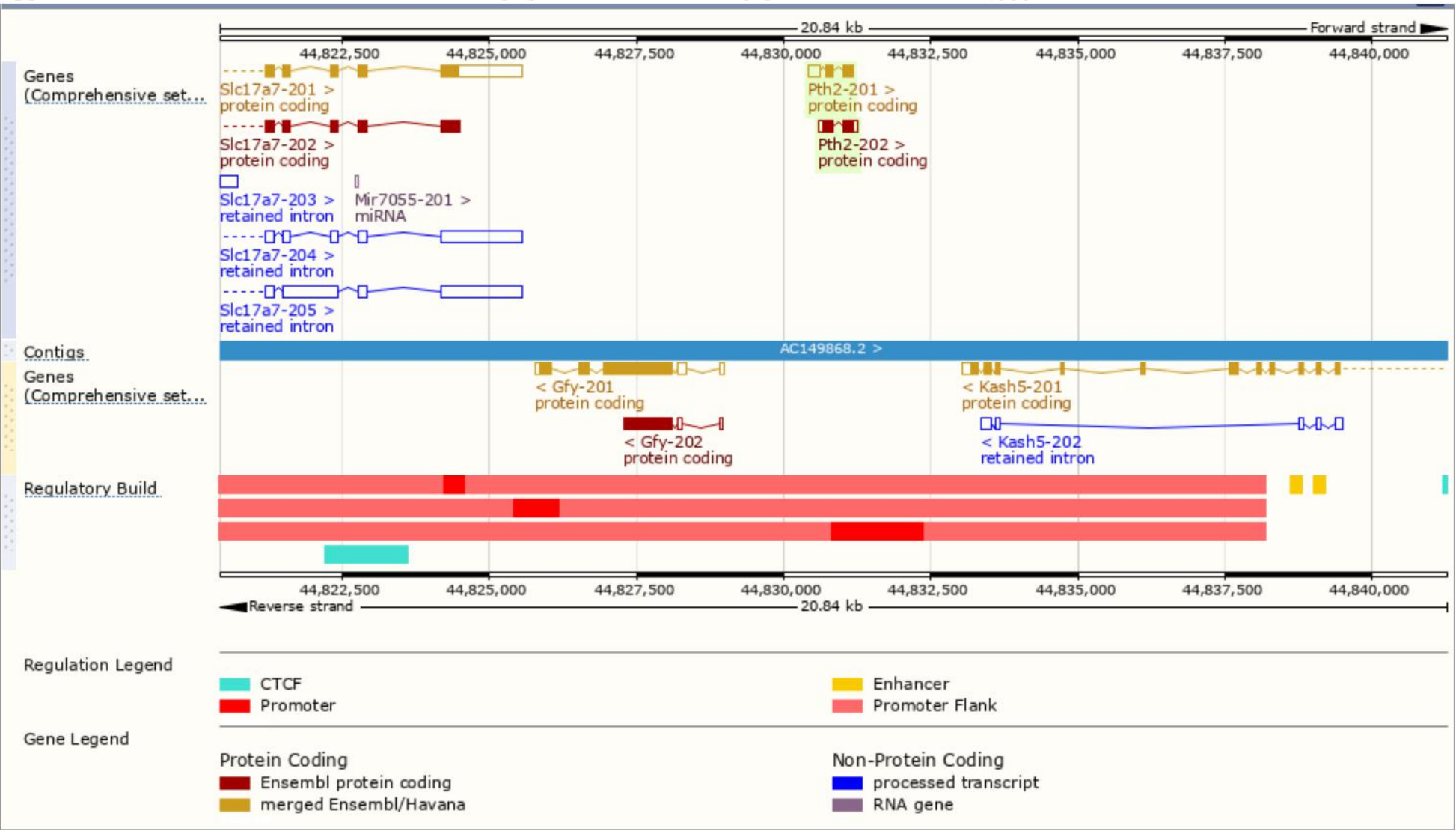
The gene has 2 transcript, and all the transcripts are shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Pth2-201	ENSMUST00000042754.7	503	100aa	Protein coding	CCDS21233	Q91W27	TSL:1 GENCODE basic APPRIS P1
Pth2-202	ENSMUST00000210086.2	481	113aa	Protein coding	-	A0A1B0GR43	TSL:2 GENCODE basic

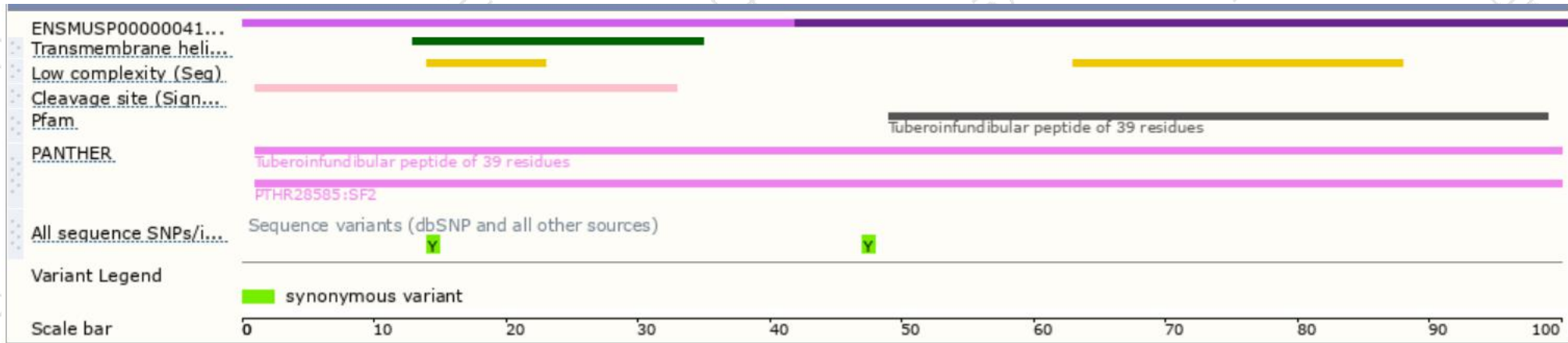
The strategy is based on the design of *Pth2-201* transcript, The transcription is shown below



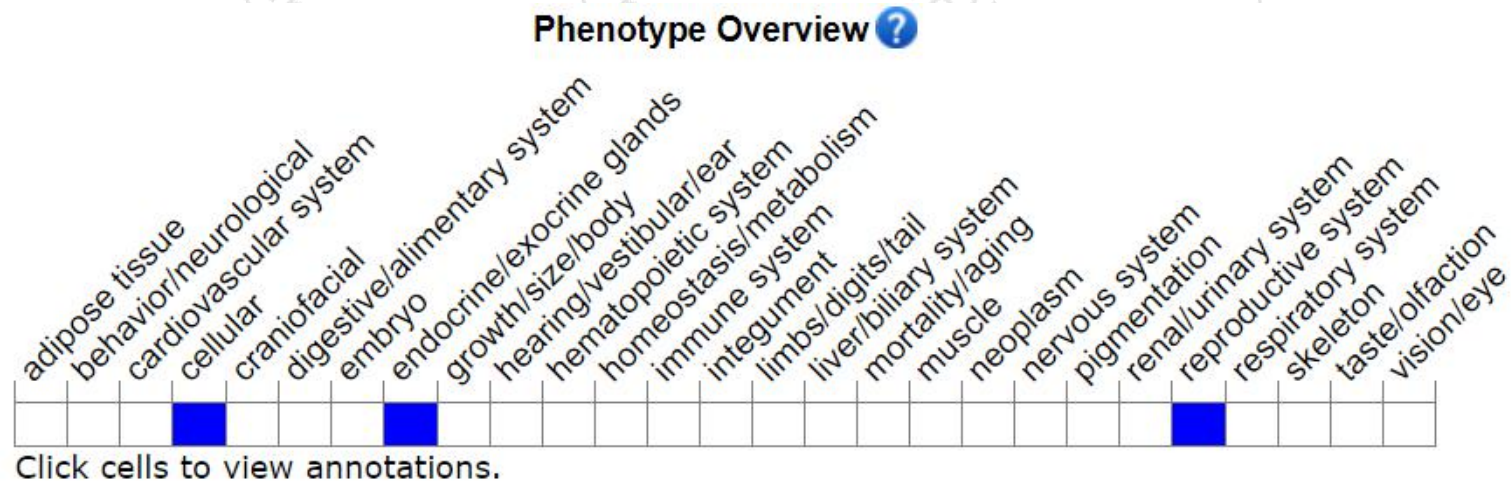
Genomic location distribution



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/marker/MGI:2152297>) .

Both sexes of mice homozygous for a null allele are sterile. Female homozygotes display very small atretic ovaries without follicles while male homozygotes lack postmeiotic germ cells due to a block during prophase of meiosis I and subsequent death of developing spermatocytes.

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