

***Calcr-P2A-iCre* cas9-ki Strategy**

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Reviewer

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Project Overview

Project Name

Calcr-P2A-iCre

Project type

Cas9-KI

Strain background

C57BL/6JGpt

- The *Calcr* gene has 5 transcripts. According to the reference and structure of *Calcr* gene, *Calcr-201*(ENSMUST00000075644.13) is selected for presentation of the recommended strategy.
- *Calcr-201* gene has 16 exons, with the ATG start codon in exon3 and TGA stop codon in exon16.
- In this project, *Calcr* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: In vitro, gRNA and donor vectors were constructed. Cas9, gRNA and donor were injected into the fertilized eggs of C57BL/6JGpt mice for homologous recombination, and obtained positive F0 mice identified by PCR, sequencing analysis. The stable inheritable positive F1 mice model was obtained by mating F0 mice with C57BL/6JGpt mice.

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 (EGFP-P2A-iCre) expression in the mouse. *Genesis*.2002 Jan.32(1):19-26.

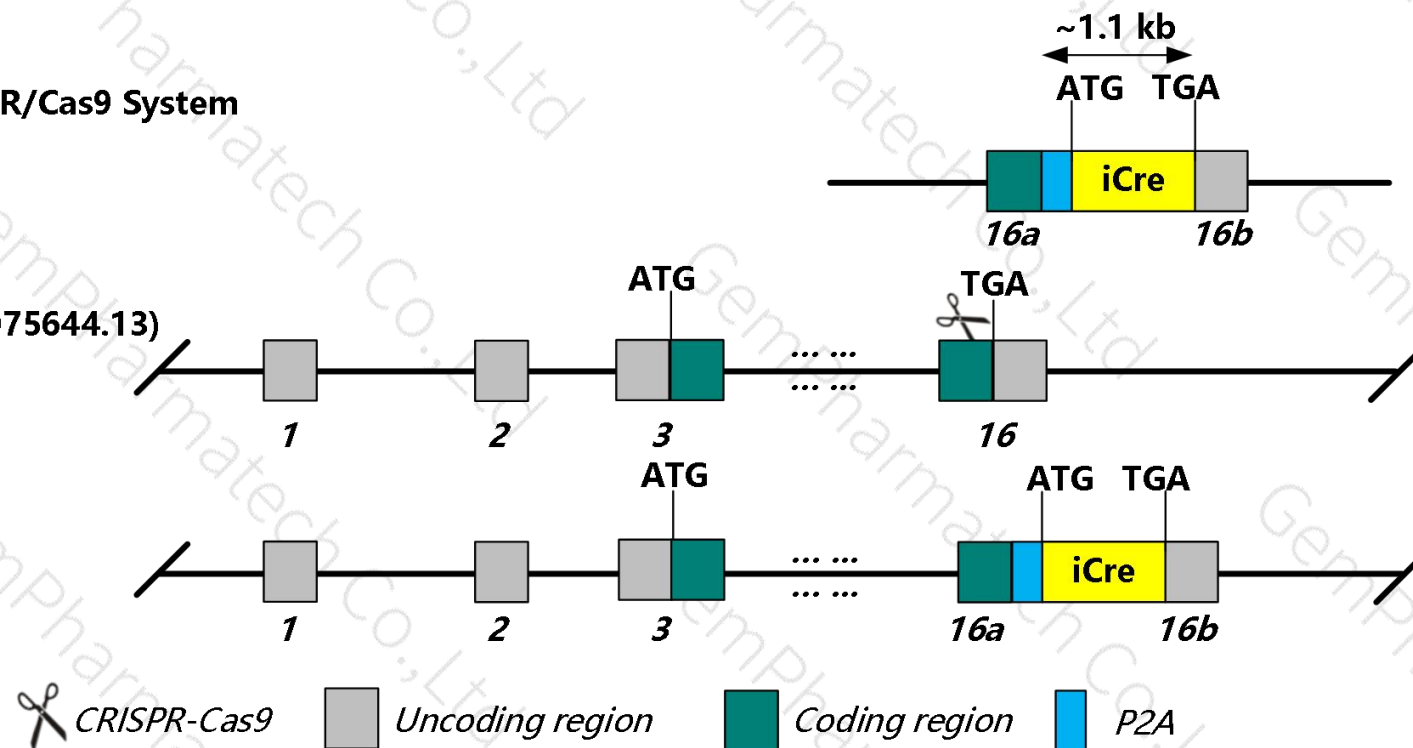
Knockin strategy

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

Donor and CRISPR/Cas9 System

Calcr-201
(ENSMUST00000075644.13)
Wild-type allele

Targeted allele



- According to the existing MGI data, haploinsufficiency may result in increased bone density due to increased bone formation. Homozygous inactivation may result in embryonic lethality. Mice homozygous for another disruption allele at this locus show a normal phenotype.
- The two genes are linked by P2A, which will be transcribed and translated together but spliced into two protein molecules, with the front protein carrying the polypeptide translated from the P2A sequence.
- Insertion of P2A-iCre may affect the regulation of the 3' end of the *Calcr* gene.
- There may be 1 to 2 amino acid synonymous mutation in exon16 of *Calcr* gene in this strategy.
- The *Calcr* gene is located on the Chr6. 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)

Calcr calcitonin receptor [*Mus musculus* (house mouse)]

Gene ID: 12311, updated on 17-Feb-2021

Download Datasets

Summary

Official Symbol	Calcr provided by MGI
Official Full Name	calcitonin receptor provided by MGI
Primary source	MGI:MGI:101950
See related	Ensembl:ENSMUSG00000023964
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	C; Clr; Ct-r
Expression	Biased expression in whole brain E14.5 (RPKM 1.5), CNS E18 (RPKM 1.2) and 5 other tissues See more
Orthologs	human all
NEW	Try the new Gene table
	Try the new Transcript table

Genomic context

Location: 6 A1; 6 1.81 cM

Exon count: 17

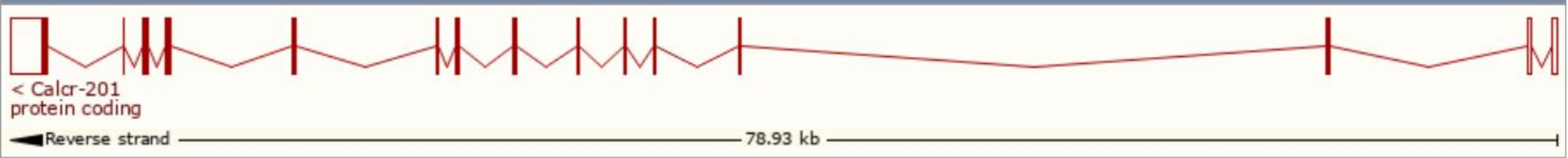
See Calcr in [Genome Data Viewer](#)

Transcript information (Ensembl)

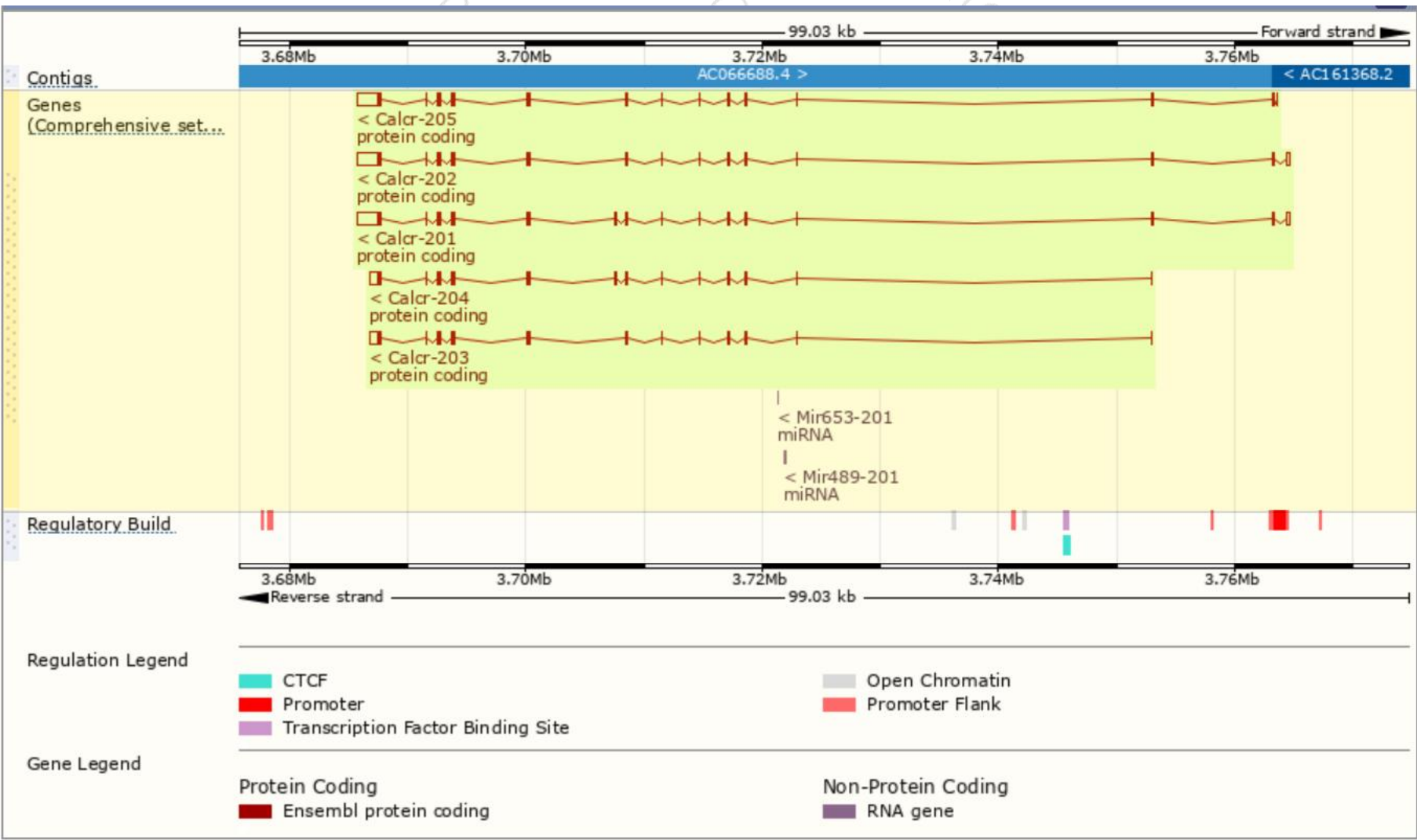
The gene has 5 transcripts, and all transcripts are shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Calcr-201	ENSMUST00000075644.13	3763	533aa	Protein coding	CCDS39417	Q60755-1	TSL:1 GENCODE basic
Calcr-202	ENSMUST00000115622.8	3721	496aa	Protein coding	CCDS39416	Q60755-2	TSL:1 GENCODE basic APPRIS P1
Calcr-205	ENSMUST00000171613.8	3518	496aa	Protein coding	CCDS39416	Q60755-2	TSL:1 GENCODE basic APPRIS P1
Calcr-204	ENSMUST00000170266.3	2175	533aa	Protein coding	CCDS39417	Q60755-1	TSL:1 GENCODE basic
Calcr-203	ENSMUST00000168592.9	2064	496aa	Protein coding	CCDS39416	Q60755-2	TSL:5 GENCODE basic APPRIS P1

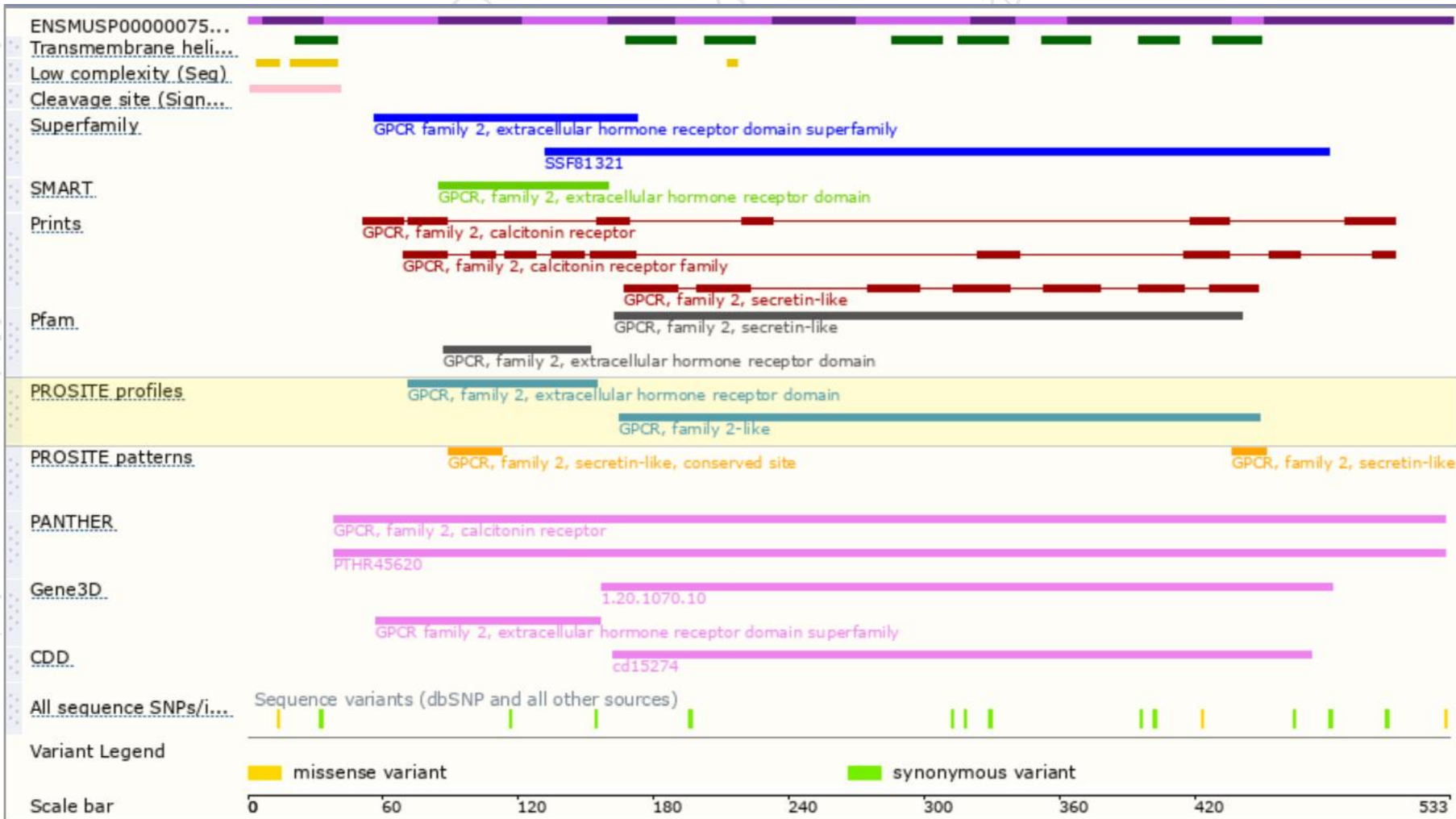
The strategy is based on the design of *Calcr-201* transcript,the transcription is shown below



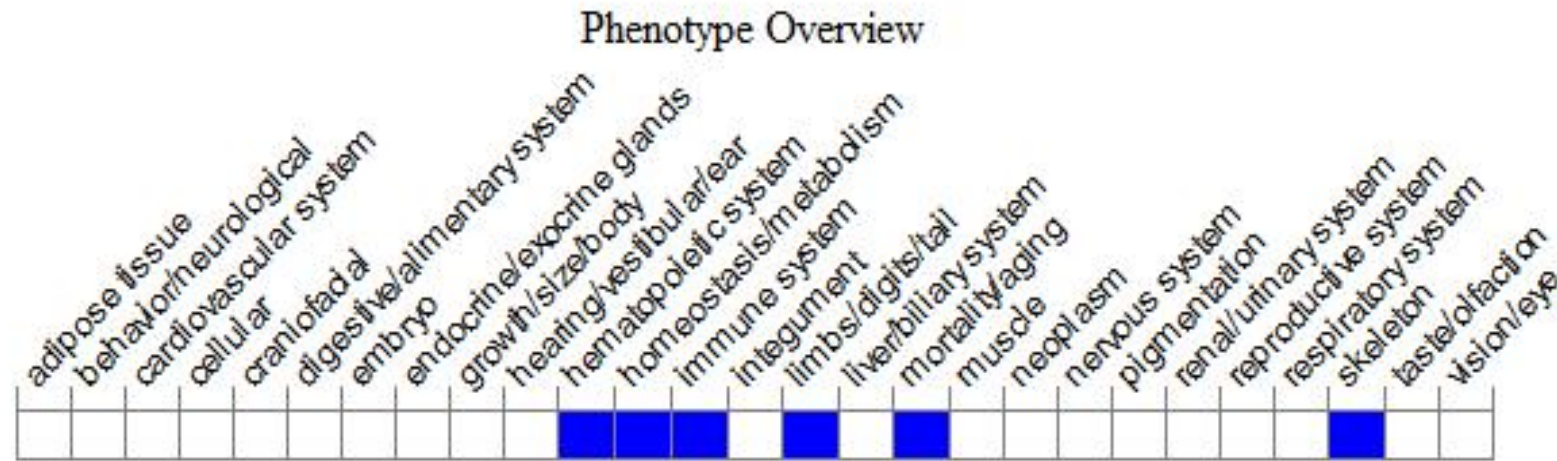
Genomic location distribution



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, haploinsufficiency may result in increased bone density due to increased bone formation. Homozygous inactivation may result in embryonic lethality. Mice homozygous for another disruption allele at this locus show a normal phenotype.

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