

Sema3b Cas9-CKO Strategy

Designer: Huan Wang

Reviewer: Lingyan Wu

Design Date: 2020-7-29

Project Overview

Project Name

Sema3b

Project type

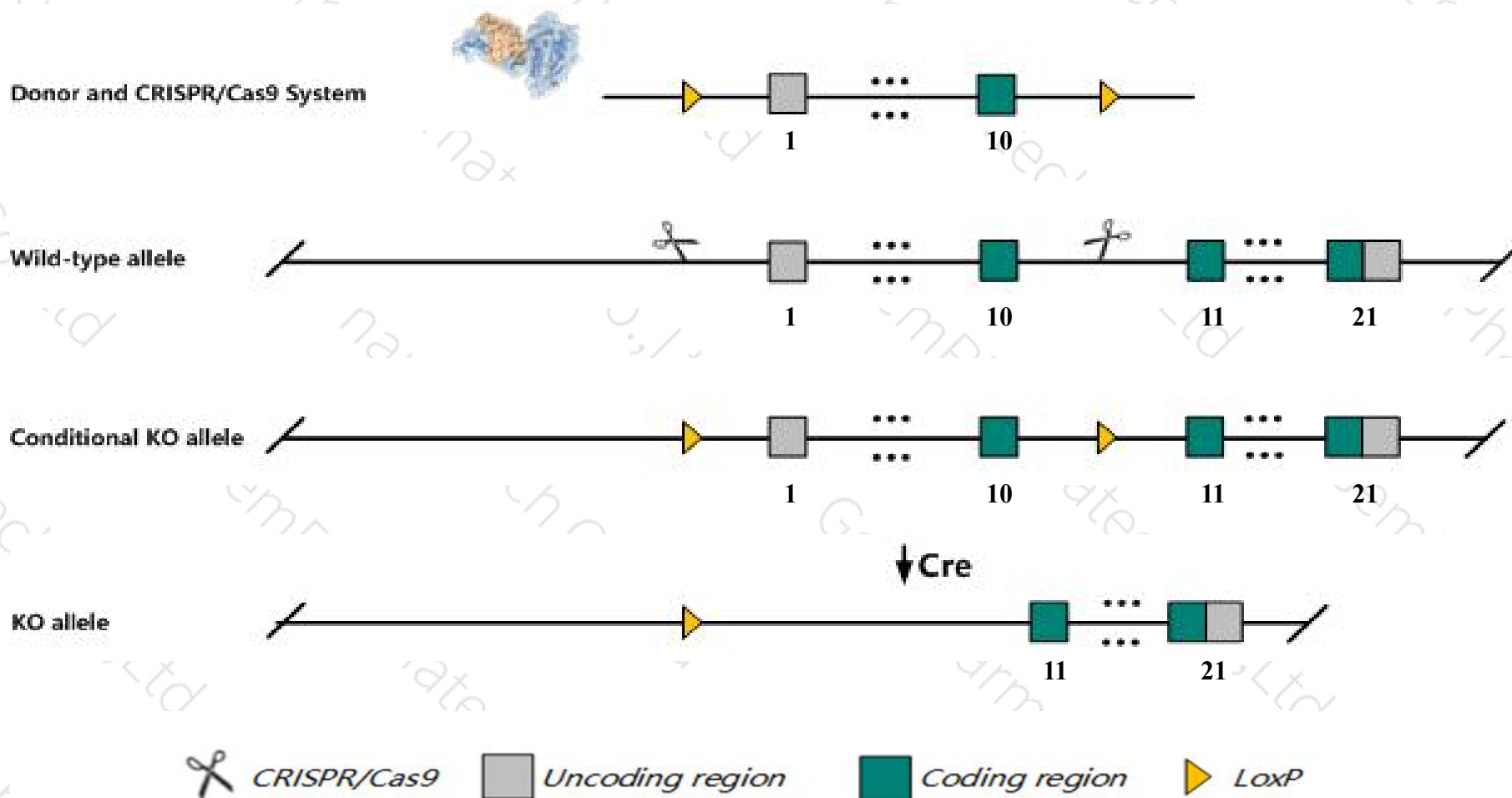
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Sema3b* gene. The schematic diagram is as follows:



Technical routes

- The *Sema3b* gene has 14 transcripts. According to the structure of *Sema3b* gene, exon1-exon10 of *Sema3b*-203(ENSMUST00000102530.7) transcript is recommended as the knockout region. The region contains start codon ATG . Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Sema3b* 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 one knock-out allele are viable and fertile with no obvious pathological abnormalities. Mice homozygous for a second knock-out allele exhibit improper positioning of a major brain commissural projection, the anterior commissure.
- The *Sema3b* gene is located on the Chr9. 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)

Sema3b sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3B [Mus musculus (house mouse)]

Gene ID: 20347, updated on 13-Mar-2020

Summary

Official Symbol Sema3b provided by [MGI](#)

Official Full Name sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3B provided by [MGI](#)

Primary source [MGI:MGI:107561](#)

See related [Ensembl:ENSMUSG00000057969](#)

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 SemA, Sema, sema5, semaV

Summary This gene encodes a secreted protein that belongs to the class 3 semaphorin/collapsin family. Members of this family play a role in growth cone guidance during neurogenesis. The encoded protein inhibits axonal extension. This protein is thought to be an osteoblast protein that regulates bone mass and affects skeletal homeostasis. A similar gene in humans functions as a tumor suppressor gene. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Apr 2014]

Expression Broad expression in colon adult (RPKM 23.8), lung adult (RPKM 21.6) and 15 other tissues [See more](#)

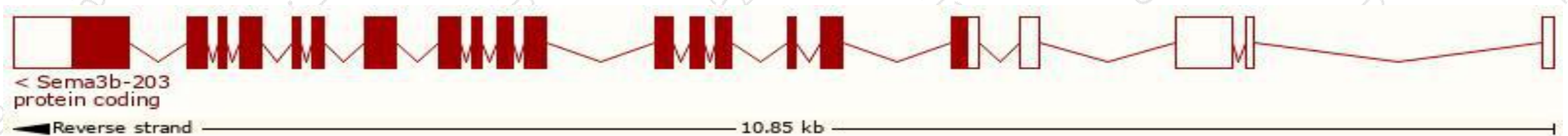
Orthologs [human](#) [all](#)

Transcript information (Ensembl)

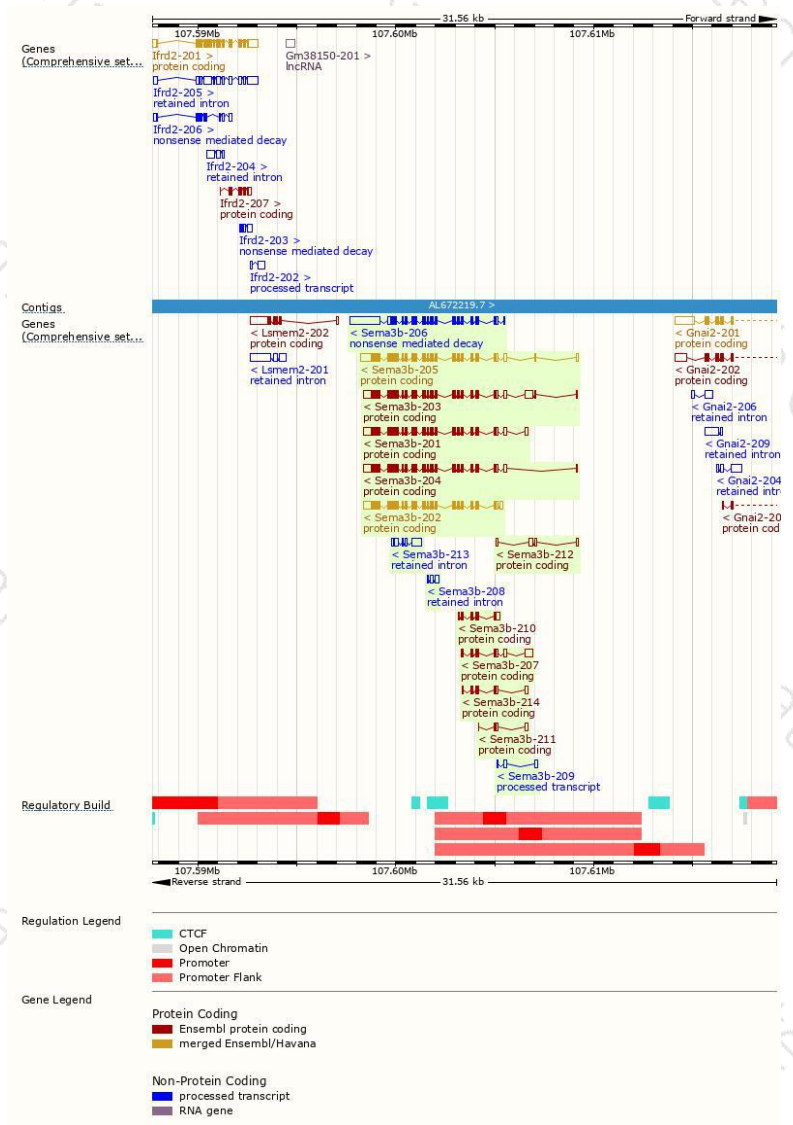
The gene has 14 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sema3b-203	ENSMUST00000102530.7	3410	749aa	Protein coding	CCDS23501	M9MMK0	TSL:5 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
Sema3b-205	ENSMUST00000102532.9	3191	749aa	Protein coding	CCDS23501	M9MMK0	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
Sema3b-201	ENSMUST00000073448.11	2987	749aa	Protein coding	CCDS23501	M9MMK0	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
Sema3b-204	ENSMUST00000102531.6	2958	749aa	Protein coding	CCDS23501	M9MMK0	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
Sema3b-202	ENSMUST00000102529.9	2875	749aa	Protein coding	CCDS23501	M9MMK0	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
Sema3b-207	ENSMUST00000193180.5	1023	147aa	Protein coding	-	A0A0A6YWW1	CDS 3' incomplete TSL:3
Sema3b-210	ENSMUST00000194433.5	709	172aa	Protein coding	-	A0A0A6YW56	CDS 3' incomplete TSL:2
Sema3b-214	ENSMUST00000195662.5	623	137aa	Protein coding	-	A0A0A6YWM9	CDS 3' incomplete TSL:3
Sema3b-212	ENSMUST00000195057.1	453	10aa	Protein coding	-	A0A0G2JDD7	CDS 3' incomplete TSL:3
Sema3b-211	ENSMUST00000194606.1	348	48aa	Protein coding	-	A0A0A6YXC1	CDS 3' incomplete TSL:3
Sema3b-206	ENSMUST00000123926.7	3433	534aa	Nonsense mediated decay	-	M0QWQ7	TSL:1
Sema3b-209	ENSMUST00000193551.1	358	No protein	Processed transcript	-	-	TSL:2
Sema3b-213	ENSMUST00000195472.1	827	No protein	Retained intron	-	-	TSL:5
Sema3b-208	ENSMUST00000193234.1	358	No protein	Retained intron	-	-	TSL:3

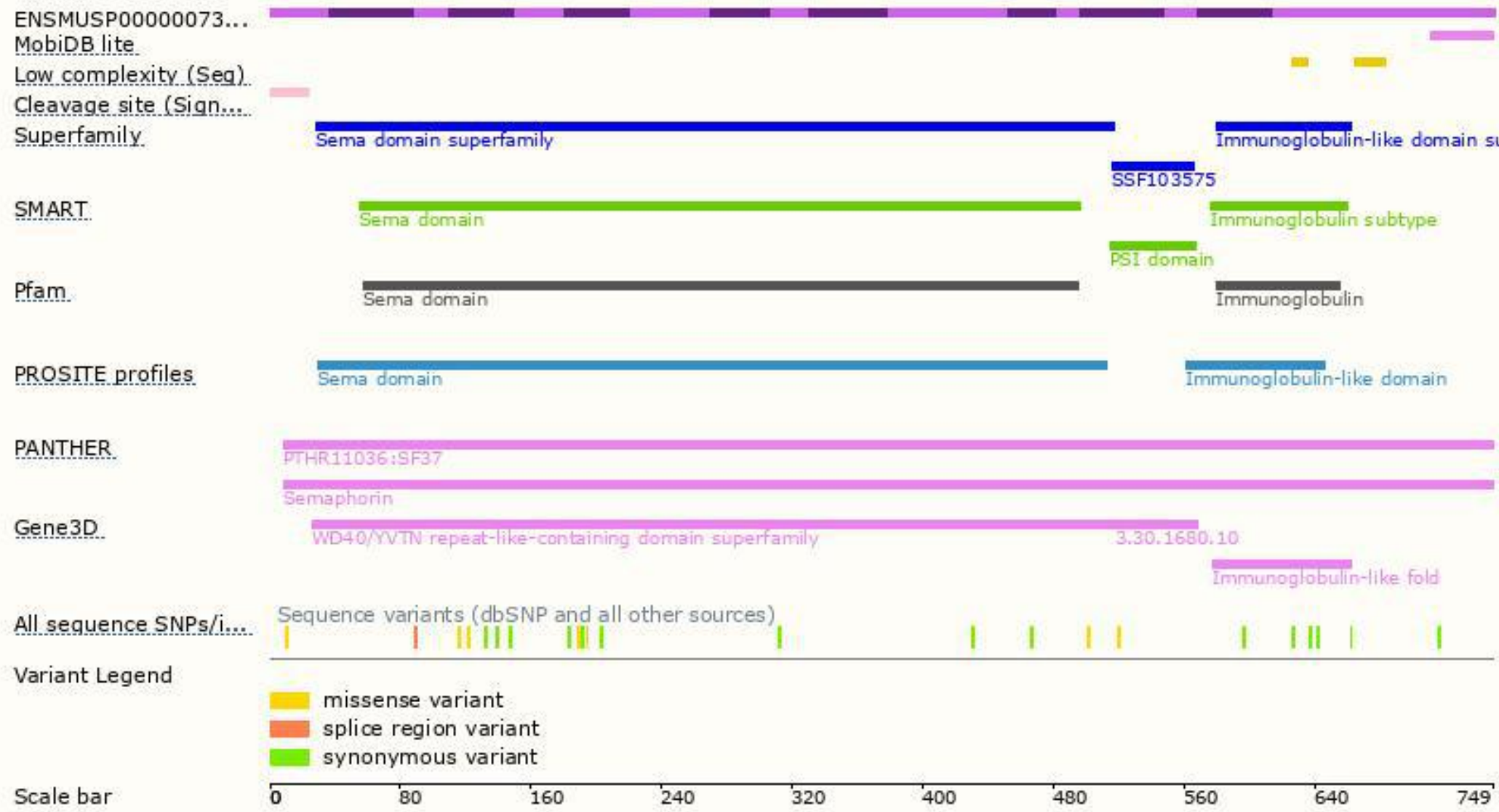
The strategy is based on the design of *Sema3b-203* transcript, the transcription is shown below:



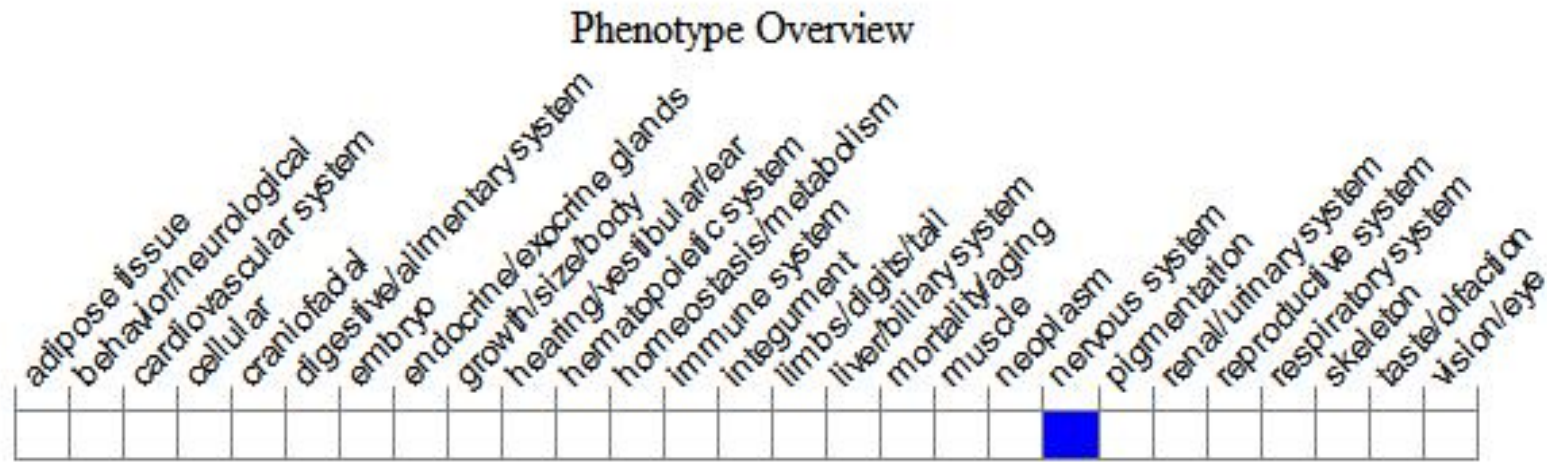
Genomic location distribution



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 one knock-out allele are viable and fertile with no obvious pathological abnormalities. Mice homozygous for a second knock-out allele exhibit improper positioning of a major brain commissural projection, the anterior commissure.

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

Tel: 400-9660890

