# *Nfib* Cas9-CKO Strategy

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**Reviewer: Daohua Xu**

**Design Date: 2021-5-8**

# Project Overview

| | |
|---|---|
| **Project Name** | ***Nfib*** |
| **Project type** | **Cas9-CKO** |
| **Strain background** | **C57BL/6JGpt** |

# Conditional Knockout strategy

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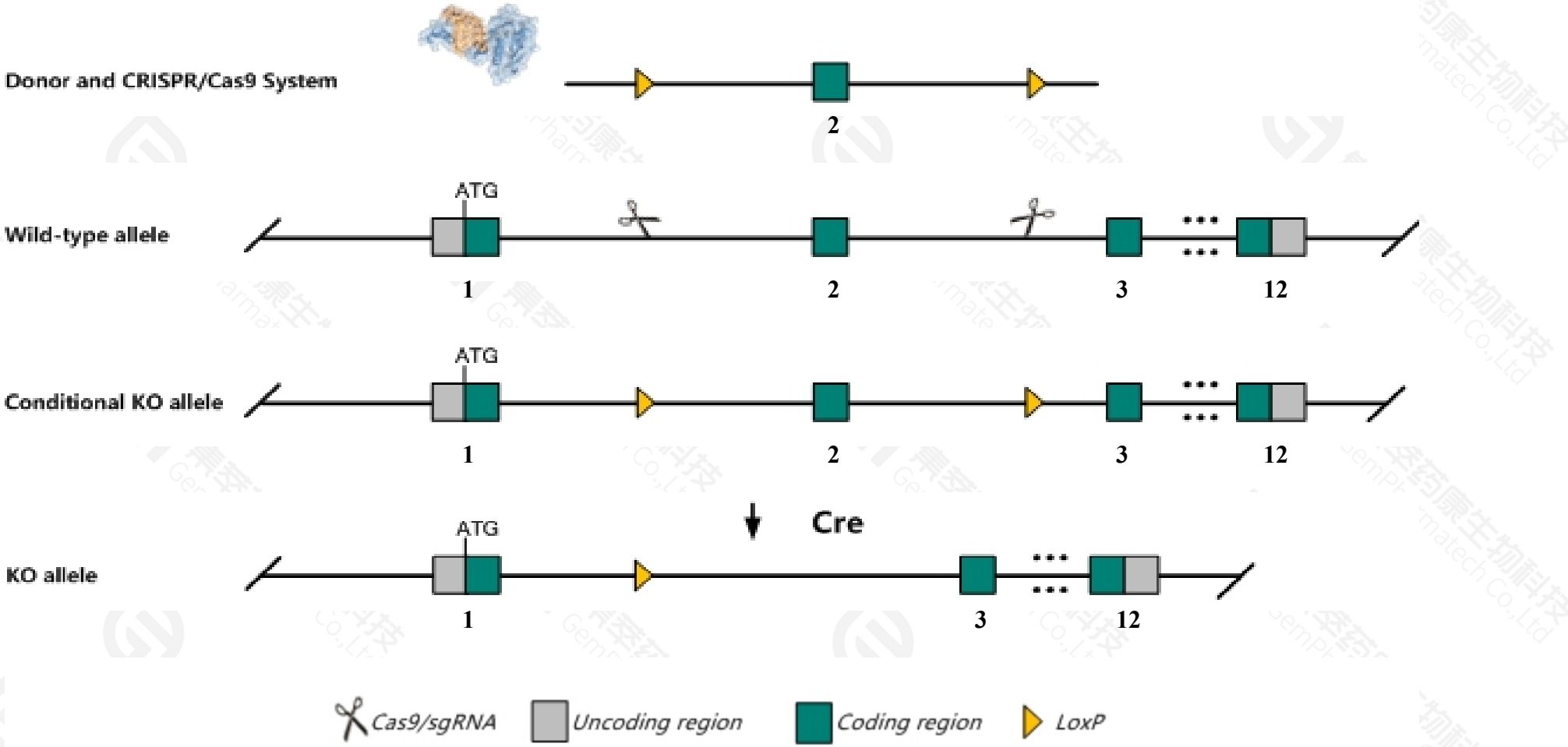
This model will use CRISPR/Cas9 technology to edit the *Nfib* gene. The schematic diagram is as follows:



# Technical routes

➢ The *Nfib* gene has 12 transcripts. According to the structure of *Nfib* gene, exon2 of *Nfib-201*(ENSMUST00000050872.15) transcript is recommended as the knockout region.The region contains 532bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Nfib* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor was constructed.Cas9, sgRNA 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 was 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.

# Notice

➢ According to the existing MGI data,homozygous inactivation of this gene causes severe lung defects and neonatal death from respiratory failure. Homozygotes for a null allele show callosal agenesis and abnormalities in forebrain, basilar pons, hippocampus, and submandibular gland development, as well as lung maturation defects.

➢ The *Nfib* gene is located on the Chr4. 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）

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## Nfib nuclear factor I/B [Mus musculus (house mouse)]

Gene ID: 18028, updated on 13-Mar-2020

### Summary

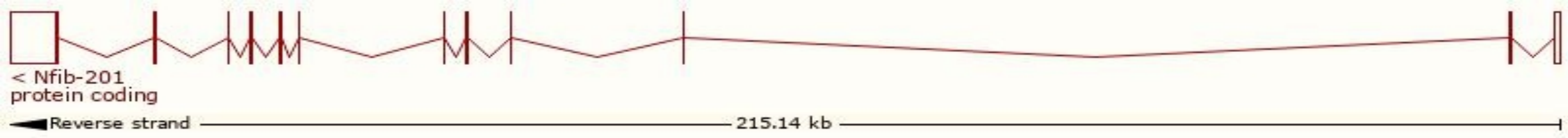
| | |
|---|---|
| **Official Symbol** | Nfib provided by MGI |
| **Official Full Name** | nuclear factor I/B provided by MGI |
| **Primary source** | MGI:MGI:103188 |
| **See related** | Ensembl:ENSMUSG00000008575 |
| **Gene type** | protein coding |
| **RefSeq status** | VALIDATED |
| **Organism** | Mus musculus |
| **Lineage** | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| **Also known as** | 6720429L07Rik, CTF, E030026I10Rik, NF-I/B, NF1-B, NFI-B |
| **Expression** | Broad expression in CNS E14 (RPKM 21.2), whole brain E14.5 (RPKM 17.6) and 21 other tissues See more |
| **Orthologs** | human all |

# Transcript information（Ensembl）

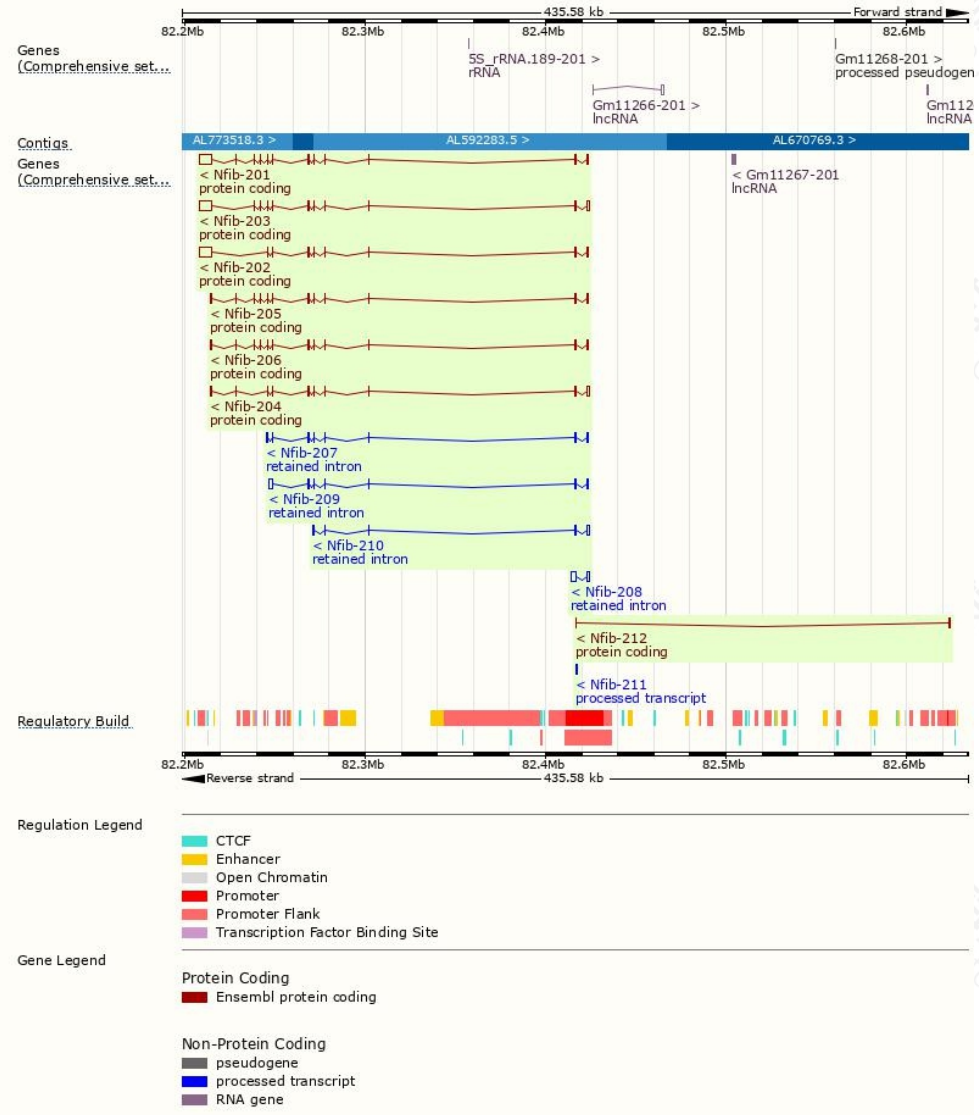
The gene has 12 transcripts,all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|---|---|---|---|---|---|---|---|
| Nfib-201 | ENSMUST00000050872.14 | 8770 | 570aa | Protein coding | CCDS51213 | P97863 | 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 P2 |
| Nfib-202 | ENSMUST00000064770.8 | 8429 | 420aa | Protein coding | CCDS38790 | P97863 Q6GSP7 | TSL:1 GENCODE basic |
| Nfib-203 | ENSMUST00000107245.8 | 9116 | 493aa | Protein coding | - | I7HIP8 | TSL:1 GENCODE basic |
| Nfib-206 | ENSMUST00000107248.7 | 3294 | 569aa | Protein coding | - | A2BG77 | 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 ALT1 |
| Nfib-205 | ENSMUST00000107247.7 | 3267 | 560aa | Protein coding | - | A2BG75 | 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 ALT1 |
| Nfib-204 | ENSMUST00000107246.1 | 3075 | 486aa | Protein coding | - | A2BG76 | TSL:1 GENCODE basic |
| Nfib-212 | ENSMUST00000155821.1 | 685 | 174aa | Protein coding | - | A2ADI3 | CDS 3' incomplete TSL:3 |
| Nfib-211 | ENSMUST00000152588.1 | 379 | No protein | Processed transcript | - | - | TSL:5 |
| Nfib-208 | ENSMUST00000135024.1 | 3835 | No protein | Retained intron | - | - | TSL:1 |
| Nfib-209 | ENSMUST00000140874.7 | 3308 | No protein | Retained intron | - | - | TSL:1 |
| Nfib-207 | ENSMUST00000122918.7 | 2699 | No protein | Retained intron | - | - | TSL:1 |
| Nfib-210 | ENSMUST00000148982.1 | 2281 | No protein | Retained intron | - | - | TSL:1 |

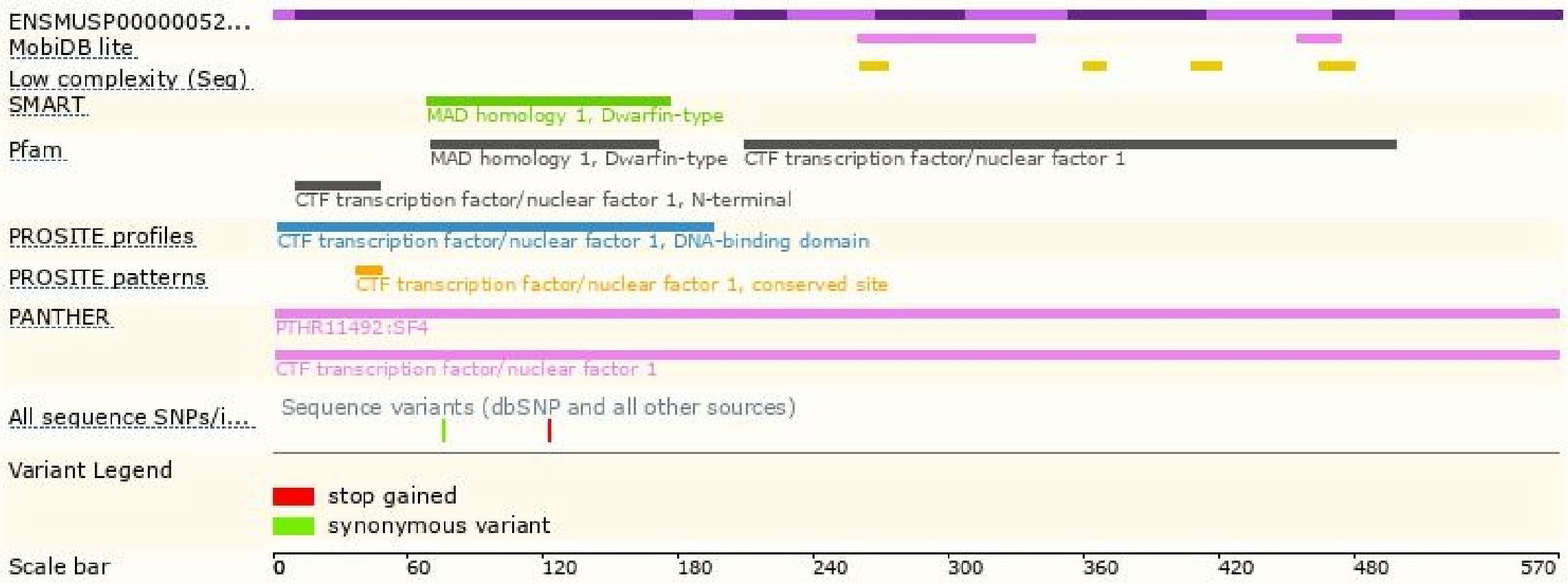
The strategy is based on the design of *Nfib-201* transcript,the transcription is shown below:



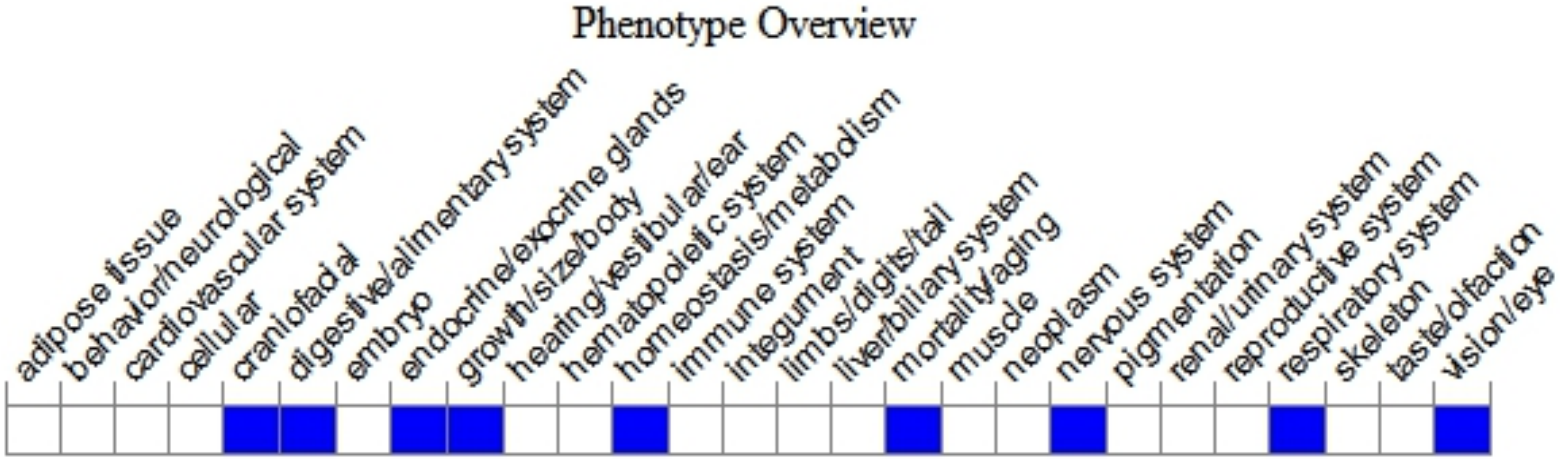
# Genomic location distribution



# 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/).*

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

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