# *Rnf165* Cas9-CKO Strategy

Designer: Huimin Su

Reviewer: Ruiuri Zhang

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# Project Overview

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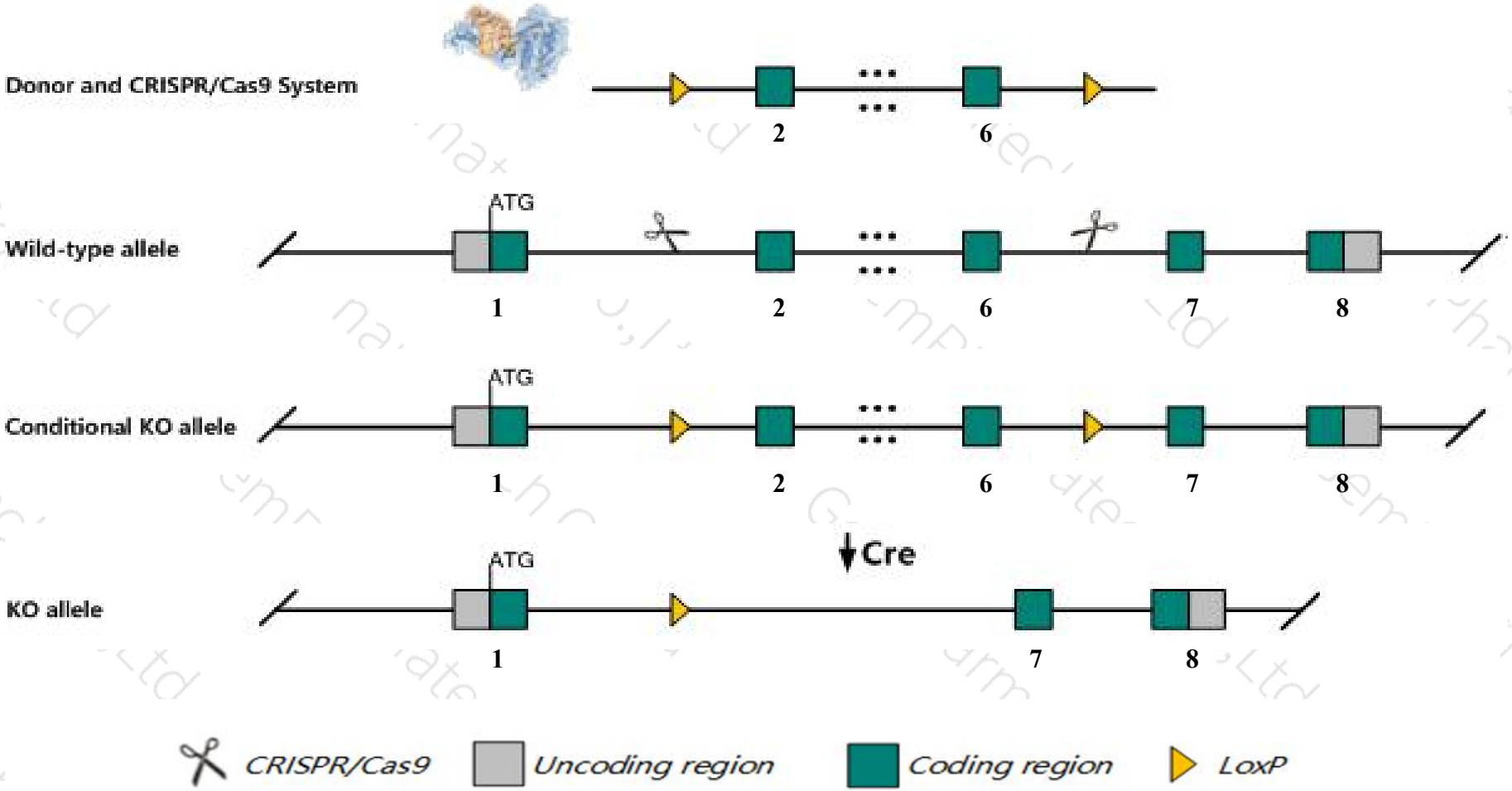
| **Project Name** | ***Rnf165*** |
| **Project type** | **Cas9-CKO** |
| **Strain background** | **C57BL/6JGpt** |

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rnf165* gene. The schematic diagram is as follows:



# Technical routes

- The *Rnf165* gene has 7 transcripts. According to the structure of *Rnf165* gene, exon2-exon6 of *Rnf165-201* (ENSMUST00000026494.13) transcript is recommended as the knockout region.The region contains 758bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Rnf165* 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.

# Notice

➢ According to the existing MGI data,mice homozygous for a gene trap allele exhibit partial neonatal lethality followed by complete postnatal lethality, growth retardation, abnormal joint mobility, cyanosis, abnormal motor neuron innervation pattern and abnormal phrenic nerve innervation pattern to diaphragm.

➢ The *Rnf165* gene is located on the Chr18. 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）

集萃药康 GemPharmatech

## Rnf165 ring finger protein 165 [ *Mus musculus* (house mouse) ]

Gene ID: 225743, updated on 13-Mar-2020

### Summary

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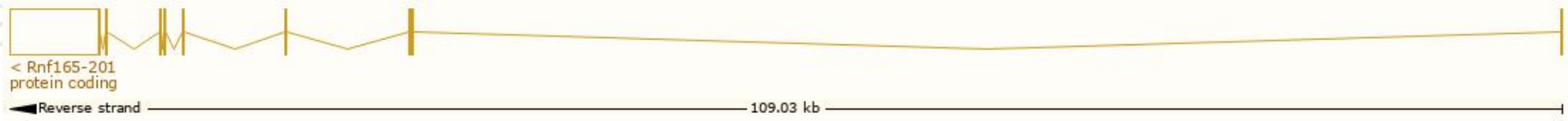
|---|---|
| **Official Symbol** | Rnf165 provided by MGI |
| **Official Full Name** | ring finger protein 165 provided by MGI |
| **Primary source** | MGI:MGI:2444521 |
| **See related** | Ensembl:ENSMUSG00000025427 |
| **Gene type** | protein coding |
| **RefSeq status** | VALIDATED |
| **Organism** | Mus musculus |
| **Lineage** | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| **Also known as** | Akd2; Gm96; Ark2c; AI427432; 2900024M11Rik; G630064H08Rik |
| **Expression** | Biased expression in whole brain E14.5 (RPKM 11.0), CNS E14 (RPKM 9.2) and 7 other tissues See more |
| **Orthologs** | human all |

# Transcript information（Ensembl）

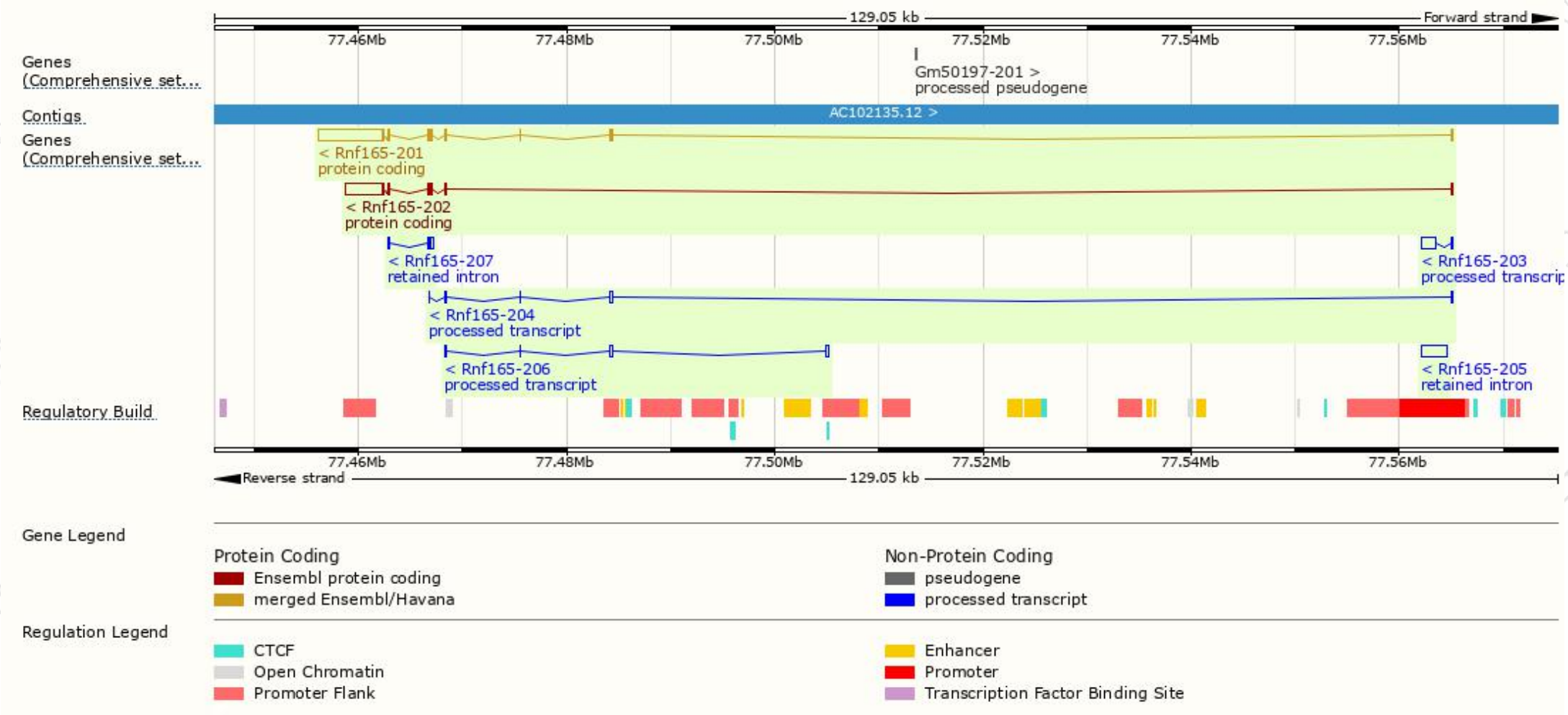
The gene has 7 transcripts,all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|---|---|---|---|---|---|---|---|
| Rnf165-201 | ENSMUST00000026494.13 | 7300 | 347aa | Protein coding | CCDS50327 | E9QAU8 | TSL:5 GENCODE basic APPRIS P1 |
| Rnf165-202 | ENSMUST00000182024.1 | 4309 | 154aa | Protein coding | - | A0A0R4J2A4 | TSL:5 GENCODE basic |
| Rnf165-203 | ENSMUST00000182146.2 | 1427 | No protein | Processed transcript | - | - | TSL:1 |
| Rnf165-206 | ENSMUST00000182348.2 | 675 | No protein | Processed transcript | - | - | TSL:5 |
| Rnf165-204 | ENSMUST00000182153.2 | 667 | No protein | Processed transcript | - | - | TSL:5 |
| Rnf165-205 | ENSMUST00000182294.1 | 2517 | No protein | Retained intron | - | - | TSL:NA |
| Rnf165-207 | ENSMUST00000182805.1 | 408 | No protein | Retained intron | - | - | TSL:3 |

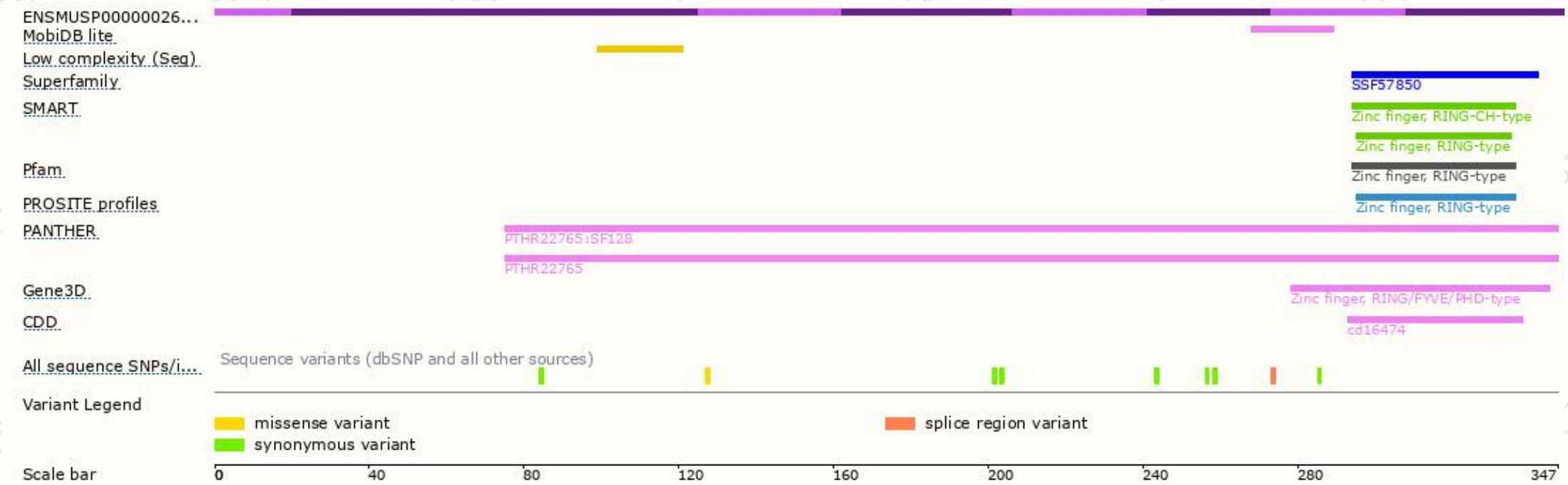
The strategy is based on the design of *Rnf165-201* transcript, the transcription is shown below



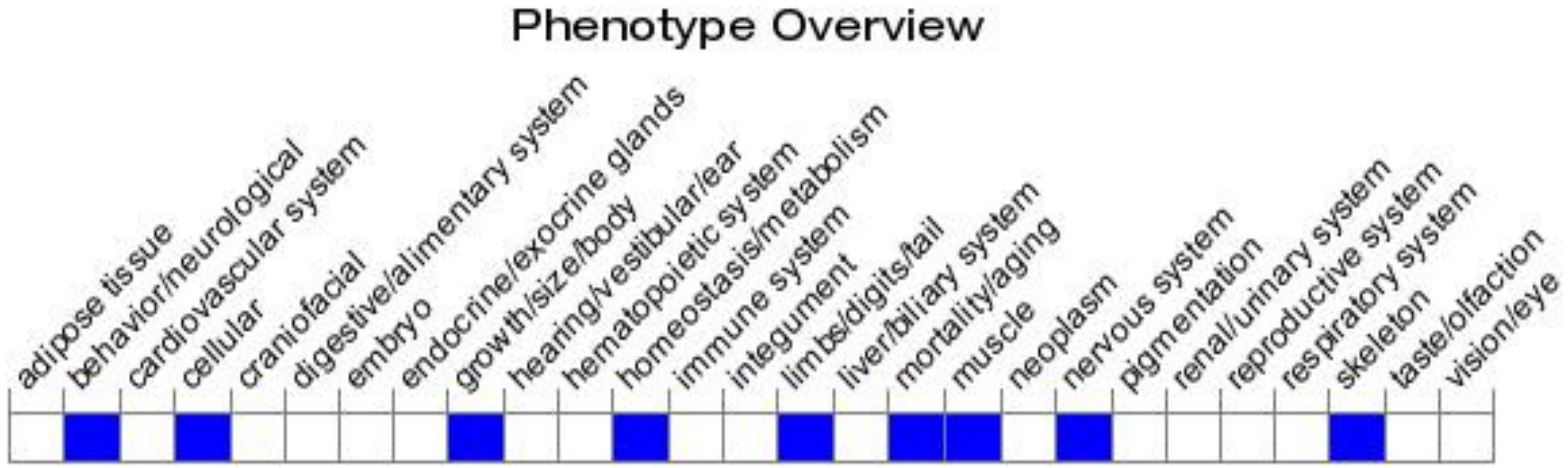
# Genomic location distribution



# 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, mice homozygous for a gene trap allele exhibit partial neonatal lethality followed by complete postnatal lethality, growth retardation, abnormal joint mobility, cyanosis, abnormal motor neuron innervation pattern and abnormal phrenic nerve innervation pattern to diaphragm.

集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd