# *Gnl3* Cas9-CKO Strategy

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**Design Date: 2020-10-13**

# Project Overview

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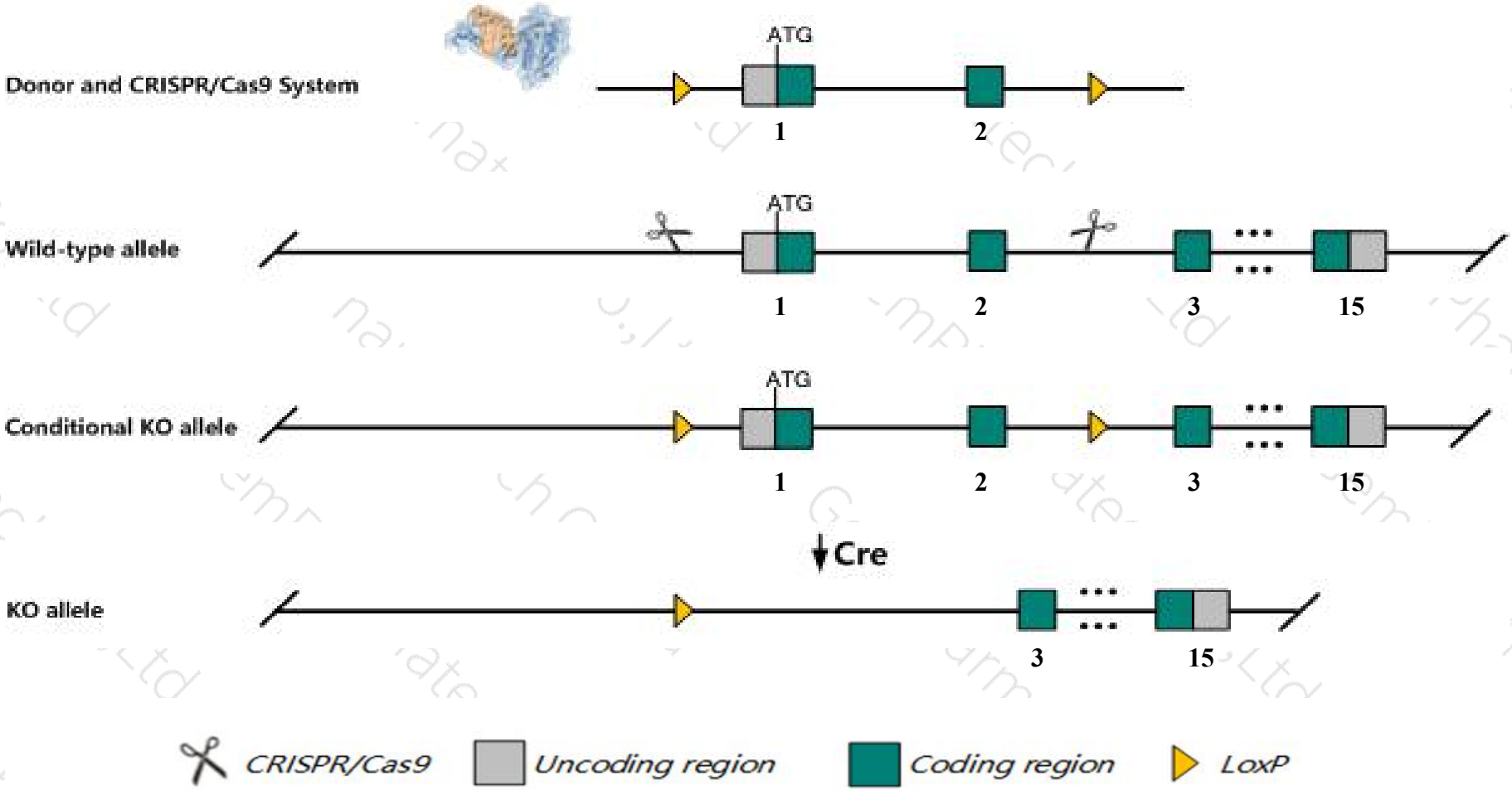
|---|---|
| **Project Name** | ***Gnl3*** |
| **Project type** | **Cas9-CKO** |
| **Strain background** | **C57BL/6JGpt** |

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Gnl3* gene. The schematic diagram is as follows:



# Technical routes

➢ The *Gnl3* gene has 14 transcripts. According to the structure of *Gnl3* gene, exon1-exon2 of *Gnl3-201*(ENSMUST00000037739.7) 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 *Gnl3* 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,homozygous disruption of this gene leads to early embryonic loss as blastocysts fail to enter the S phase. MEFs heterozygous for a gene trap allele have reduced proliferative capacity while MEFs heterozygous for a null allele show reduced doubling rates,increased apoptosis and premature senescence.

➢ Transcript *Gnl3*-203 may not be affected.

➢ The KO region contains functional region of the *Gm24916, Snord69* and *Snord19* gene.Knockout the region may affect the function of *Gm24916, Snord69* and *Snord19* gene.

➢ The KO region contains functional region of the *Pbrm1* gene.Knockout the region may affect the function of *Pbrm1* gene.

➢ The Intron2 is only 394bp,loxp insertion may affect mRNA splicing.

➢ The *Gnl3* gene is located on the Chr14. 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

## Gnl3 guanine nucleotide binding protein-like 3 (nucleolar) [Mus musculus (house mouse)]

Gene ID: 30877, updated on 13-Mar-2020

### Summary

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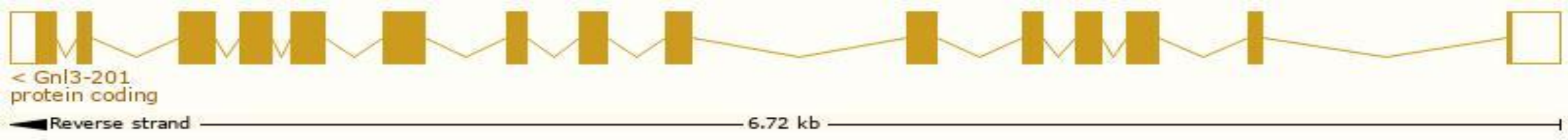
|---|---|
| **Official Symbol** | Gnl3 provided by MGI |
| **Official Full Name** | guanine nucleotide binding protein-like 3 (nucleolar) provided byMGI |
| **Primary source** | MGI:MGI:1353651 |
| **See related** | Ensembl:ENSMUSG00000042354 |
| **Gene type** | protein coding |
| **RefSeq status** | 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** | Ns |
| **Expression** | Broad expression in liver E14 (RPKM 33.9), liver E14.5 (RPKM 25.2) and 22 other tissuesSee more |
| **Orthologs** | human all |

# Transcript information（Ensembl）

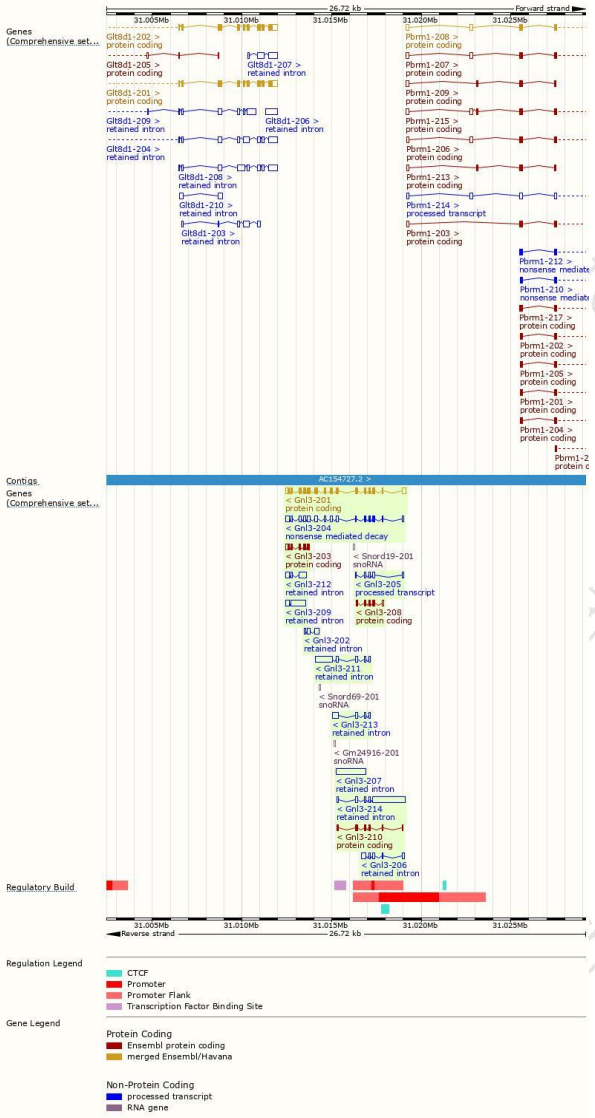
The gene has 14 transcripts,all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|---|---|---|---|---|---|---|---|
| **Gnl3-201** | ENSMUST00000037739.7 | 1947 | 538aa | Protein coding | CCDS26905 | Q8CI11 | TSL:1 GENCODE basic APPRIS P1 |
| **Gnl3-203** | ENSMUST00000226379.1 | 597 | 159aa | Protein coding | - | A0A2I3BR32 | CDS 5' incomplete |
| **Gnl3-210** | ENSMUST00000228341.1 | 516 | 156aa | Protein coding | - | A0A2I3BPZ6 | CDS 3' incomplete |
| **Gnl3-208** | ENSMUST00000227467.1 | 494 | 146aa | Protein coding | - | A0A2I3BRV9 | CDS 3' incomplete |
| **Gnl3-204** | ENSMUST00000226740.1 | 1765 | 136aa | Nonsense mediated decay | - | A0A2I3BR82 | |
| **Gnl3-205** | ENSMUST00000227087.1 | 466 | No protein | Processed transcript | - | - | |
| **Gnl3-214** | ENSMUST00000228774.1 | 2264 | No protein | Retained intron | - | - | |
| **Gnl3-207** | ENSMUST00000227389.1 | 1656 | No protein | Retained intron | - | - | |
| **Gnl3-211** | ENSMUST00000228427.1 | 1360 | No protein | Retained intron | - | - | |
| **Gnl3-209** | ENSMUST00000227869.1 | 1033 | No protein | Retained intron | - | - | |
| **Gnl3-212** | ENSMUST00000228713.1 | 718 | No protein | Retained intron | - | - | |
| **Gnl3-213** | ENSMUST00000228739.1 | 693 | No protein | Retained intron | - | - | |
| **Gnl3-206** | ENSMUST00000227170.1 | 666 | No protein | Retained intron | - | - | |
| **Gnl3-202** | ENSMUST00000226220.1 | 508 | No protein | Retained intron | - | - | |

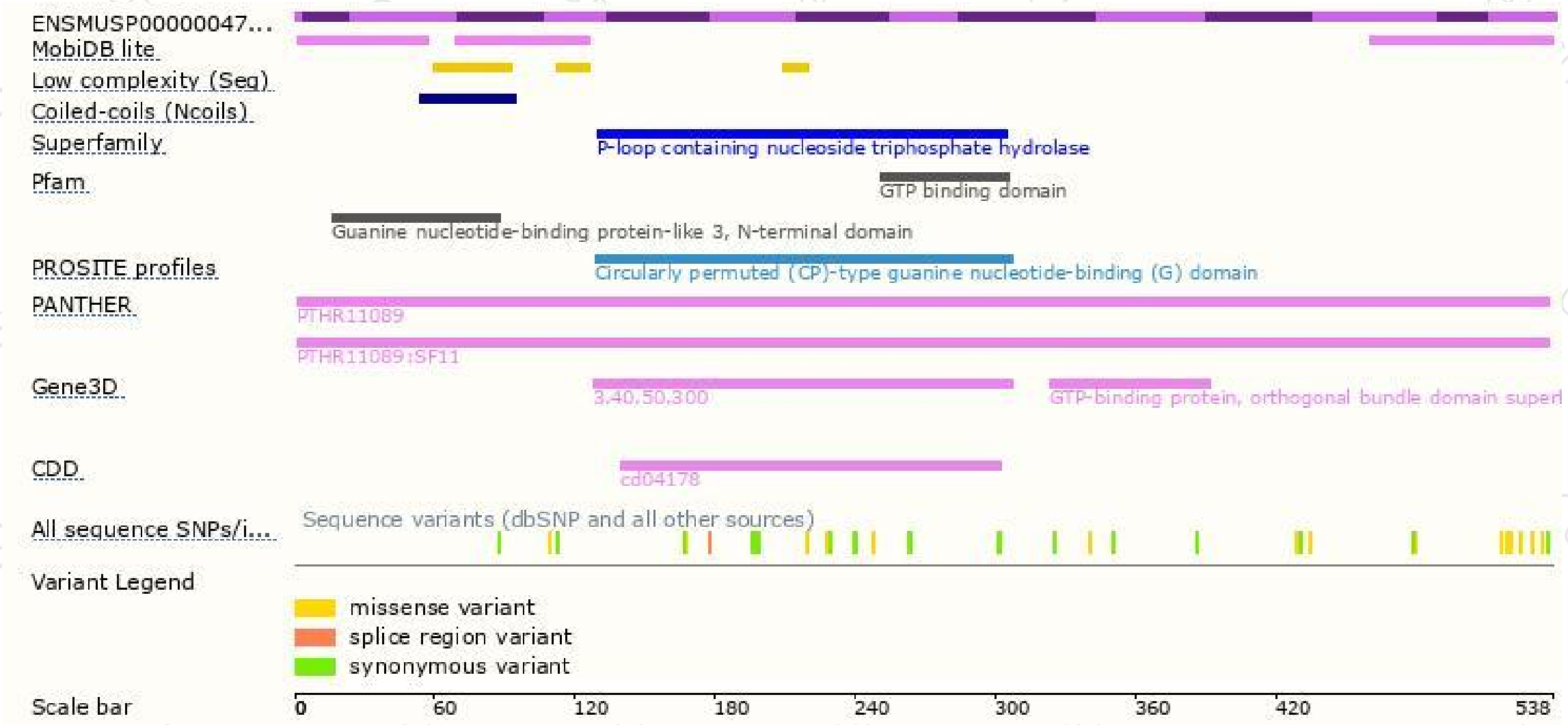
The strategy is based on the design of *Gnl3-201* transcript,the transcription is shown below:



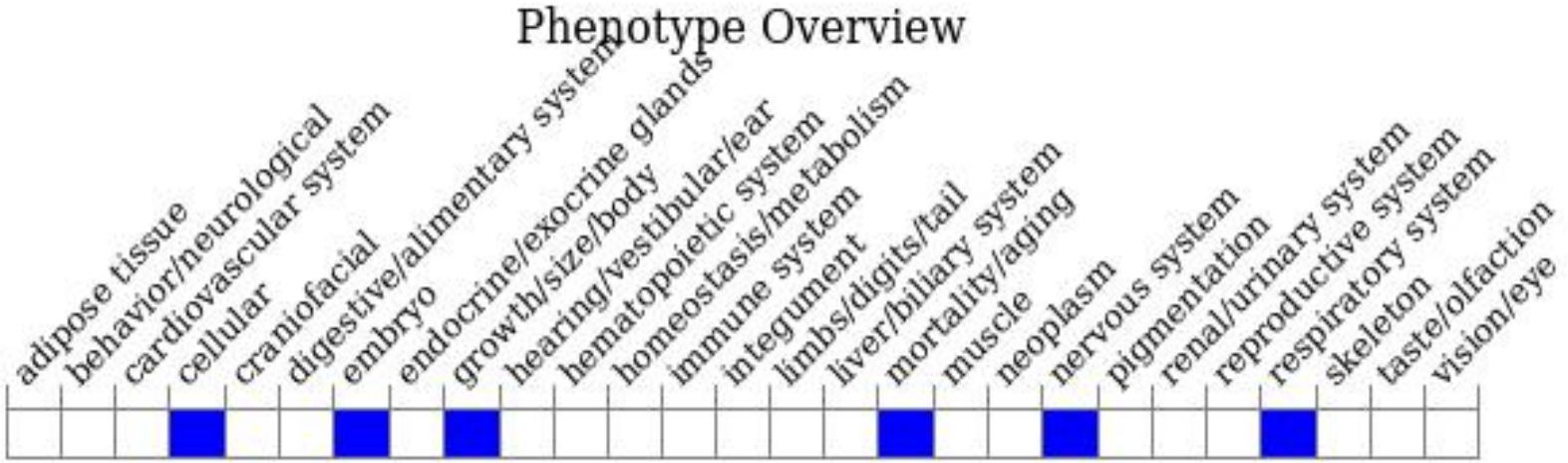
# Genomic location distribution



# 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,homozygous disruption of this gene leads to early embryonic loss as blastocysts fail to enter the S phase. MEFs heterozygous for a gene trap allele have reduced proliferative capacity while MEFs heterozygous for a null allele show reduced doubling rates,increased apoptosis and premature senescence.

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

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