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GemPharmatech

# *Pax5* Cas9-CKO Strategy

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**Reviewer: Jing Jin**

**Design Date: 2019/7/31**

# Project Overview

| | |
|---|---|
| **Project Name** | ***Pax5*** |
| **Project type** | **Cas9-CKO** |
| **Strain background** | **C57BL/6JGpt** |

# Conditional Knockout strategy

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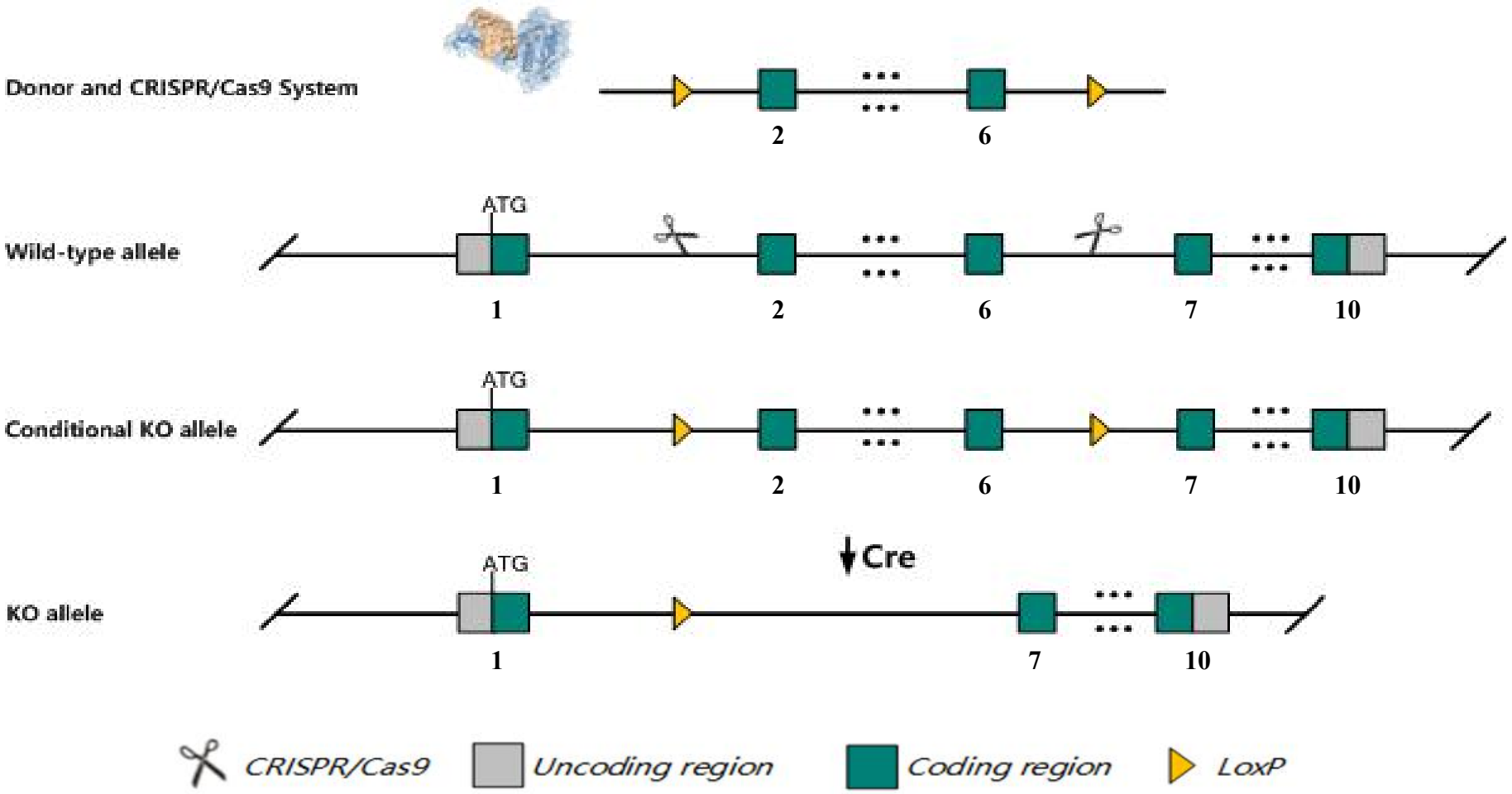
This model will use CRISPR/Cas9 technology to edit the *Pax5* gene. The schematic diagram is as follows:



# Technical routes



The *Pax5* gene has 20 transcripts. According to the structure of *Pax5* gene, exon2-exon6 of *Pax5-201*(ENSMUST00000014174.13) transcript is recommended as the knockout region.The region contains 734bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Pax5* 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,null mutants exhibit impaired development of the midbrain resulting in a reduced inferior colliculus and an altered cerebellar folial pattern, failure of B cell differentiation, runting, and high postnatal mortality with few survivors.

The KO region contains functional region of the Pax5 gene.Knockout the region may affect the function of LOC108254674 gene.

The *Pax5* 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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## Pax5 paired box 5 [Mus musculus (house mouse)]

Gene ID: 18507, updated on 13-Mar-2020

### Summary

| | |
|---|---|
| **Official Symbol** | Pax5 provided by MGI |
| **Official Full Name** | paired box 5 provided byMGI |
| **Primary source** | MGI:MGI:97489 |
| **See related** | Ensembl:ENSMUSG00000014030 |
| **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** | BSAP, EBB-1, KLP, Pax-5 |
| **Expression** | Biased expression in spleen adult (RPKM 27.3), mammary gland adult (RPKM 4.5) and 3 other tissuesSee more |
| **Orthologs** | human all |

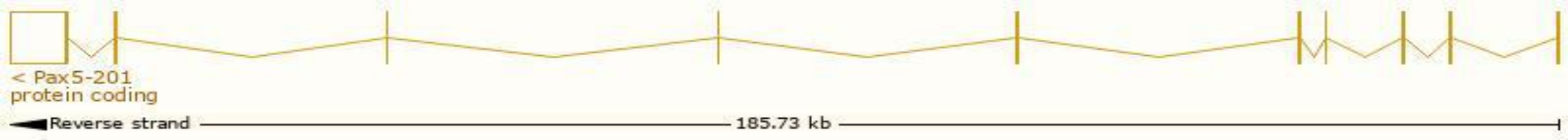
# Transcript information Ensembl



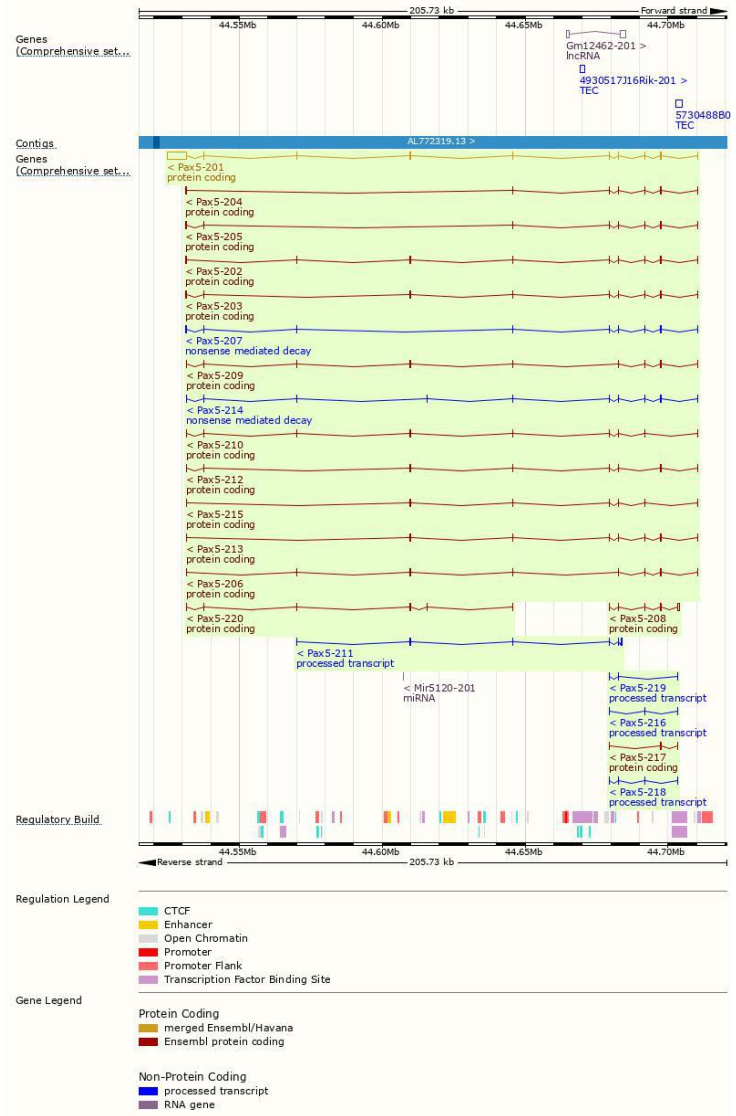
The gene has 20 transcripts,all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|---|---|---|---|---|---|---|---|
| Pax5-201 | ENSMUST00000014174.13 | 8004 | 391aa | Protein coding | CCDS18125 | Q02650 | 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 |
| Pax5-202 | ENSMUST00000102932.9 | 1460 | 362aa | Protein coding | - | A2AKM6 | TSL:5 GENCODE basic |
| Pax5-203 | ENSMUST00000107825.8 | 1445 | 357aa | Protein coding | - | A2AKM5 | TSL:5 GENCODE basic |
| Pax5-205 | ENSMUST00000107827.8 | 1315 | 355aa | Protein coding | - | A2AKM7 | TSL:5 GENCODE basic |
| Pax5-204 | ENSMUST00000107826.8 | 1228 | 326aa | Protein coding | - | A2AKM8 | TSL:5 GENCODE basic |
| Pax5-208 | ENSMUST00000146335.7 | 1194 | 200aa | Protein coding | - | V9GXS5 | CDS 3' incomplete TSL:5 |
| Pax5-209 | ENSMUST00000165417.8 | 1056 | 348aa | Protein coding | - | G3UW65 | TSL:5 GENCODE basic |
| Pax5-210 | ENSMUST00000172866.7 | 1019 | 336aa | Protein coding | - | G3UYK4 | CDS 5' incomplete TSL:5 |
| Pax5-213 | ENSMUST00000173821.7 | 996 | 328aa | Protein coding | - | G3V008 | TSL:5 GENCODE basic |
| Pax5-206 | ENSMUST00000134968.8 | 969 | 319aa | Protein coding | - | G3UX39 | TSL:5 GENCODE basic |
| Pax5-212 | ENSMUST00000173733.7 | 885 | 291aa | Protein coding | - | G3UXF6 | TSL:5 GENCODE basic |
| Pax5-215 | ENSMUST00000174319.7 | 830 | 273aa | Protein coding | - | G3UY80 | CDS 5' incomplete TSL:5 |
| Pax5-220 | ENSMUST00000194075.1 | 631 | 209aa | Protein coding | - | A0A0A6YVX2 | CDS 5' incomplete TSL:5 |
| Pax5-217 | ENSMUST00000186542.1 | 324 | 108aa | Protein coding | - | A0A087WST9 | CDS 3' incomplete TSL:5 |
| Pax5-207 | ENSMUST00000143235.7 | 1417 | 288aa | Nonsense mediated decay | - | G3UZ71 | TSL:5 |
| Pax5-214 | ENSMUST00000174242.7 | 1112 | 307aa | Nonsense mediated decay | - | G3UZ86 | TSL:5 |
| Pax5-211 | ENSMUST00000172949.7 | 758 | No protein | Processed transcript | - | - | TSL:5 |
| Pax5-218 | ENSMUST00000187197.6 | 421 | No protein | Processed transcript | - | - | TSL:5 |
| Pax5-216 | ENSMUST00000174761.7 | 356 | No protein | Processed transcript | - | - | TSL:5 |
| Pax5-219 | ENSMUST00000191036.1 | 223 | No protein | Processed transcript | - | - | TSL:5 |

The strategy is based on the design of *Pax5-201* transcript,the transcription is shown below:

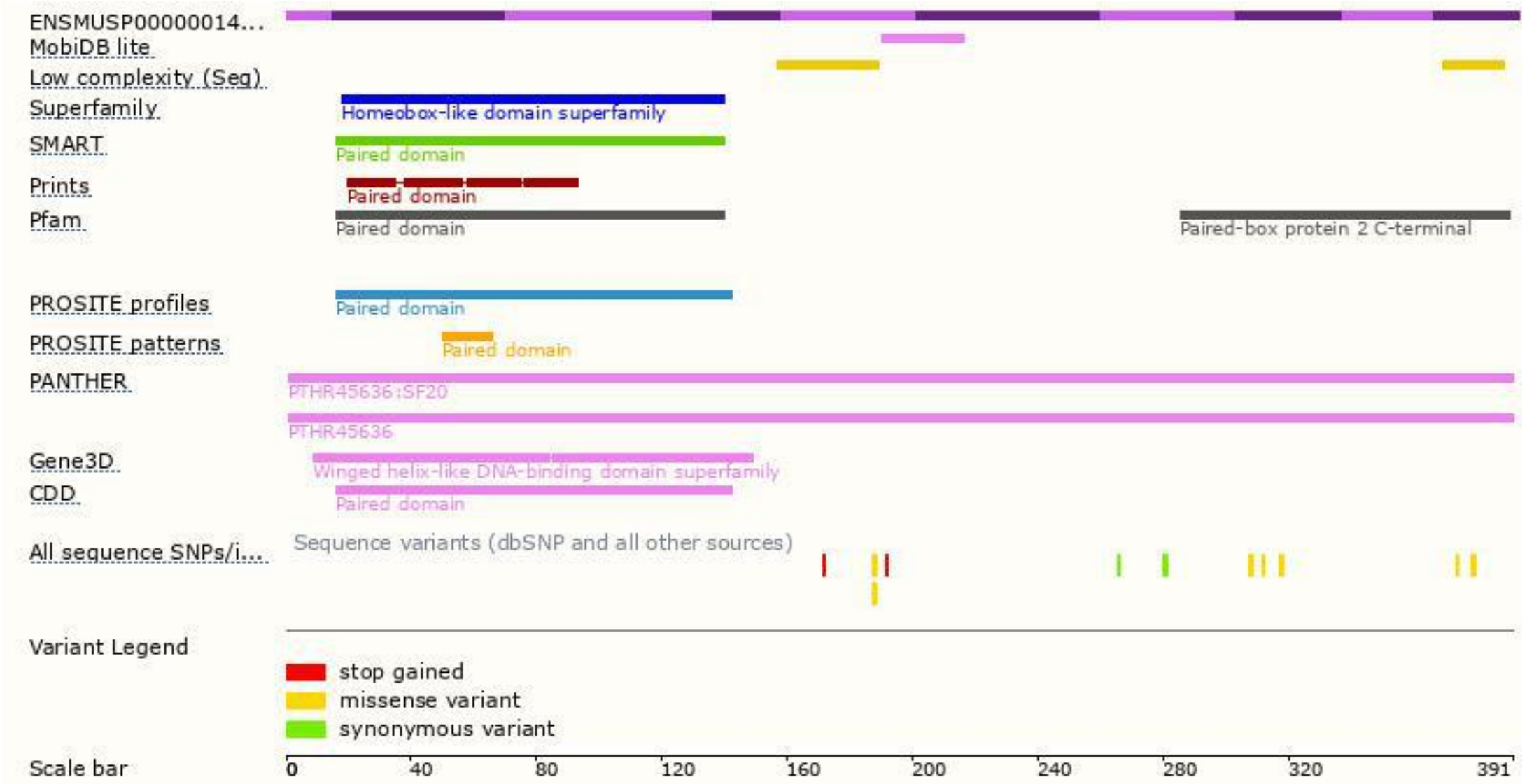


# Genomic location distribution

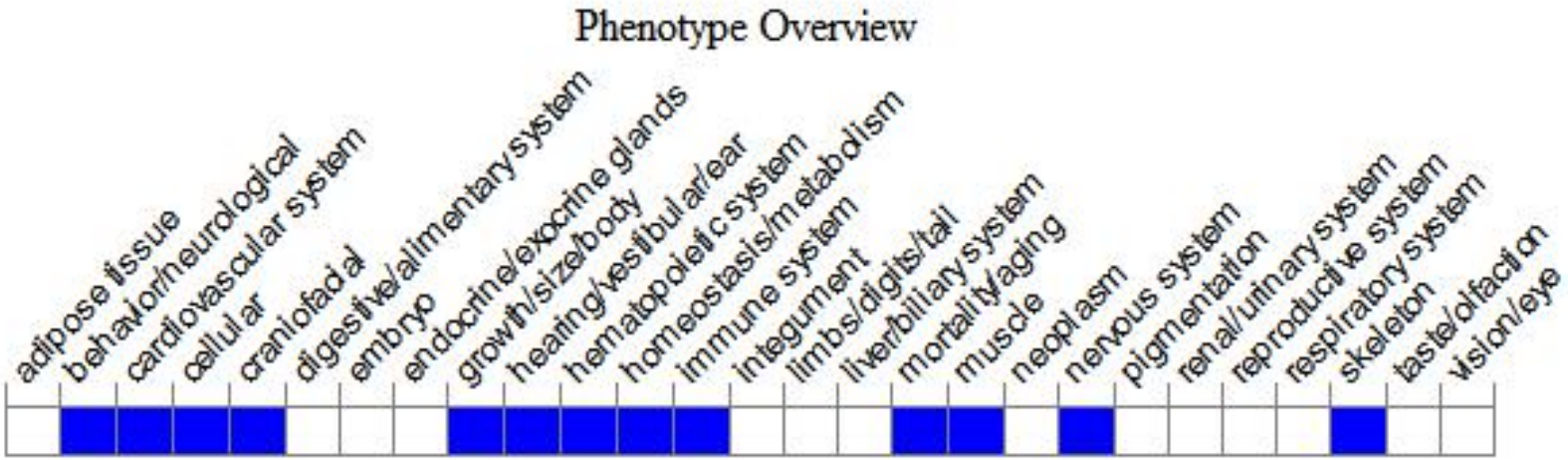


# Protein domain





# 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,null mutants exhibit impaired development of the midbrain resulting in a reduced inferior colliculus and an altered cerebellar folial pattern, failure of B cell differentiation, runting, and high postnatal mortality with few survivors.

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

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