

# *Car4* Cas9-CKO Strategy

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**Reviewer:**

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# Project Overview

**Project Name**

*Car4*

**Project type**

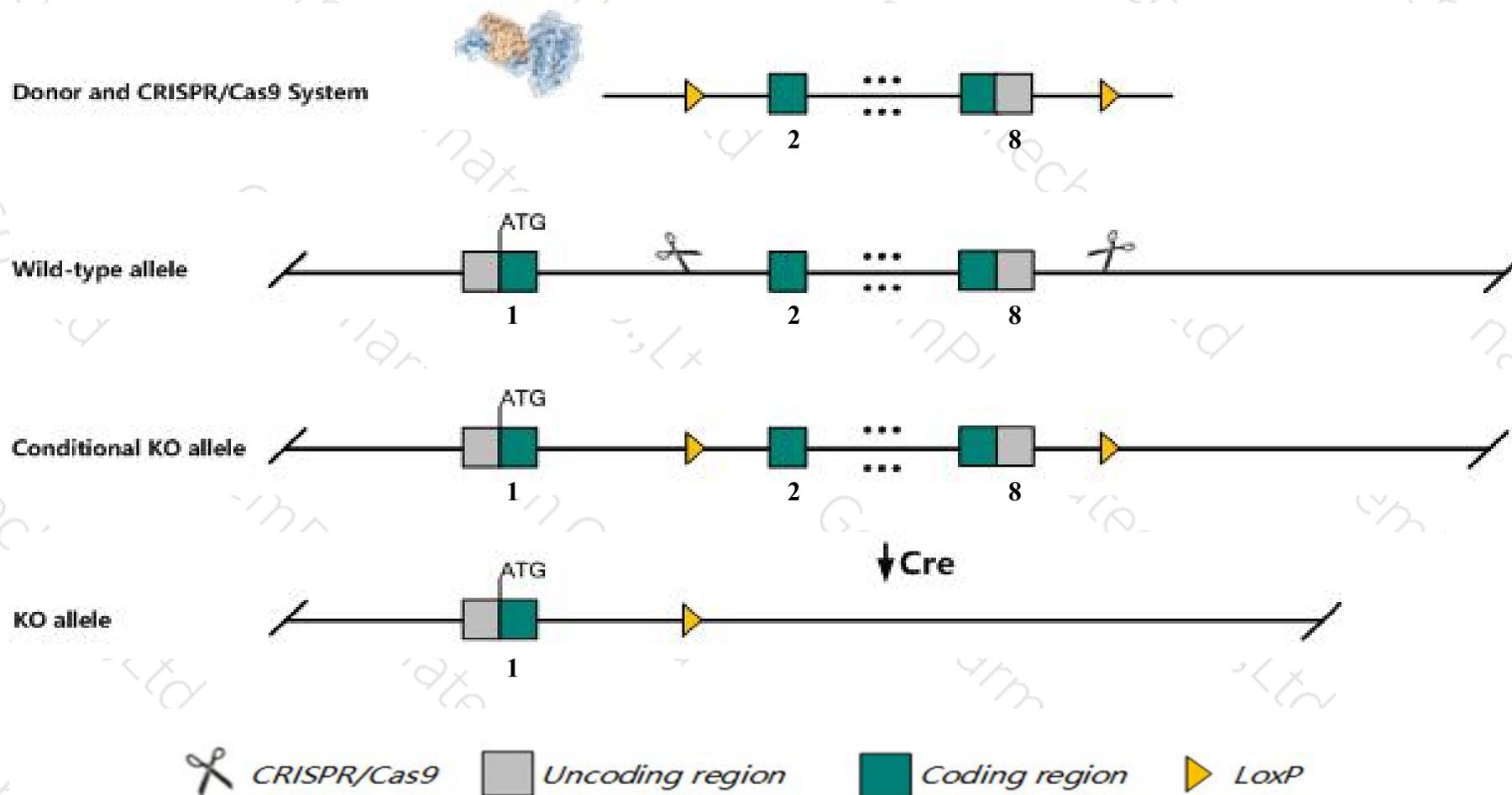
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Car4* gene. The schematic diagram is as follows:



# Technical routes

- The *Car4* gene has 6 transcripts. According to the structure of *Car4* gene, exon2-exon8 of *Car4-201* (ENSMUST00000103194.9) transcript is recommended as the knockout region. The region contains most of coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Car4* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA 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, Homozygous null mice are produced in lower than expected numbers, with females preferentially lost in the fetal or early postnatal period. Surviving homozygotes are healthy and fertile when crossed with wild-type partners; however, homozygous intercrosses yield small litters and pups do not survive.
- The *Car4* gene is located on the Chr11. 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)

## Car4 carbonic anhydrase 4 [ *Mus musculus* (house mouse) ]

Gene ID: 12351, updated on 12-Aug-2019

### Summary

|                           |   |
|---------------------------|---|
| <b>Official Symbol</b>    | Car4 provided by <a href="#">MGI</a>  |
| <b>Official Full Name</b> | carbonic anhydrase 4 provided by <a href="#">MGI</a>  |
| <b>Primary source</b>     | <a href="#">MGI:MGI:1096574</a>   |
| <b>See related</b>        | <a href="#">Ensembl:ENSMUSG00000000805</a>  |
| <b>Gene type</b>          | protein coding  |
| <b>RefSeq status</b>      | VALIDATED   |
| <b>Organism</b>           | <a href="#">Mus musculus</a>  |
| <b>Lineage</b>            | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| <b>Also known as</b>      | Ca4; AW456718   |
| <b>Expression</b>         | Biased expression in colon adult (RPKM 148.4), placenta adult (RPKM 86.4) and 10 other tissues <a href="#">See more</a>   |
| <b>Orthologs</b>          | <a href="#">human</a> <a href="#">all</a>   |

# Transcript information (Ensembl)

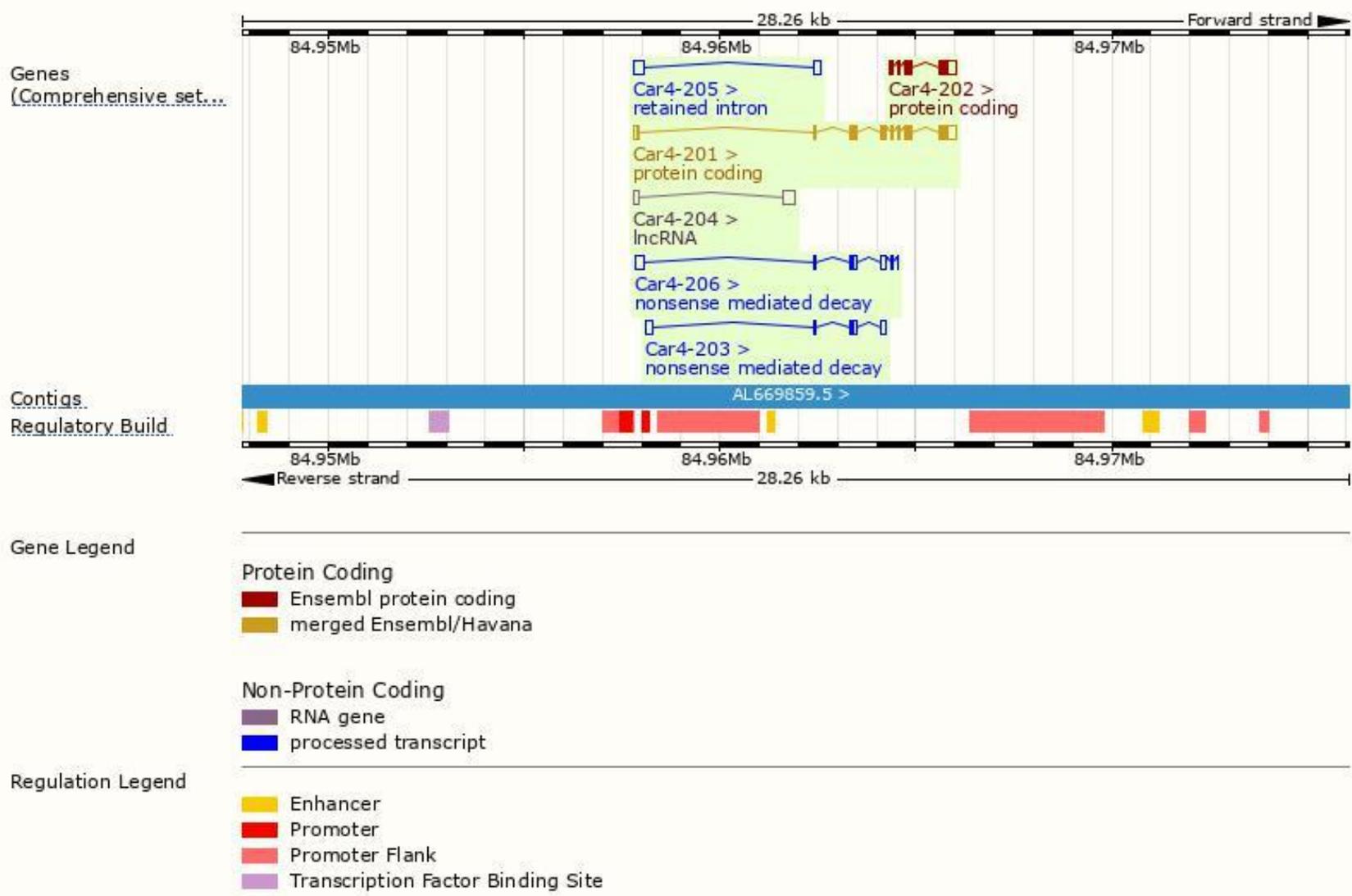
The gene has 6 transcripts, all transcripts are shown below:

| Name     | Transcript ID                        | bp   | Protein               | Biotype                 | CCDS                      | UniProt                | Flags                         |
|----------|--------------------------------------|------|-----------------------|-------------------------|---------------------------|------------------------|-------------------------------|
| Car4-201 | <a href="#">ENSMUST00000103194.9</a> | 1256 | <a href="#">305aa</a> | Protein coding          | <a href="#">CCDS25190</a> | <a href="#">Q64444</a> | TSL:1 GENCODE basic APPRIS P1 |
| Car4-202 | <a href="#">ENSMUST00000108076.2</a> | 732  | <a href="#">164aa</a> | Protein coding          | -                         | <a href="#">F6ST32</a> | CDS 5' incomplete TSL:3       |
| Car4-206 | <a href="#">ENSMUST00000150596.7</a> | 692  | <a href="#">38aa</a>  | Nonsense mediated decay | -                         | <a href="#">D6RCZ3</a> | TSL:5                         |
| Car4-203 | <a href="#">ENSMUST00000127827.1</a> | 516  | <a href="#">38aa</a>  | Nonsense mediated decay | -                         | <a href="#">D6RCZ3</a> | TSL:2                         |
| Car4-204 | <a href="#">ENSMUST00000138331.1</a> | 443  | No protein            | Processed transcript    | -                         | -                      | TSL:3                         |
| Car4-205 | <a href="#">ENSMUST00000139416.1</a> | 435  | No protein            | Retained intron         | -                         | -                      | TSL:3                         |

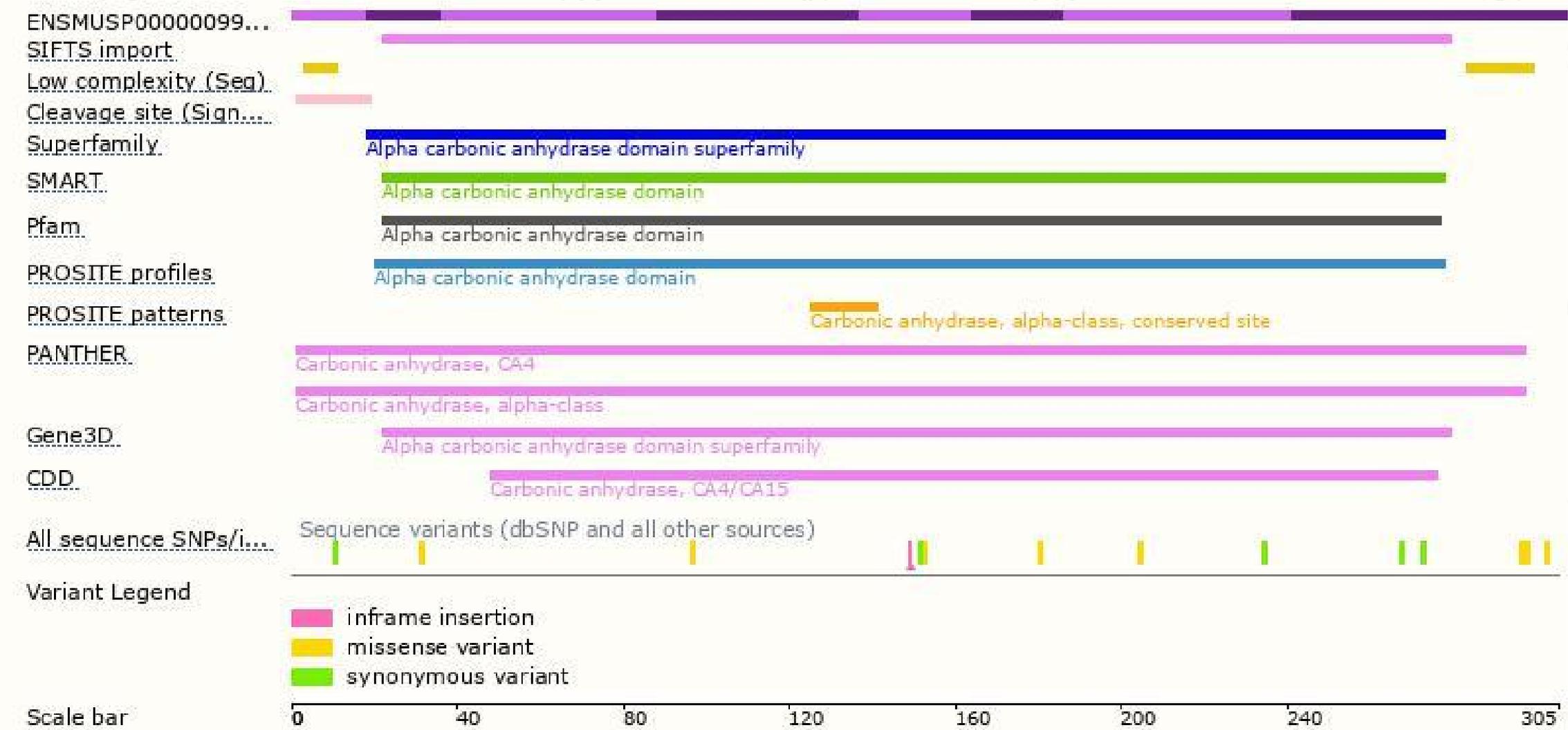
The strategy is based on the design of *Car4-201* transcript, The transcription is shown below



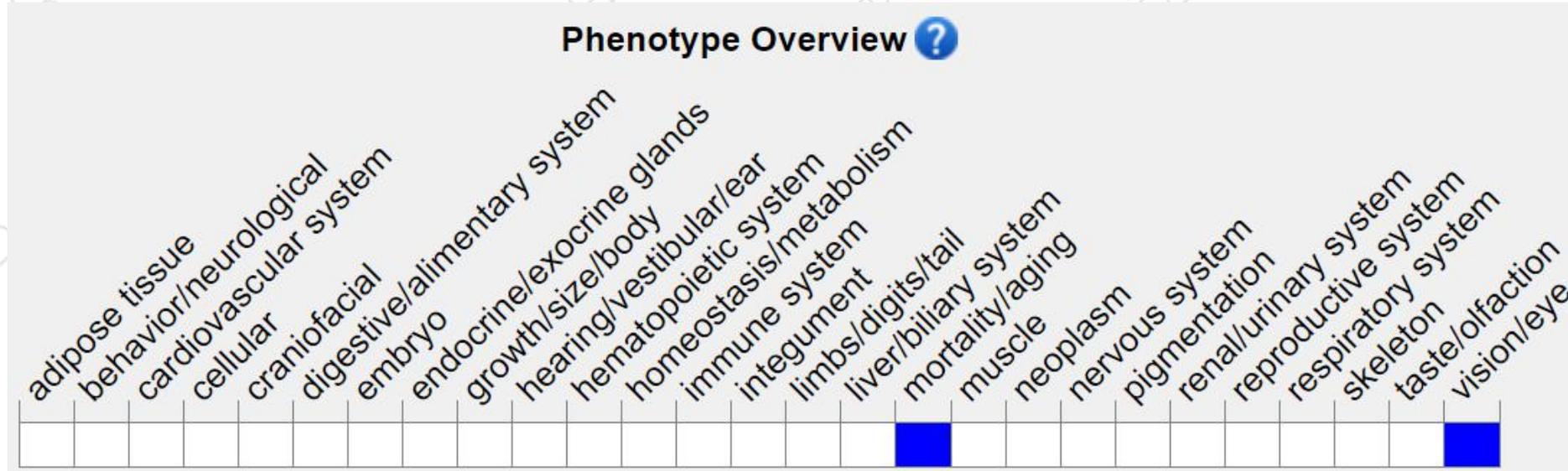
# Genomic location distribution



# 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 null mice are produced in lower than expected numbers, with females preferentially lost in the fetal or early postnatal period. Surviving homozygotes are healthy and fertile when crossed with wild-type partners; however, homozygous intercrosses yield small litters and pups do not survive.

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

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