

# ***Raf1*** Cas9-CKO Strategy

**Designer:**

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**Design Date:**

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# Project Overview

**Project Name**

*Raf1*

**Project type**

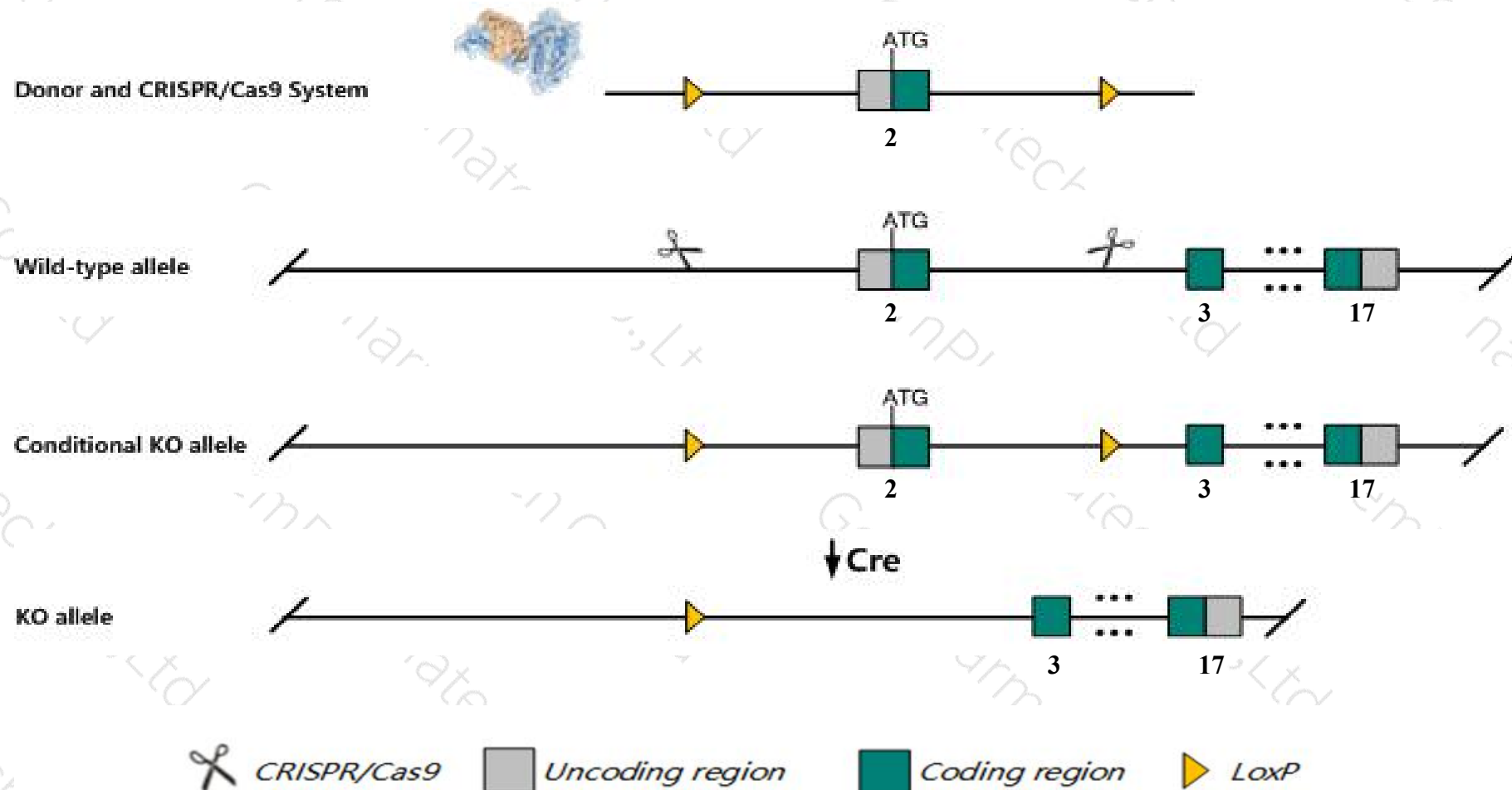
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Raf1* gene. The schematic diagram is as follows:



- The *Raf1* gene has 11 transcripts. According to the structure of *Raf1* gene, exon2 of *Raf1-201* (ENSMUST00000000451.13) 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 *Raf1* 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.

- According to the existing MGI data, Homozygotes for targeted null mutations are growth retarded, with hypocellular fetal livers, placental anomalies, and defects of skin and lungs, resulting in lethality around mid-gestation. Mice heterozygous for a knock-in allele exhibit hypertrophic cardiomyopathy.
- Transcript *Raf1-206,209* may not be affected.
- The *Raf1* gene is located on the Chr6. 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)

## Raf1 v-raf-leukemia viral oncogene 1 [Mus musculus (house mouse)]

Gene ID: 110157, updated on 31-Jan-2019

### Summary



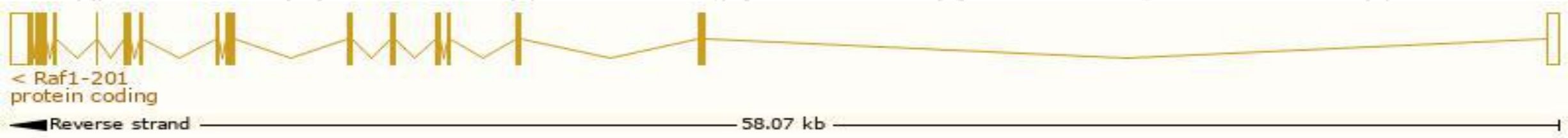
|                           |   |
|---------------------------|---|
| <b>Official Symbol</b>    | Raf1 provided by <a href="#">MGI</a>  |
| <b>Official Full Name</b> | v-raf-leukemia viral oncogene 1 provided by <a href="#">MGI</a>   |
| <b>Primary source</b>     | <a href="#">MGI:MGI:97847</a>   |
| <b>See related</b>        | <a href="#">Ensembl:ENSMUSG00000000441</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>      | 6430402F14Rik, AA990557, BB129353, Cra1, D830050J10Rik, Raf-1, c-Raf, cRaf, v-Raf   |
| <b>Expression</b>         | Ubiquitous expression in CNS E14 (RPKM 50.6), whole brain E14.5 (RPKM 48.5) and 28 other tissues <a href="#">See more</a>   |
| <b>Orthologs</b>          | <a href="#">human</a> <a href="#">all</a>   |

# Transcript information (Ensembl)

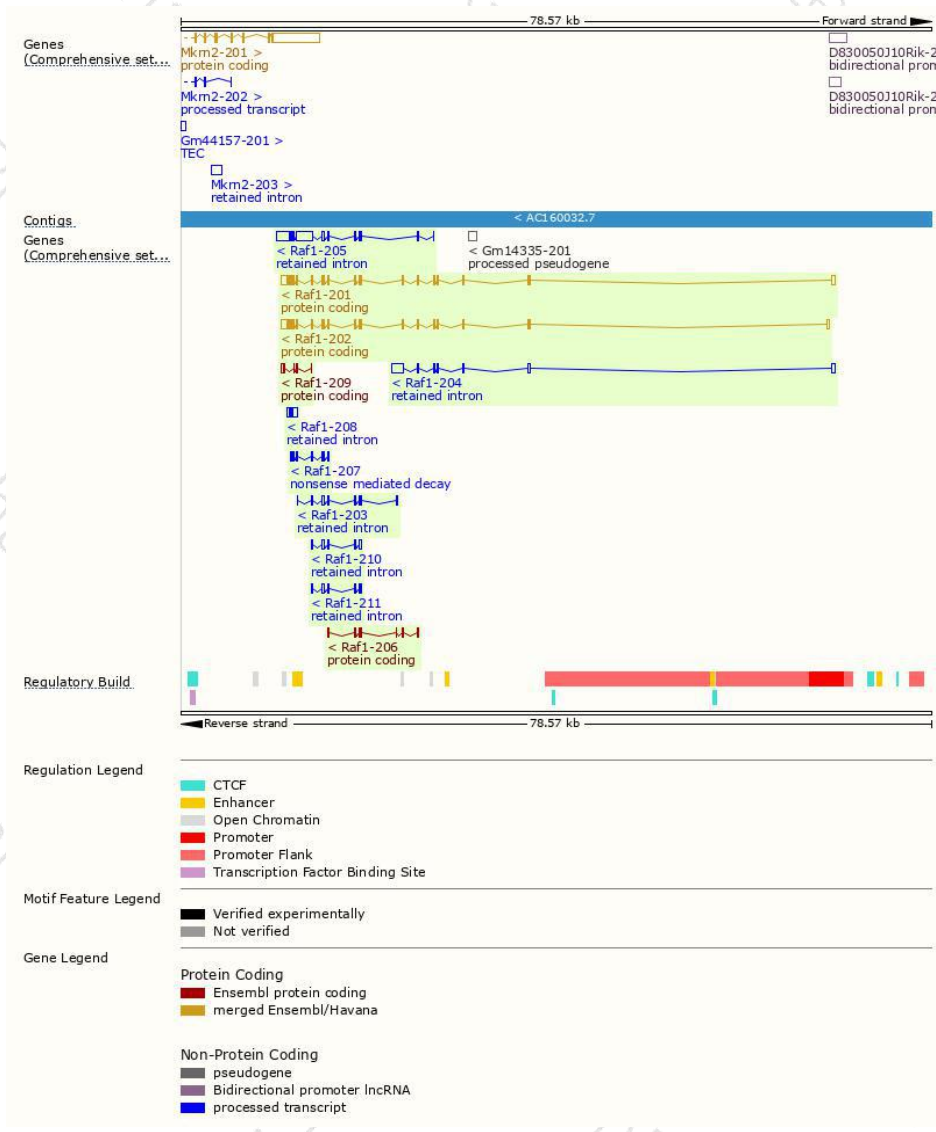
The gene has 11 transcripts,all transcripts are shown below:

| Name     | Transcript ID                         | bp   | Protein               | Biotype                 | CCDS                      | UniProt                    | Flags   |
|----------|---------------------------------------|------|-----------------------|-------------------------|---------------------------|----------------------------|---|
| Raf1-201 | <a href="#">ENSMUST00000000451.13</a> | 3070 | <a href="#">648aa</a> | Protein coding          | <a href="#">CCDS20441</a> | <a href="#">Q99N57</a>     | TSL:1 GENCODE basic APPRIS P1   |
| Raf1-202 | <a href="#">ENSMUST00000112949.7</a>  | 2946 | <a href="#">648aa</a> | Protein coding          | <a href="#">CCDS20441</a> | <a href="#">Q99N57</a>     | TSL:1 GENCODE basic APPRIS P1   |
| Raf1-206 | <a href="#">ENSMUST00000147979.2</a>  | 613  | <a href="#">205aa</a> | Protein coding          | -                         | <a href="#">F6TUC4</a>     | 5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3 |
| Raf1-209 | <a href="#">ENSMUST00000203759.1</a>  | 538  | <a href="#">96aa</a>  | Protein coding          | -                         | <a href="#">A0A0N4SWH5</a> | CDS 5' incomplete TSL:5   |
| Raf1-207 | <a href="#">ENSMUST00000203142.1</a>  | 676  | <a href="#">118aa</a> | Nonsense mediated decay | -                         | <a href="#">A0A0N4SV36</a> | CDS 5' incomplete TSL:3   |
| Raf1-205 | <a href="#">ENSMUST00000130528.7</a>  | 3968 | No protein            | Retained intron         | -                         | -                          | TSL:1   |
| Raf1-204 | <a href="#">ENSMUST00000127503.1</a>  | 2277 | No protein            | Retained intron         | -                         | -                          | TSL:1   |
| Raf1-208 | <a href="#">ENSMUST00000203276.1</a>  | 838  | No protein            | Retained intron         | -                         | -                          | TSL:1   |
| Raf1-210 | <a href="#">ENSMUST00000203826.2</a>  | 745  | No protein            | Retained intron         | -                         | -                          | TSL:5   |
| Raf1-203 | <a href="#">ENSMUST00000124553.3</a>  | 732  | No protein            | Retained intron         | -                         | -                          | TSL:5   |
| Raf1-211 | <a href="#">ENSMUST00000204512.1</a>  | 674  | No protein            | Retained intron         | -                         | -                          | TSL:3   |

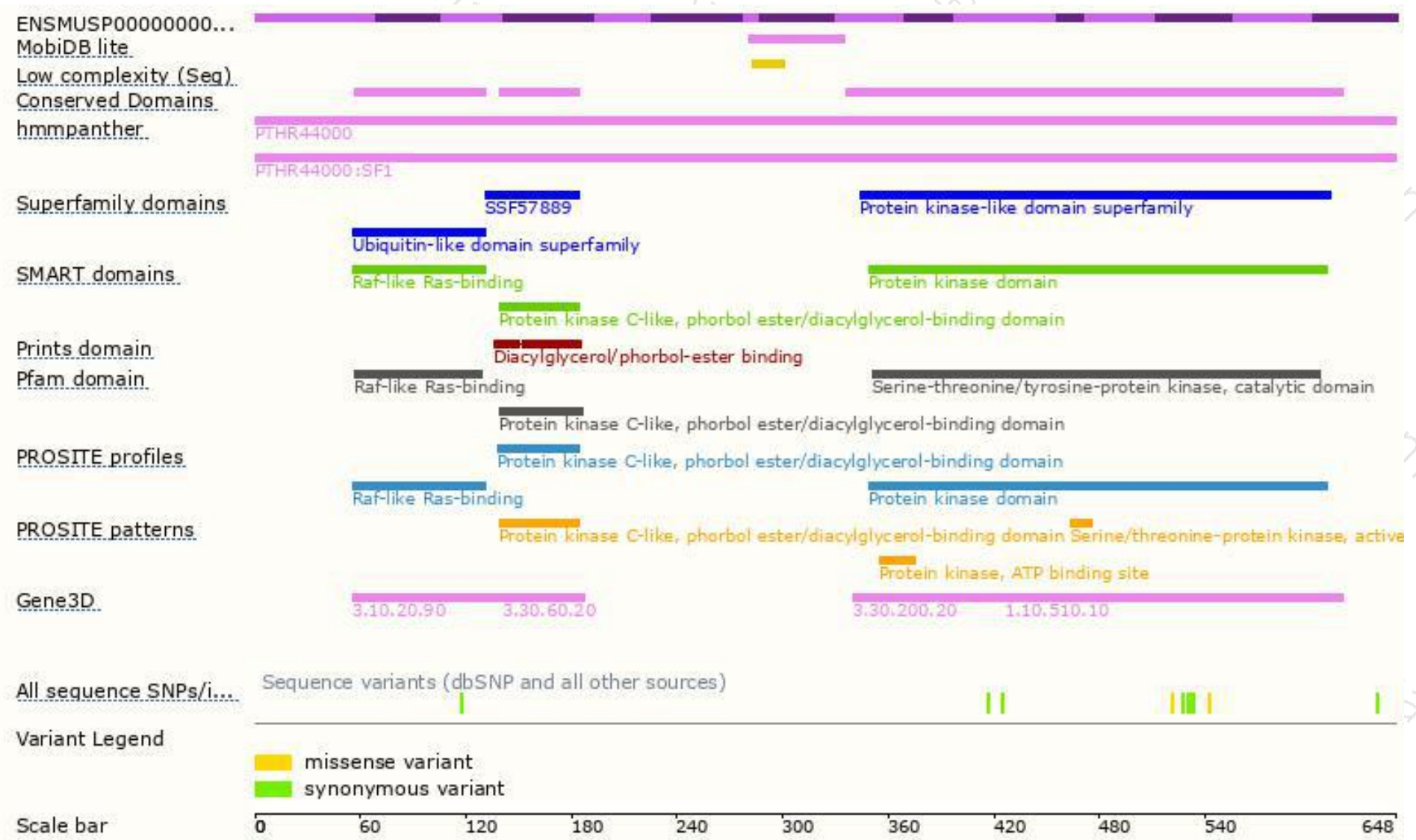
The strategy is based on the design of *Raf1-201* transcript,The transcription is shown below



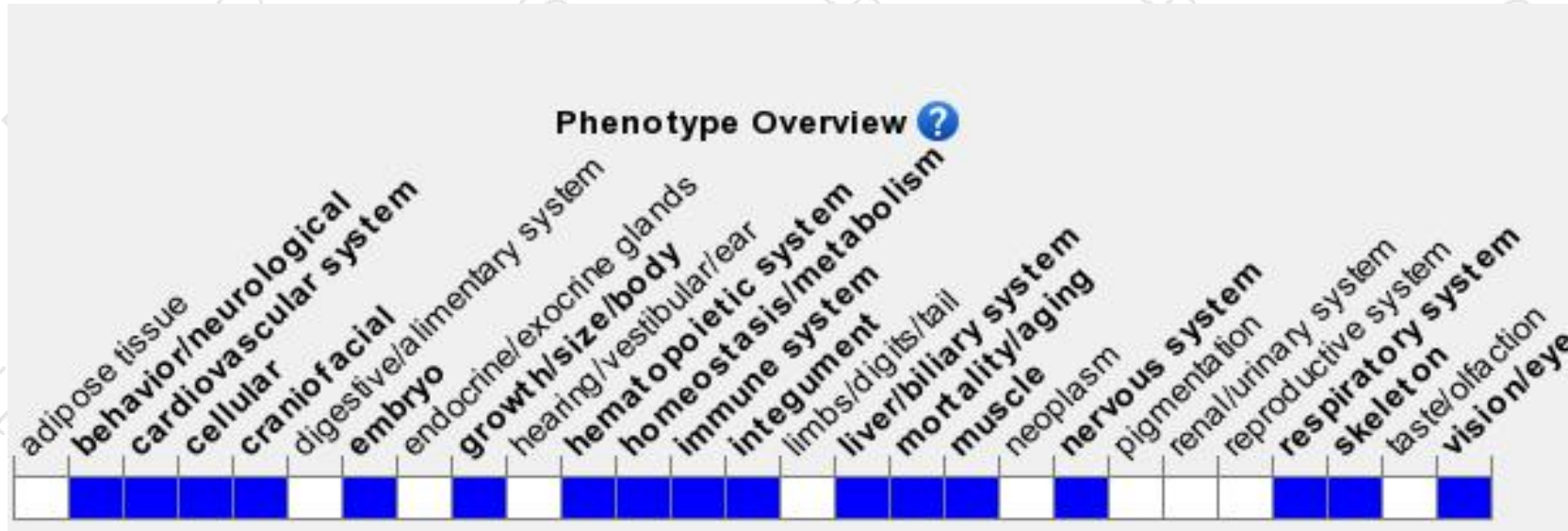
# Genomic location distribution



# 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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