

# ***Ripor2*** Cas9-CKO Strategy

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# Project Overview

**Project Name**

***Ripor2***

**Project type**

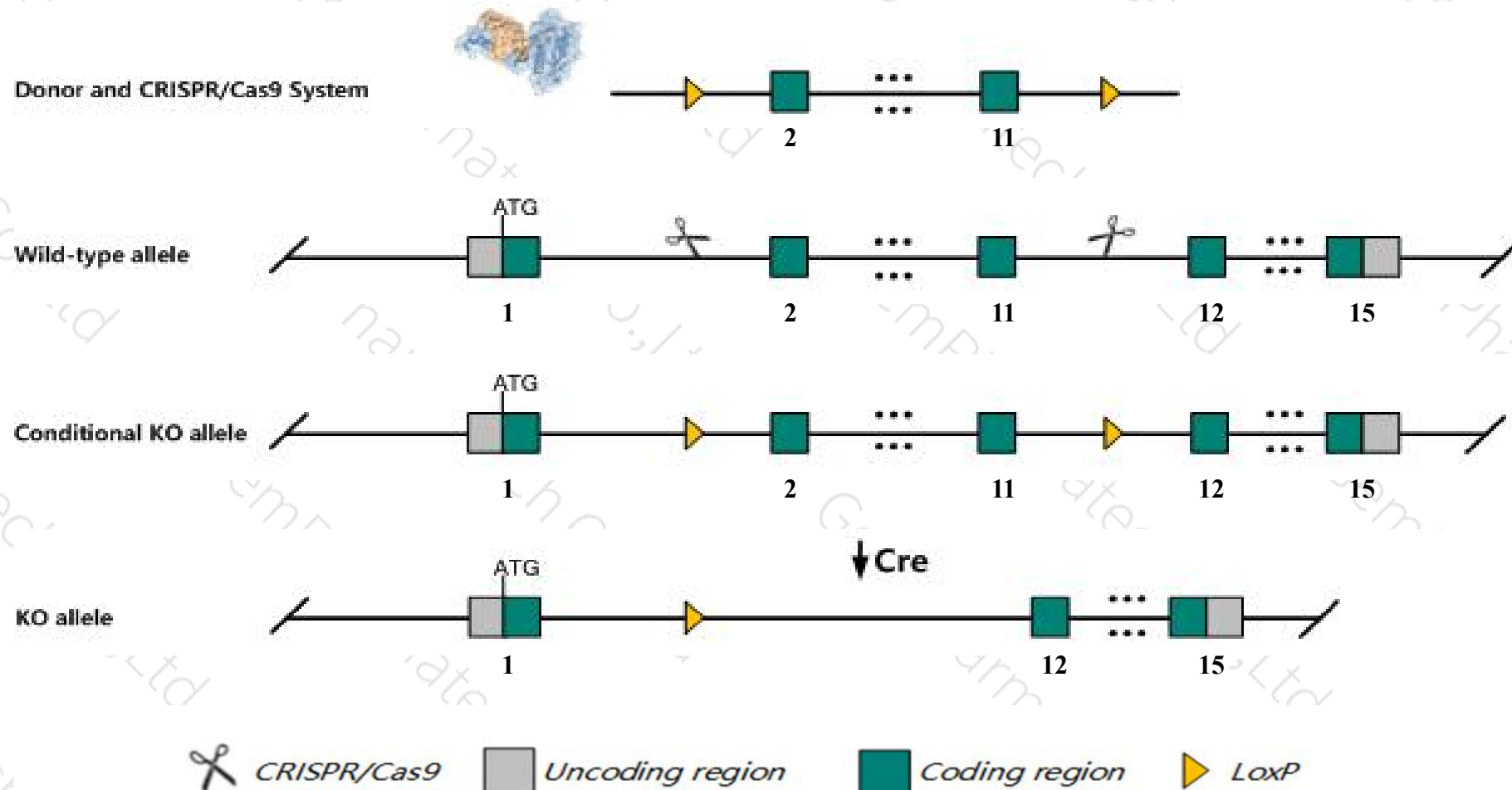
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ripor2* gene. The schematic diagram is as follows:



- The *Ripor2* gene has 14 transcripts. According to the structure of *Ripor2* gene, exon2-exon11 of *Ripor2-201* (ENSMUST00000038477.6) transcript is recommended as the knockout region. The region contains 973bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ripor2* 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, homozygous knockout mice are deaf. the gene product is expressed in the basal region of cochlear hair cell stereocilia, which are disorganized and malformed in null mice.
- The *Ripor2* gene is located on the Chr13. 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)

## Ripor2 RHO family interacting cell polarization regulator 2 [Mus musculus (house mouse)]

Gene ID: 193385, updated on 13-Mar-2020

### Summary



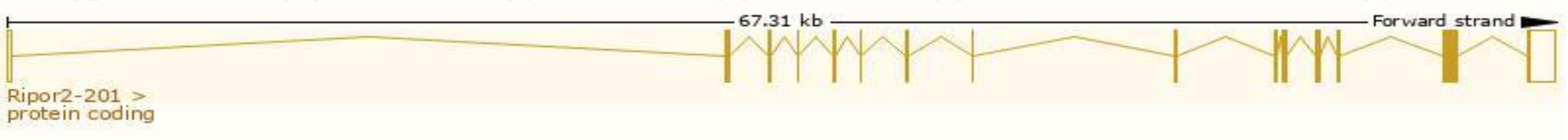
|                           |                                                                                                                                                                           |
|---------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Official Symbol</b>    | Ripor2 provided by <a href="#">MGI</a>                                                                                                                                    |
| <b>Official Full Name</b> | RHO family interacting cell polarization regulator 2 provided by <a href="#">MGI</a>                                                                                      |
| <b>Primary source</b>     | <a href="#">MGI:MGI:2444879</a>                                                                                                                                           |
| <b>See related</b>        | <a href="#">Ensembl:ENSMUSG00000036006</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>      | Fam65b                                                                                                                                                                    |
| <b>Expression</b>         | Broad expression in spleen adult (RPKM 12.9), CNS E18 (RPKM 6.6) and 18 other tissues <a href="#">See more</a>                                                            |
| <b>Orthologs</b>          | <a href="#">human</a> <a href="#">all</a>                                                                                                                                 |

# Transcript information (Ensembl)

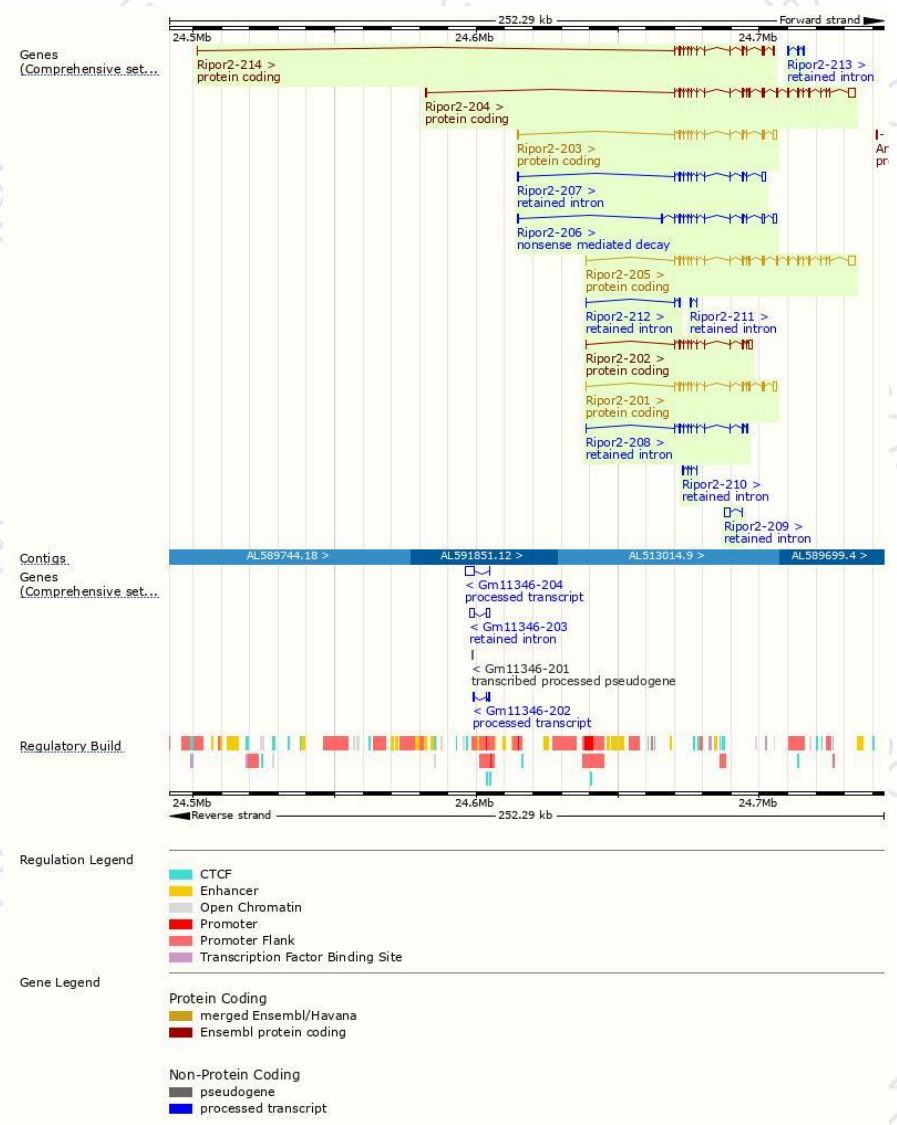
The gene has 14 transcripts,all transcripts are shown below:

| Name       | Transcript ID                         | bp   | Protein                | Biotype                 | CCDS                      | UniProt                | Flags                           |
|------------|---------------------------------------|------|------------------------|-------------------------|---------------------------|------------------------|---------------------------------|
| Ripor2-205 | <a href="#">ENSMUST00000110384.8</a>  | 5608 | <a href="#">1078aa</a> | Protein coding          | <a href="#">CCDS36622</a> | <a href="#">Q80U16</a> | TSL:1 GENCODE basic APPRIS ALT2 |
| Ripor2-204 | <a href="#">ENSMUST00000110383.7</a>  | 5476 | <a href="#">1053aa</a> | Protein coding          | <a href="#">CCDS70438</a> | <a href="#">A6PW28</a> | TSL:1 GENCODE basic APPRIS ALT2 |
| Ripor2-201 | <a href="#">ENSMUST00000038477.6</a>  | 3371 | <a href="#">672aa</a>  | Protein coding          | <a href="#">CCDS36623</a> | <a href="#">Q80U16</a> | TSL:1 GENCODE basic APPRIS ALT2 |
| Ripor2-203 | <a href="#">ENSMUST00000091694.9</a>  | 3142 | <a href="#">633aa</a>  | Protein coding          | <a href="#">CCDS26377</a> | <a href="#">Q80U16</a> | TSL:1 GENCODE basic APPRIS P3   |
| Ripor2-202 | <a href="#">ENSMUST00000058009.15</a> | 2550 | <a href="#">389aa</a>  | Protein coding          | -                         | <a href="#">Q80U16</a> | TSL:2 GENCODE basic APPRIS ALT2 |
| Ripor2-214 | <a href="#">ENSMUST00000238974.1</a>  | 2364 | <a href="#">639aa</a>  | Protein coding          | -                         | -                      | GENCODE basic APPRIS ALT2       |
| Ripor2-206 | <a href="#">ENSMUST00000132689.7</a>  | 3261 | <a href="#">40aa</a>   | Nonsense mediated decay | -                         | <a href="#">F2Z3V4</a> | TSL:1                           |
| Ripor2-207 | <a href="#">ENSMUST00000134370.7</a>  | 2342 | No protein             | Retained intron         | -                         | -                      | TSL:2                           |
| Ripor2-209 | <a href="#">ENSMUST00000175986.1</a>  | 2004 | No protein             | Retained intron         | -                         | -                      | TSL:1                           |
| Ripor2-208 | <a href="#">ENSMUST00000138547.7</a>  | 1350 | No protein             | Retained intron         | -                         | -                      | TSL:1                           |
| Ripor2-213 | <a href="#">ENSMUST00000177489.1</a>  | 760  | No protein             | Retained intron         | -                         | -                      | TSL:5                           |
| Ripor2-212 | <a href="#">ENSMUST00000177298.7</a>  | 665  | No protein             | Retained intron         | -                         | -                      | TSL:2                           |
| Ripor2-211 | <a href="#">ENSMUST00000177174.1</a>  | 649  | No protein             | Retained intron         | -                         | -                      | TSL:3                           |
| Ripor2-210 | <a href="#">ENSMUST00000176303.1</a>  | 546  | No protein             | Retained intron         | -                         | -                      | TSL:5                           |

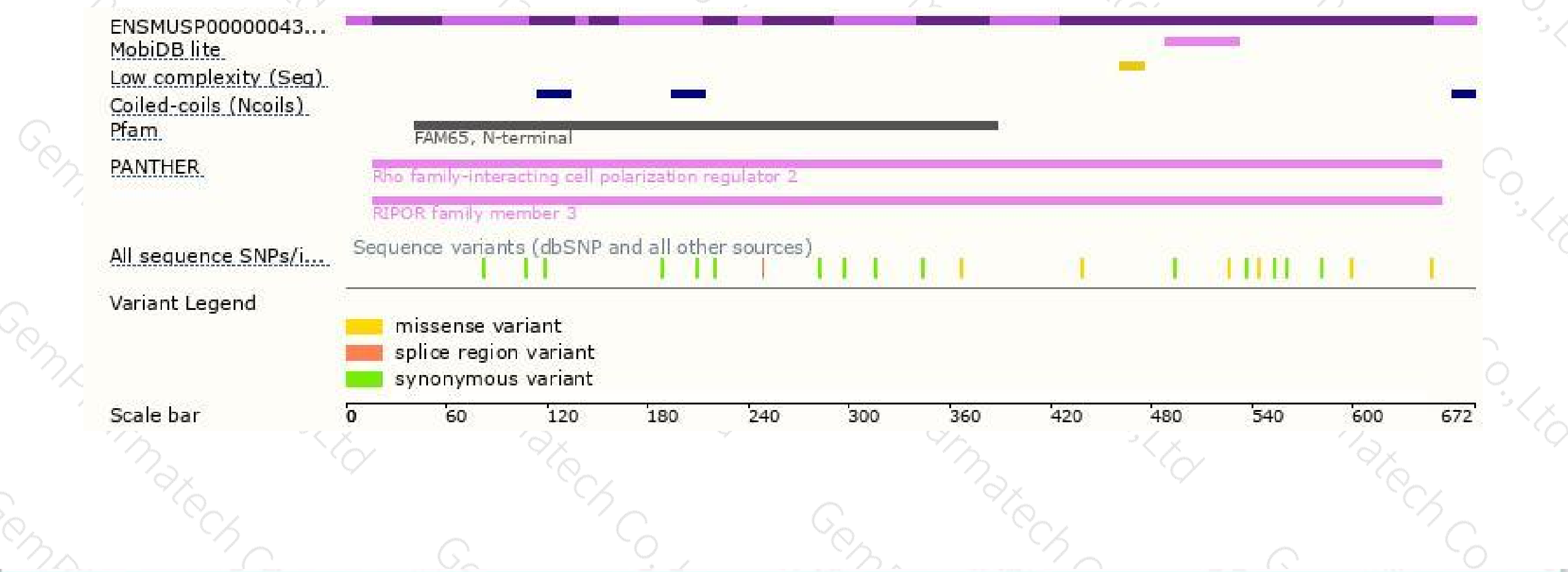
The strategy is based on the design of *Ripor2-201* transcript,The transcription is shown below



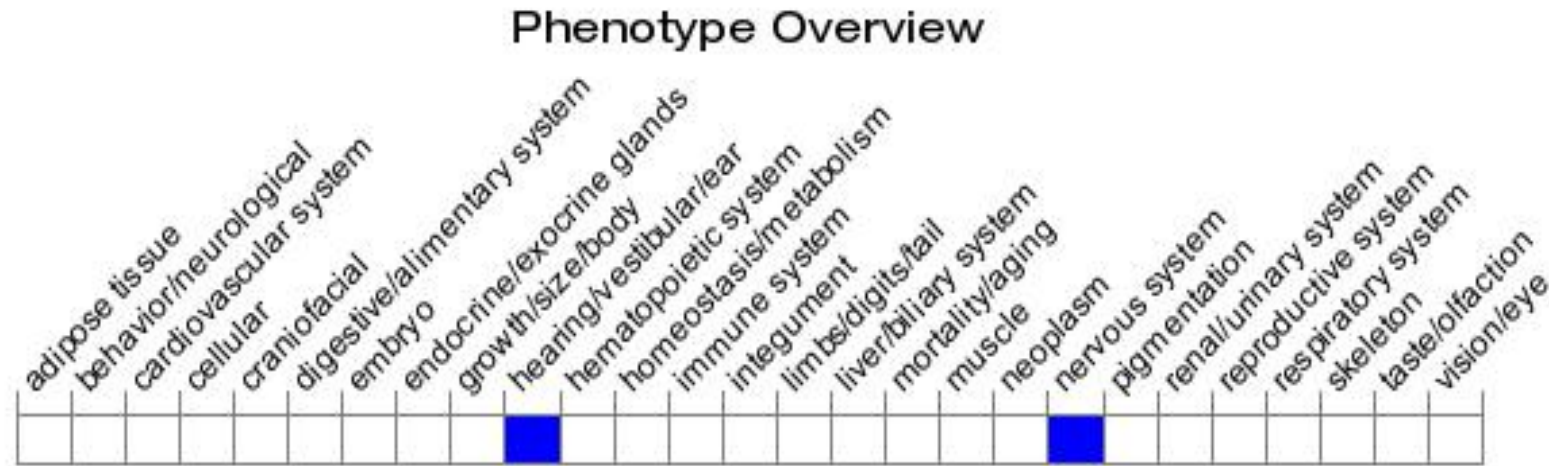
# Genomic location distribution



# 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 knockout mice are deaf. The gene product is expressed in the basal region of cochlear hair cell stereocilia, which are disorganized and malformed in null mice.

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

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