

***Rttn* Cas9-KO Strategy**

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Project Overview

Project Name

Rttn

Project type

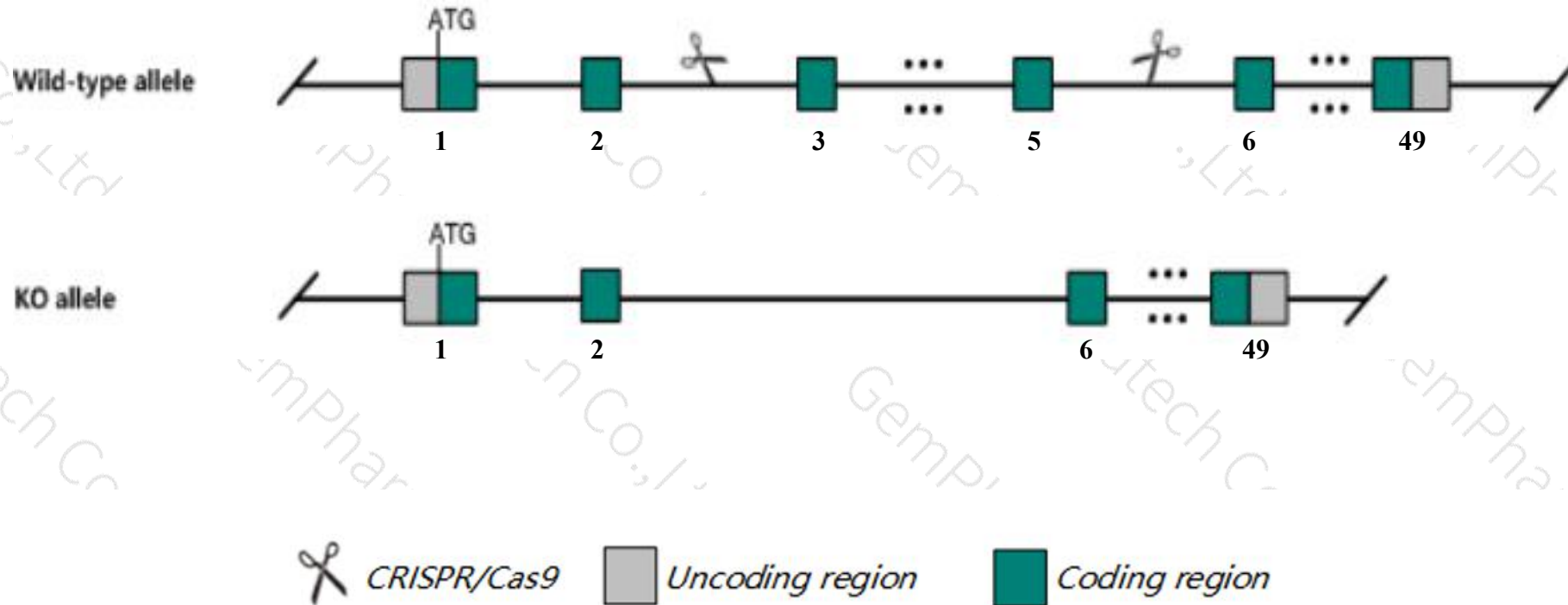
Cas9-KO

Strain background

C57BL/6JGpt

Knockout strategy

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



- The *Rttm* gene has 10 transcripts. According to the structure of *Rttm* gene, exon3-exon5 of *Rttm*-201(ENSMUST00000023828.8) transcript is recommended as the knockout region. The region contains 359bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rttm* gene. The brief process is as follows: CRISPR/Cas9 system 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.

- According to the existing MGI data, mice homozygous for an insertional mutation exhibit embryonic lethality and neurulation defects resulting in the arrest of gastrulation movements and abnormal left-right specification in the heart.
- The floxed region is near to the C-terminal of *Mir6359* gene, this strategy may influence the regulatory function of the C-terminal of *Mir6359* gene.
- Transcript *Rttm*-203&204&205&206&207&208&209&210 may not be affected.
- The *Rttm* gene is located on the Chr18. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)

Rtn rotatin [Mus musculus (house mouse)]

Gene ID: 246102, updated on 13-Mar-2020

Summary



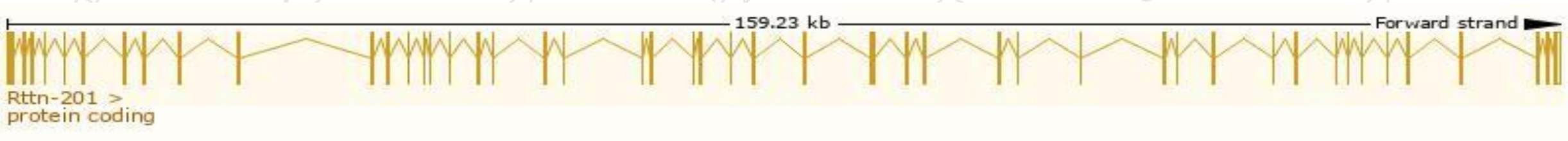
| | |
|---------------------------|---|
| Official Symbol | Rtn provided by MGI |
| Official Full Name | rotatin provided by MGI |
| Primary source | MGI:MGI:2179288 |
| See related | Ensembl:ENSMUSG00000023066 |
| Gene type | protein coding |
| RefSeq status | VALIDATED |
| Organism | Mus musculus |
| Lineage | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| Also known as | 4921538A15Rik, A1666264, C530033I08Rik, D230040K24 |
| Expression | Ubiquitous expression in limb E14.5 (RPKM 2.5), CNS E11.5 (RPKM 2.4) and 24 other tissues See more |
| Orthologs | human all |

Transcript information (Ensembl)

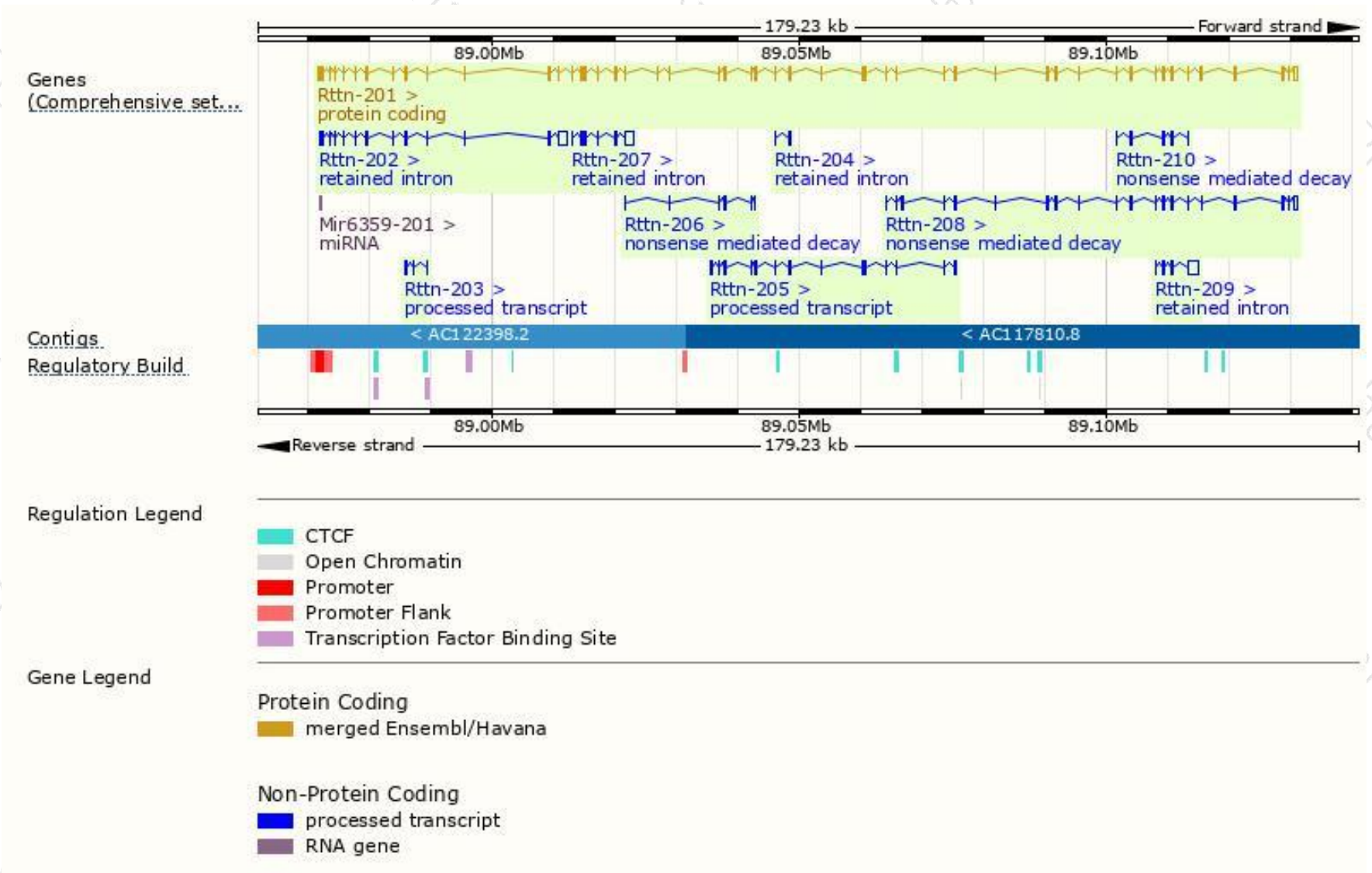
The gene has 10 transcripts,all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|----------|--------------------------------------|------|------------------------|-------------------------|---------------------------|----------------------------|-------------------------------|
| Rttm-201 | ENSMUST00000023828.8 | 7271 | 2226aa | Protein coding | CCDS29390 | Q8R4Y8 | TSL:5 GENCODE basic APPRIS P1 |
| Rttm-208 | ENSMUST00000236676.1 | 2844 | 44aa | Nonsense mediated decay | - | A0A494BBH4 | CDS 5' incomplete |
| Rttm-210 | ENSMUST00000237640.1 | 654 | 123aa | Nonsense mediated decay | - | A0A494B9F2 | CDS 5' incomplete |
| Rttm-206 | ENSMUST00000236313.1 | 615 | 45aa | Nonsense mediated decay | - | A0A494B9L6 | CDS 5' incomplete |
| Rttm-205 | ENSMUST00000235882.1 | 2123 | No protein | Processed transcript | - | - | |
| Rttm-203 | ENSMUST00000235314.1 | 363 | No protein | Processed transcript | - | - | |
| Rttm-202 | ENSMUST00000235272.1 | 3260 | No protein | Retained intron | - | - | |
| Rttm-207 | ENSMUST00000236531.1 | 2209 | No protein | Retained intron | - | - | |
| Rttm-209 | ENSMUST00000237196.1 | 1798 | No protein | Retained intron | - | - | |
| Rttm-204 | ENSMUST00000235728.1 | 488 | No protein | Retained intron | - | - | |

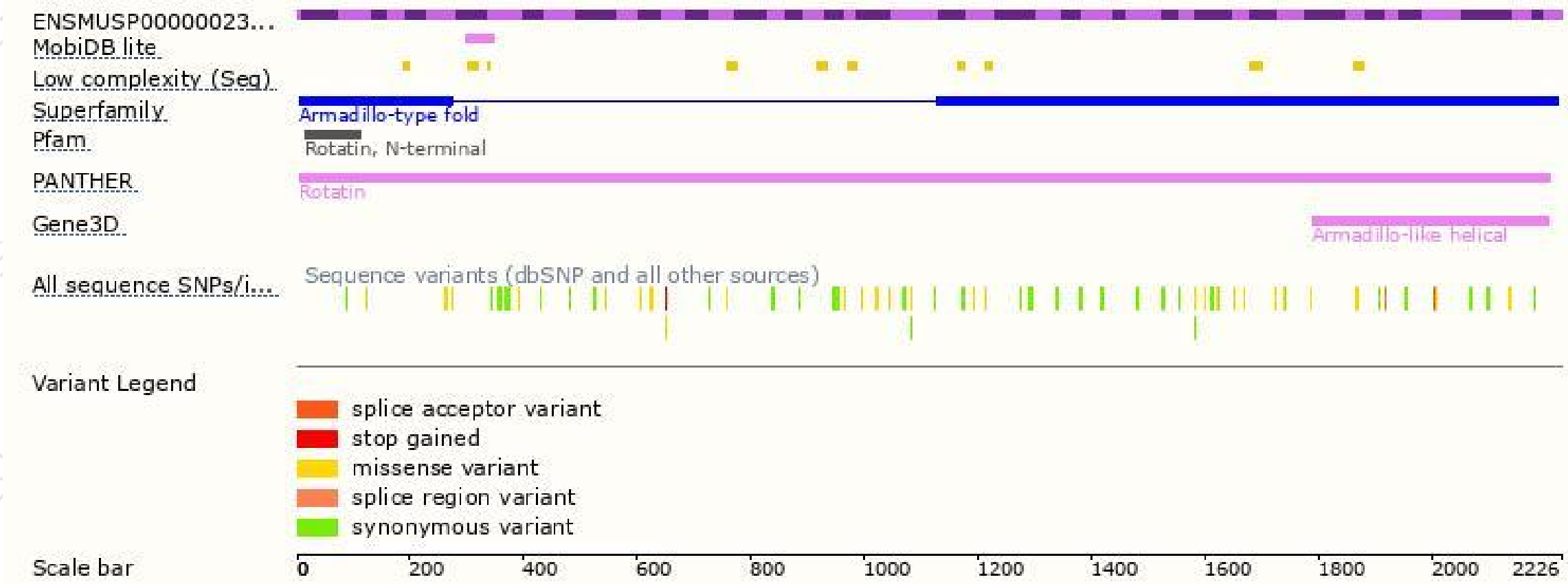
The strategy is based on the design of *Rttm-201* transcript,the transcription is shown below:



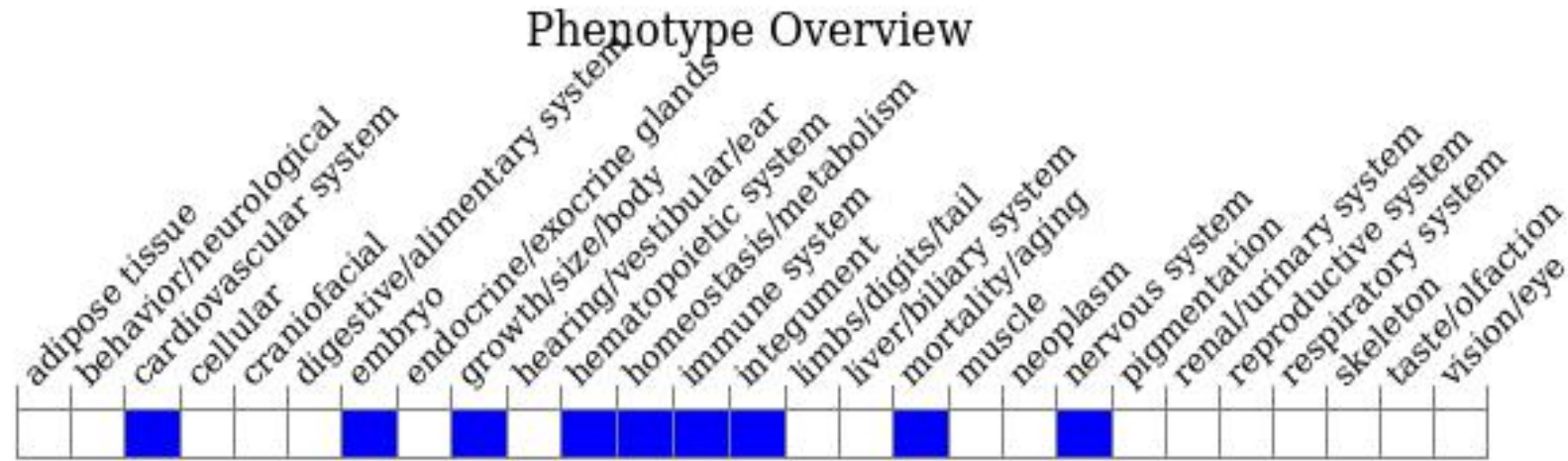
Genomic location distribution



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, mice homozygous for an insertional mutation exhibit embryonic lethality and neurulation defects resulting in the arrest of gastrulation movements and abnormal left-right specification in the heart.

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

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