Rttn Cas9-CKO Strategy

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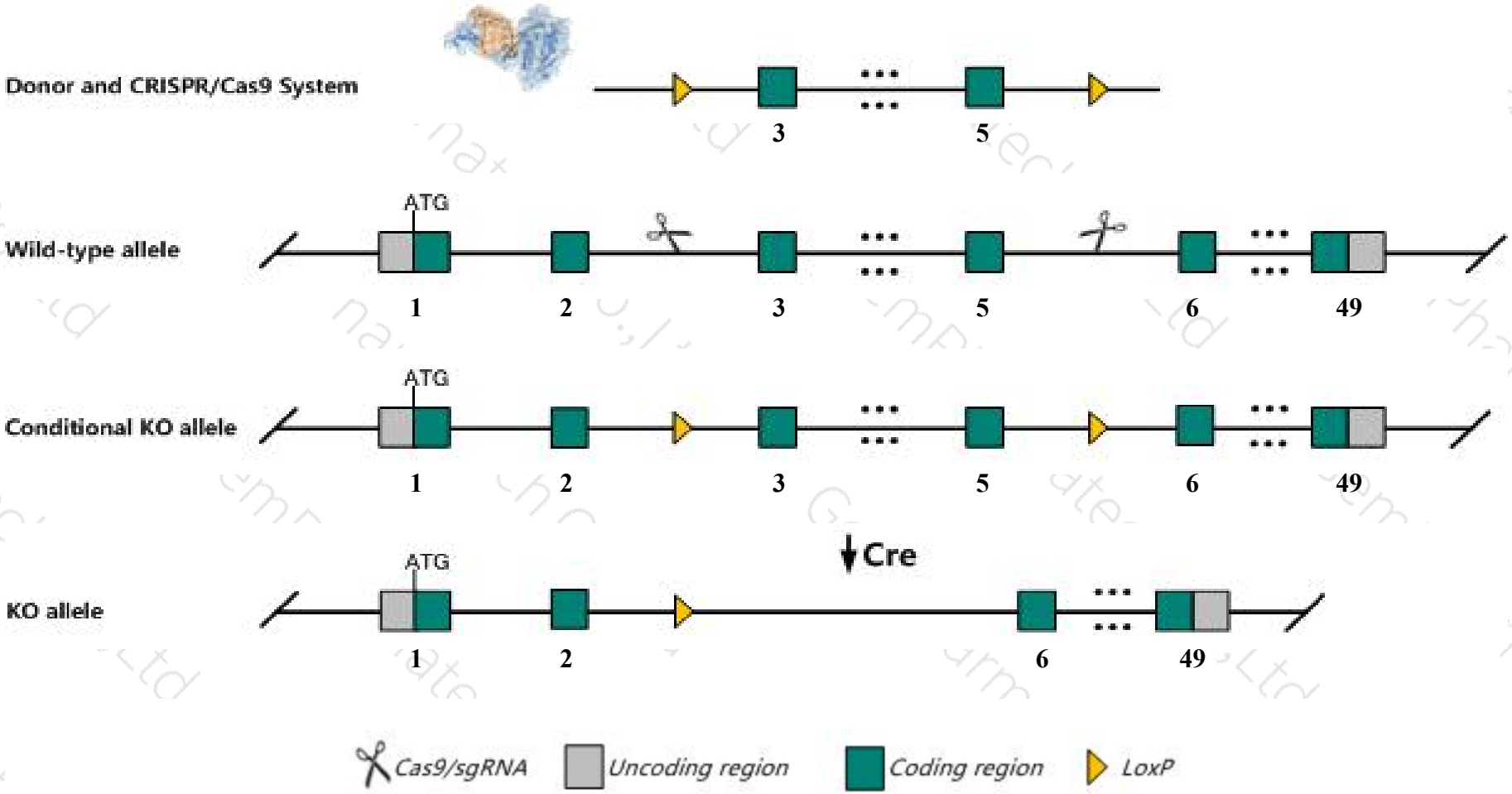
Project Overview

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

Conditional Knockout strategy

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



Technical routes

➢ The *Rttn* gene has 10 transcripts. According to the structure of *Rttn* gene, exon3-exon5 of *Rttn-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 *Rttn* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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,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 knockout 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 *Rttn*-203&204&205&206&207&208&209&210 may not be affected.

➢ The *Rttn* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

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Rttn rotatin [Mus musculus (house mouse)]

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

Summary

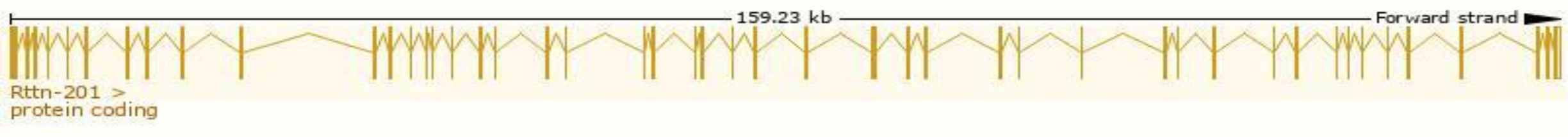
Official Symbol	Rttn provided by MGI
Official Full Name	rotatin provided by MGI
Primary source	MGI:MGI:2179288
See related	Ensembl:ENSMUSG00000023066
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	4921538A15Rik, AI666264, 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）

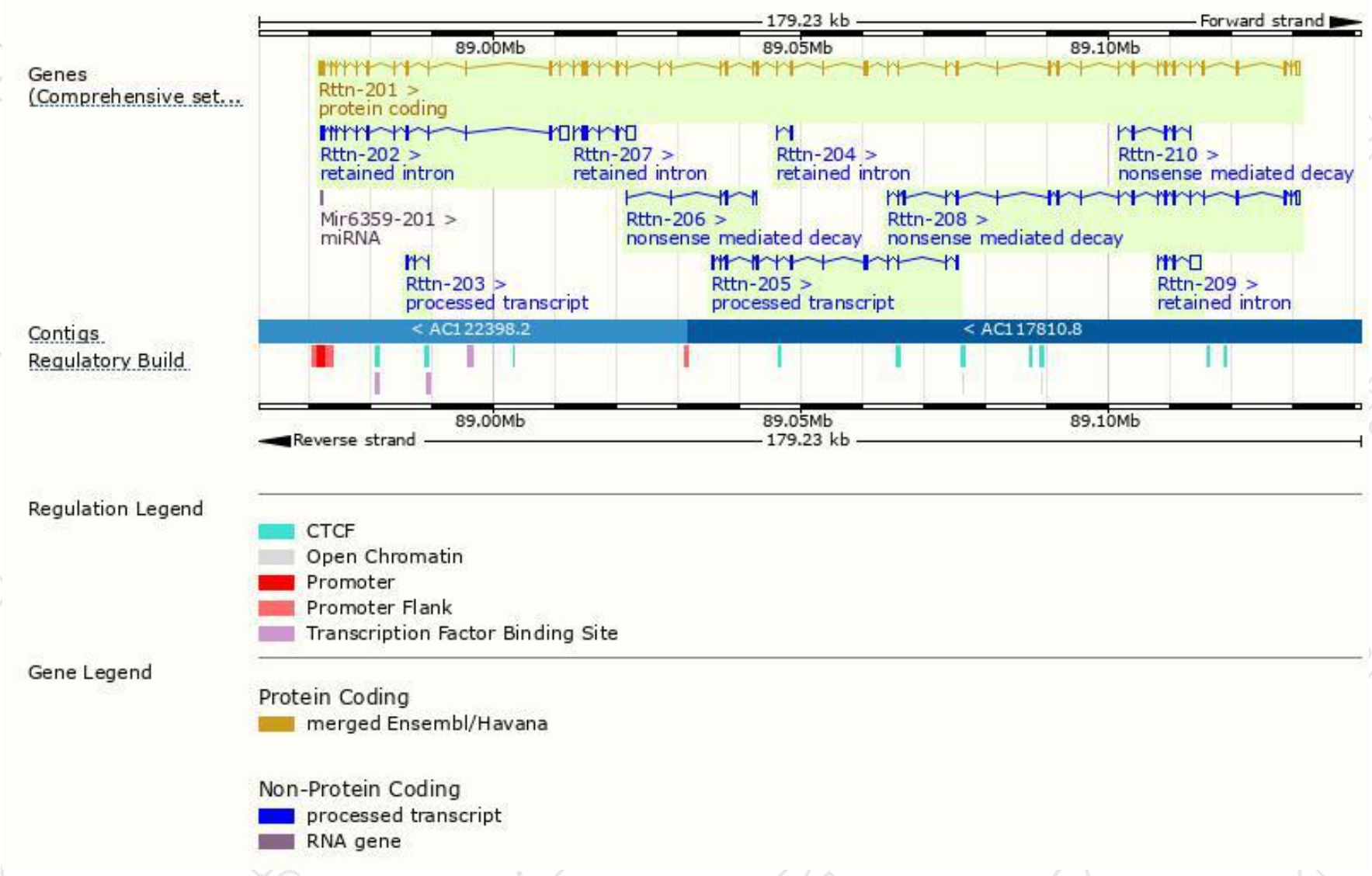
The gene has 10 transcripts,all transcripts are shown below:

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

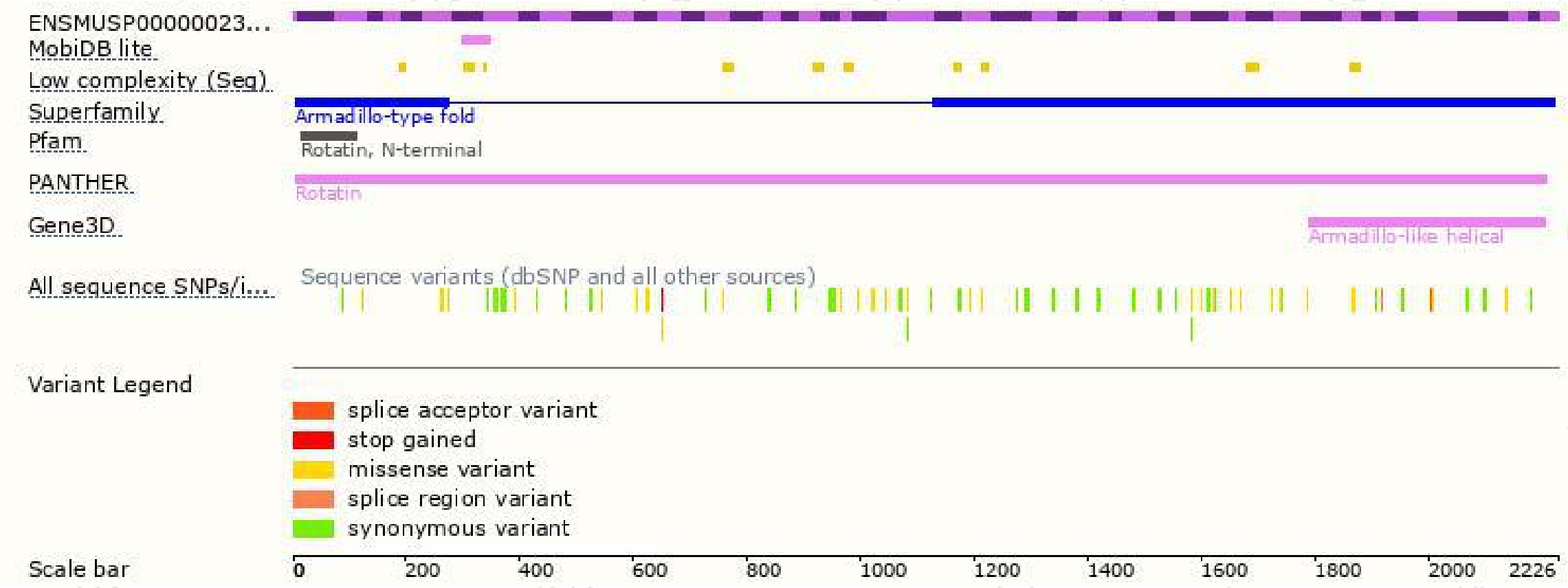
The strategy is based on the design of *Rttn-201* transcript,the transcription is shown below:



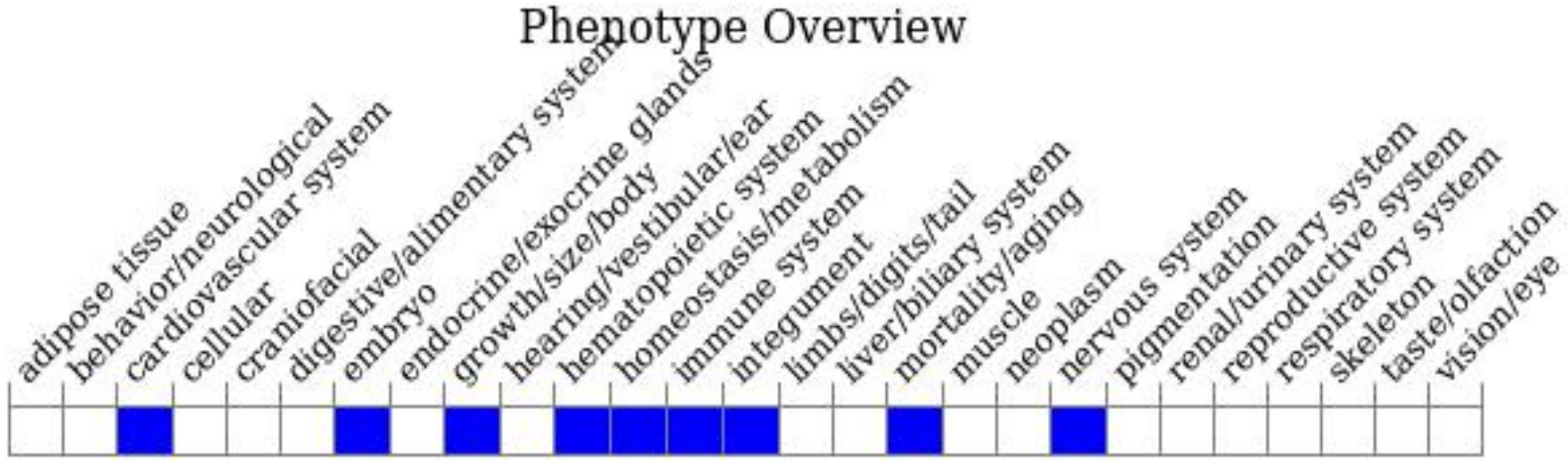
Genomic location distribution



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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