Rab3a Cas9-KO Strategy

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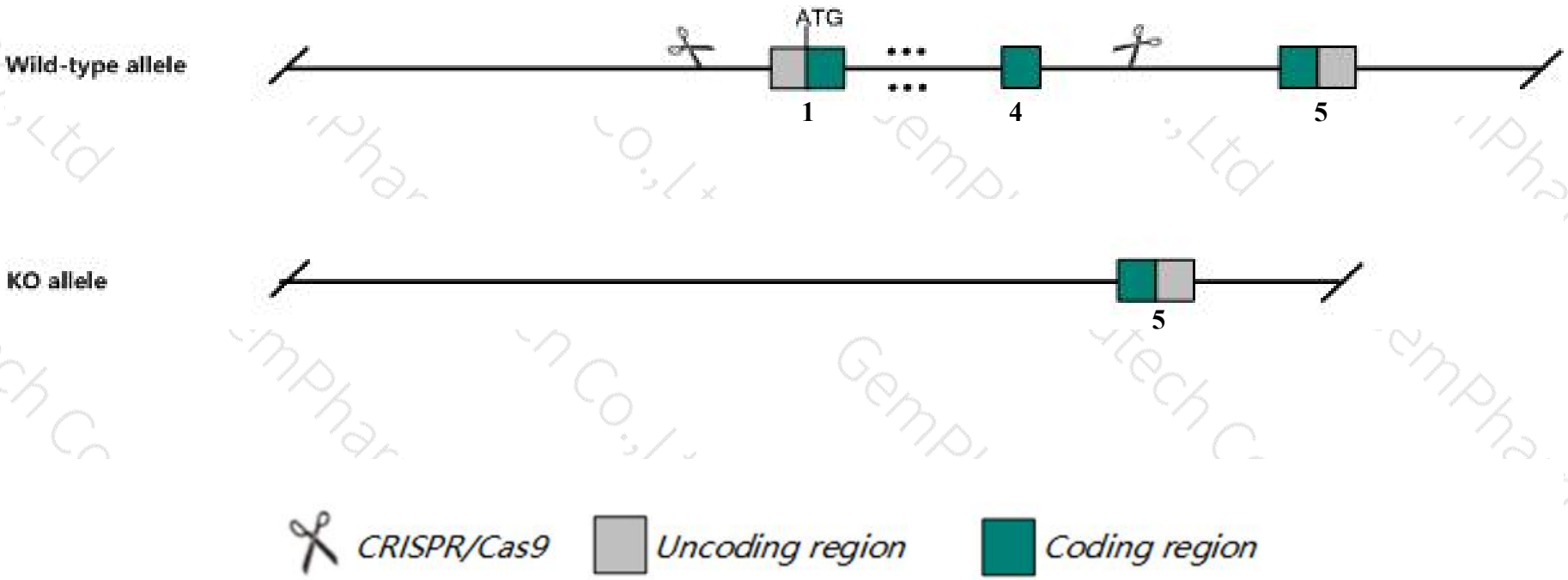
Design Date: 2019-7-29

Project Overview

Project Name	***Rab3a***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rab3a* gene. The schematic diagram is as follows:



Technical routes

➢ The *Rab3a* gene has 6 transcripts. According to the structure of *Rab3a* gene, exon1-exon4 of *Rab3a-204* (ENSMUST00000110093.8) 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 *Rab3a* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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.

Notice

- The floxed region is near to the C-terminal of *Mpv17l2* gene,this strategy may influence the regulatory function of the C-terminal of *Mpv17l2* gene.
- The insertion of 3' loxp may destroy the transcript *Rab3a*-205.
- According to the existing MGI data,Homozygous null mutants show impaired synaptic transmission, insulin secretion and glucose intolerance. This mutation and another chemically induced allele affect circadian period and sleep patterns. Heterozygotes show milder circadian rhythm anomalies.
- The *Rab3a* gene is located on the Chr8. 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）

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Rab3a RAB3A, member RAS oncogene family [Mus musculus (house mouse)]

Gene ID: 19339, updated on 19-Mar-2019

Summary

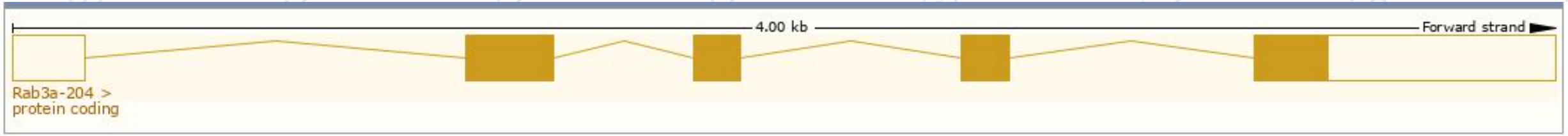
Official Symbol	Rab3a provided by MGI
Official Full Name	RAB3A, member RAS oncogene family provided by MGI
Primary source	MGI:MGI:97843
See related	Ensembl:ENSMUSG00000031840
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Biased expression in cerebellum adult (RPKM 223.6), cortex adult (RPKM 207.3) and 13 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

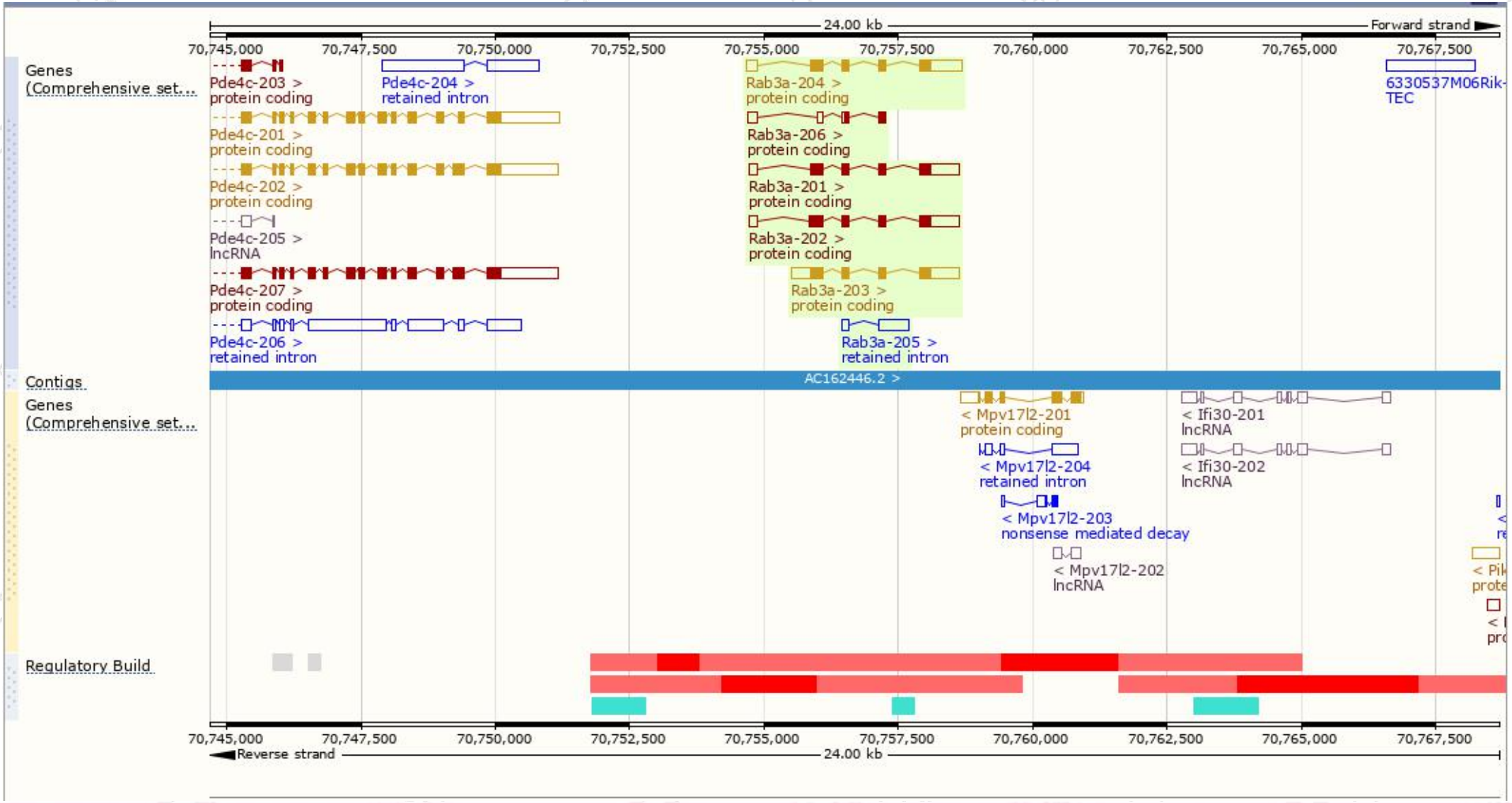
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rab3a-203	ENSMUST00000110092.3	1556	220aa	Protein coding	CCDS22379	P63011 Q0PD63	TSL:1 GENCODE basic APPRIS P1
Rab3a-204	ENSMUST00000110093.8	1442	220aa	Protein coding	CCDS22379	P63011 Q0PD63	TSL:1 GENCODE basic APPRIS P1
Rab3a-202	ENSMUST00000110090.7	1375	220aa	Protein coding	CCDS22379	P63011 Q0PD63	TSL:3 GENCODE basic APPRIS P1
Rab3a-201	ENSMUST00000034301.11	1363	220aa	Protein coding	CCDS22379	P63011 Q0PD63	TSL:1 GENCODE basic APPRIS P1
Rab3a-206	ENSMUST00000143118.2	508	62aa	Protein coding	-	D3YZP5	CDS 3' incomplete TSL:3
Rab3a-205	ENSMUST00000130468.1	672	No protein	Retained intron	-	-	TSL:3

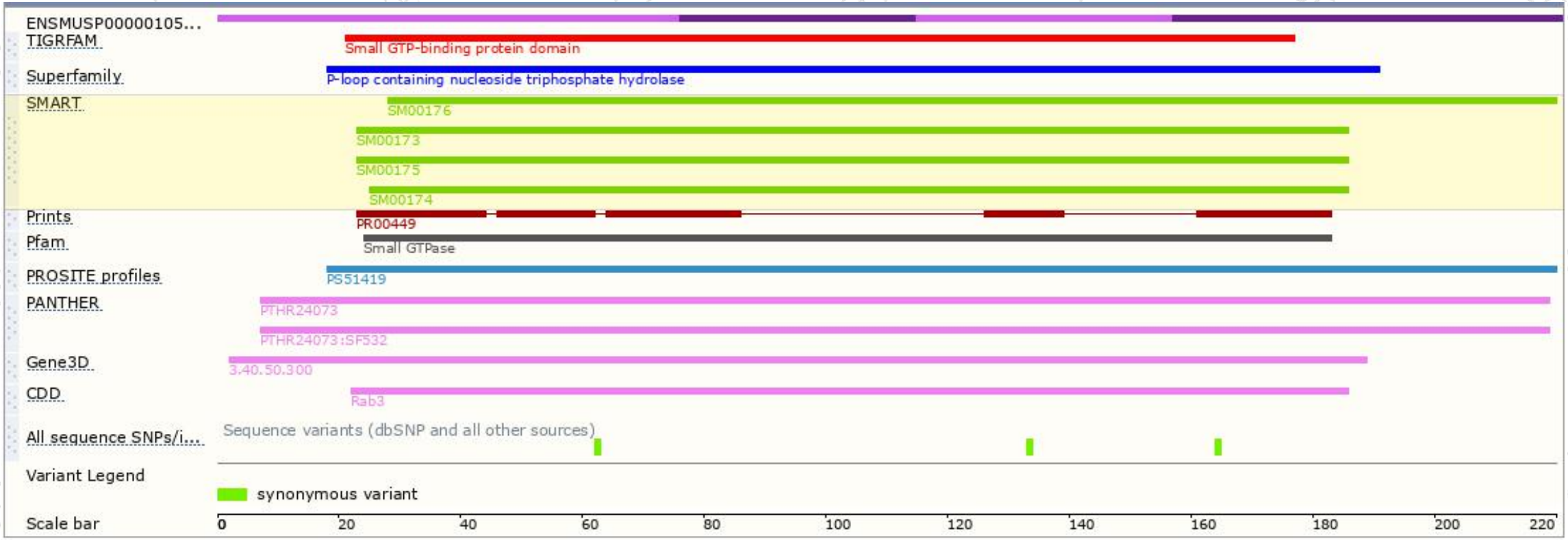
The strategy is based on the design of *Rab3a-204* transcript,The transcription is shown below



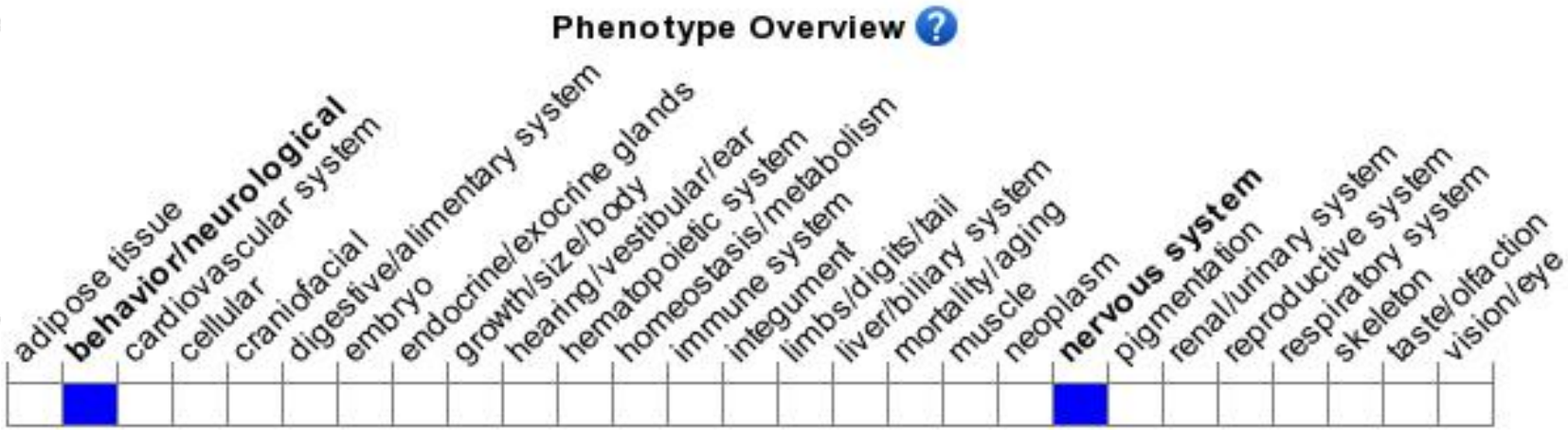
Genomic location distribution



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 mutants show impaired synaptic transmission, insulin secretion and glucose intolerance. This mutation and another chemically induced allele affect circadian period and sleep patterns. Heterozygotes show milder circadian rhythm anomalies.

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

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