Lrrn2 Cas9-CKO Strategy

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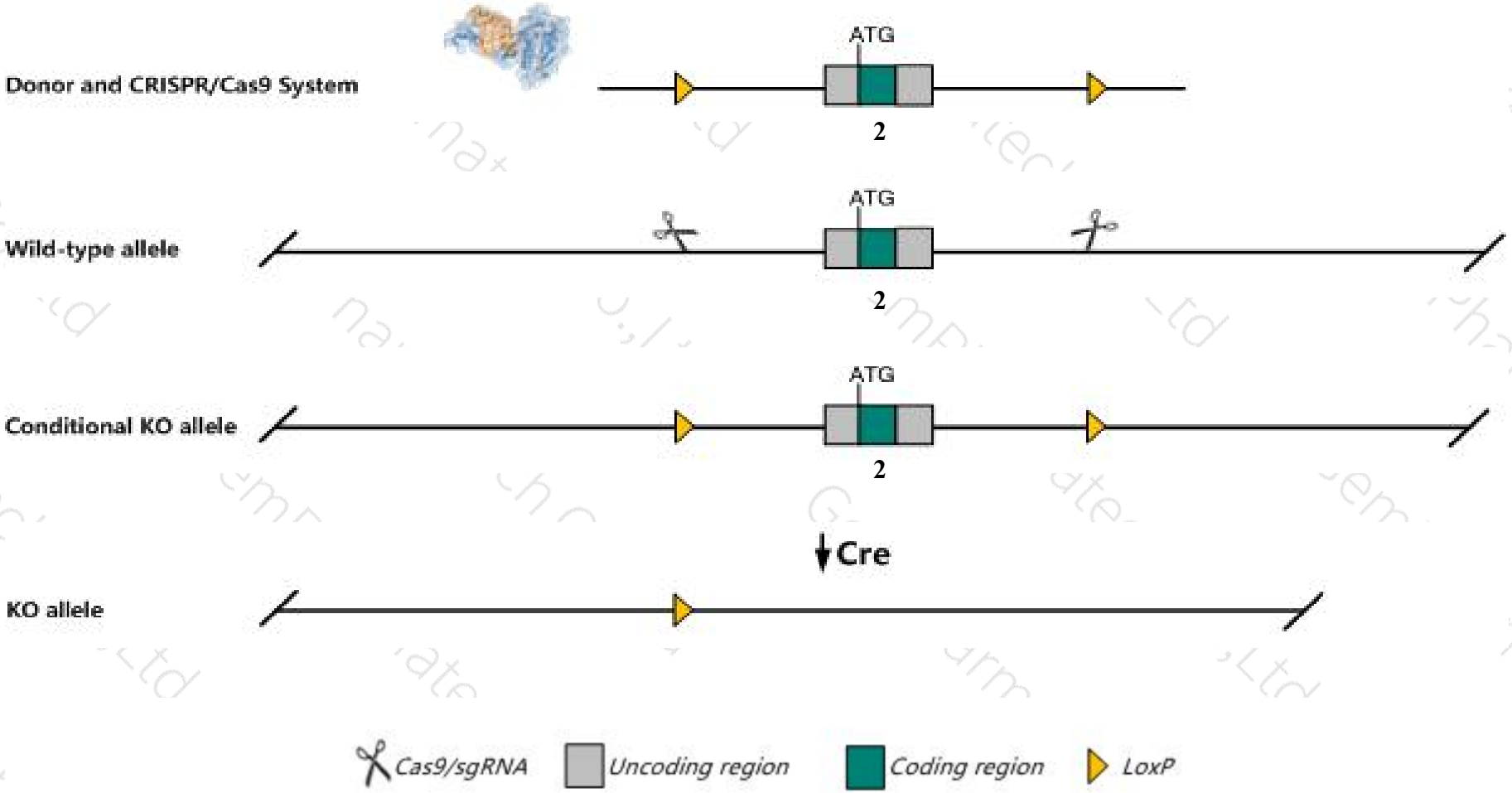
Project Overview

Project Name	***Lrrn2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Lrrn2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Lrrn2* gene has 2 transcripts. According to the structure of *Lrrn2* gene, exon2 of *Lrrn2-201*(ENSMUST00000027706.3) transcript is recommended as the knockout region.The region contains all of the coding sequence.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Lrrn2* 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,homozygous mutant mice exhibited numerous neurological abnormalities when compared with controls.

➢ The *Lrrn2* gene is located on the Chr1. 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）

Lrrn2 leucine rich repeat protein 2, neuronal [Mus musculus (house mouse)]

Gene ID: 16980, updated on 13-Mar-2020

Summary

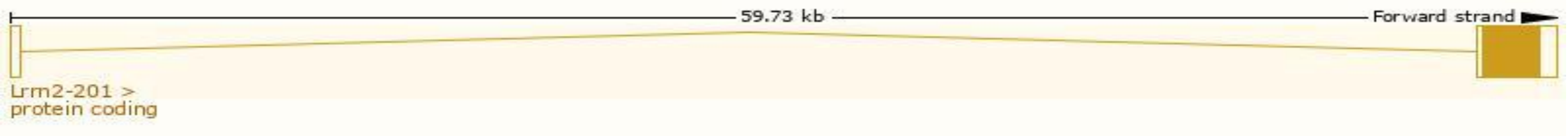
Official Symbol	Lrrn2 provided by MGI
Official Full Name	leucine rich repeat protein 2, neuronal provided byMGI
Primary source	MGI:MGI:106037
See related	Ensembl:ENSMUSG00000026443
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	5730406J09Rik, NLRR-2
Expression	Broad expression in cerebellum adult (RPKM 20.2), CNS E18 (RPKM 17.7) and 16 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

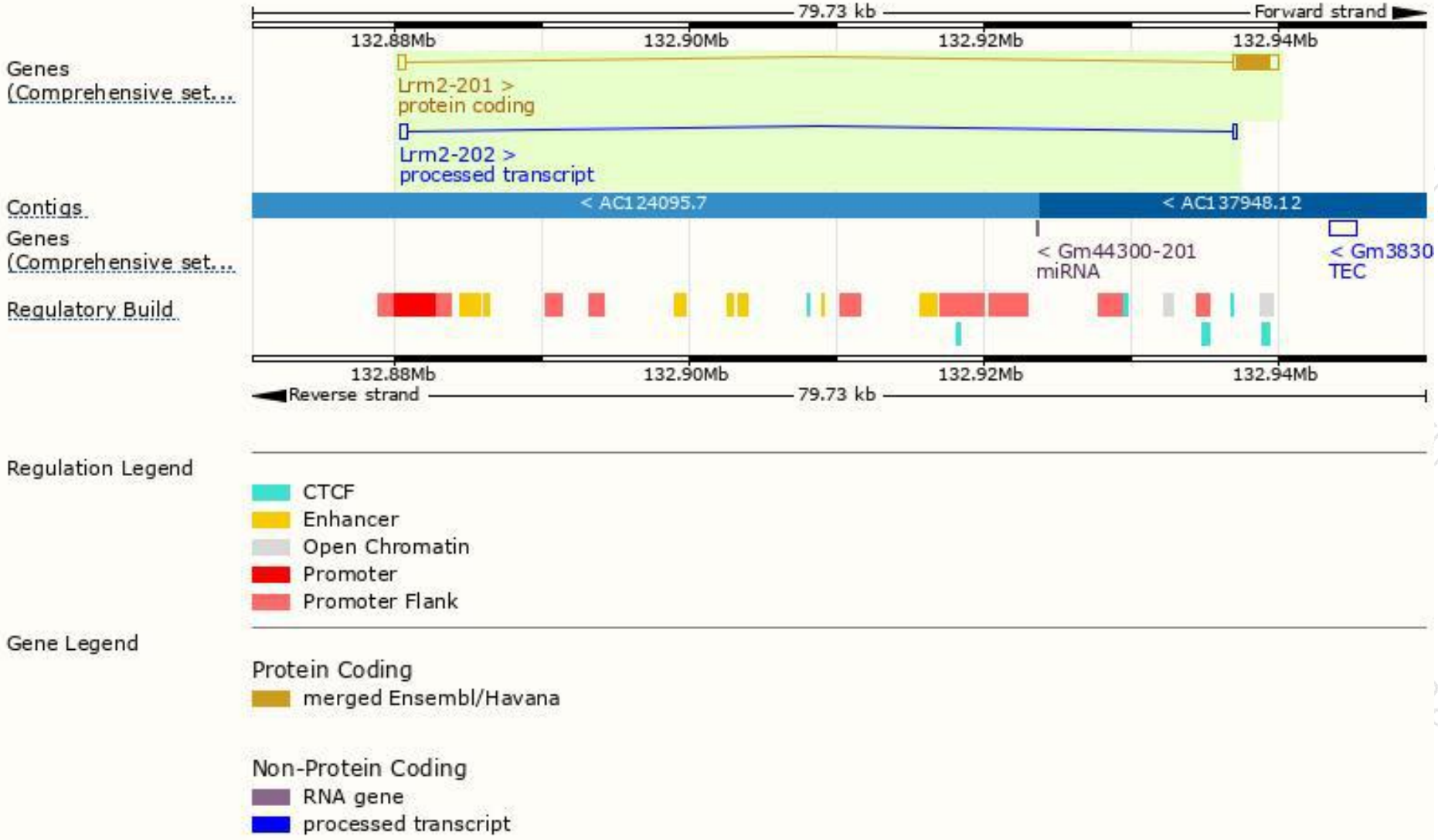
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lrrn2-201	ENSMUST00000027706.3	3428	730aa	Protein coding	CCDS15289	Q6PHP6	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Lrrn2-202	ENSMUST00000159088.1	658	No protein	Processed transcript	-	-	TSL:3

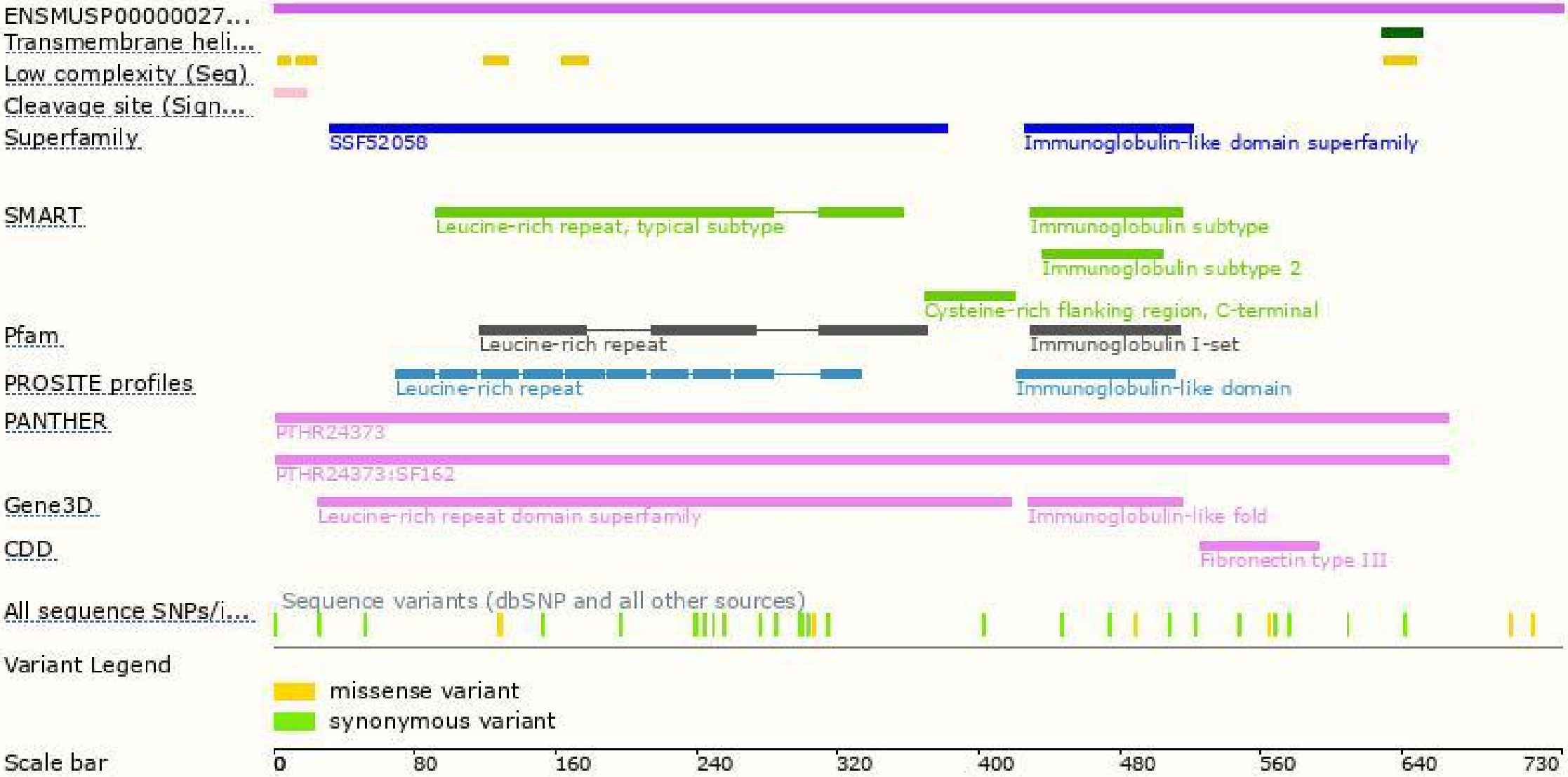
The strategy is based on the design of *Lrrn2-201* transcript,the transcription is shown below:



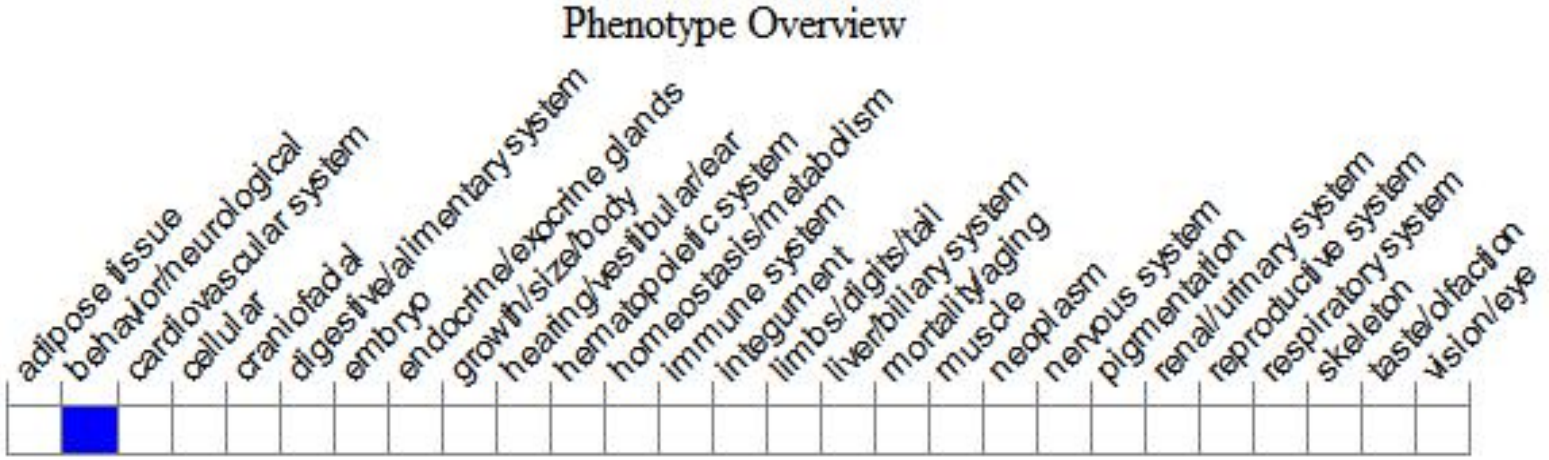
Genomic location distribution



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 mutant mice exhibited numerous neurological abnormalities when compared with controls.

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

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