Rbfox3 Cas9-CKO Strategy

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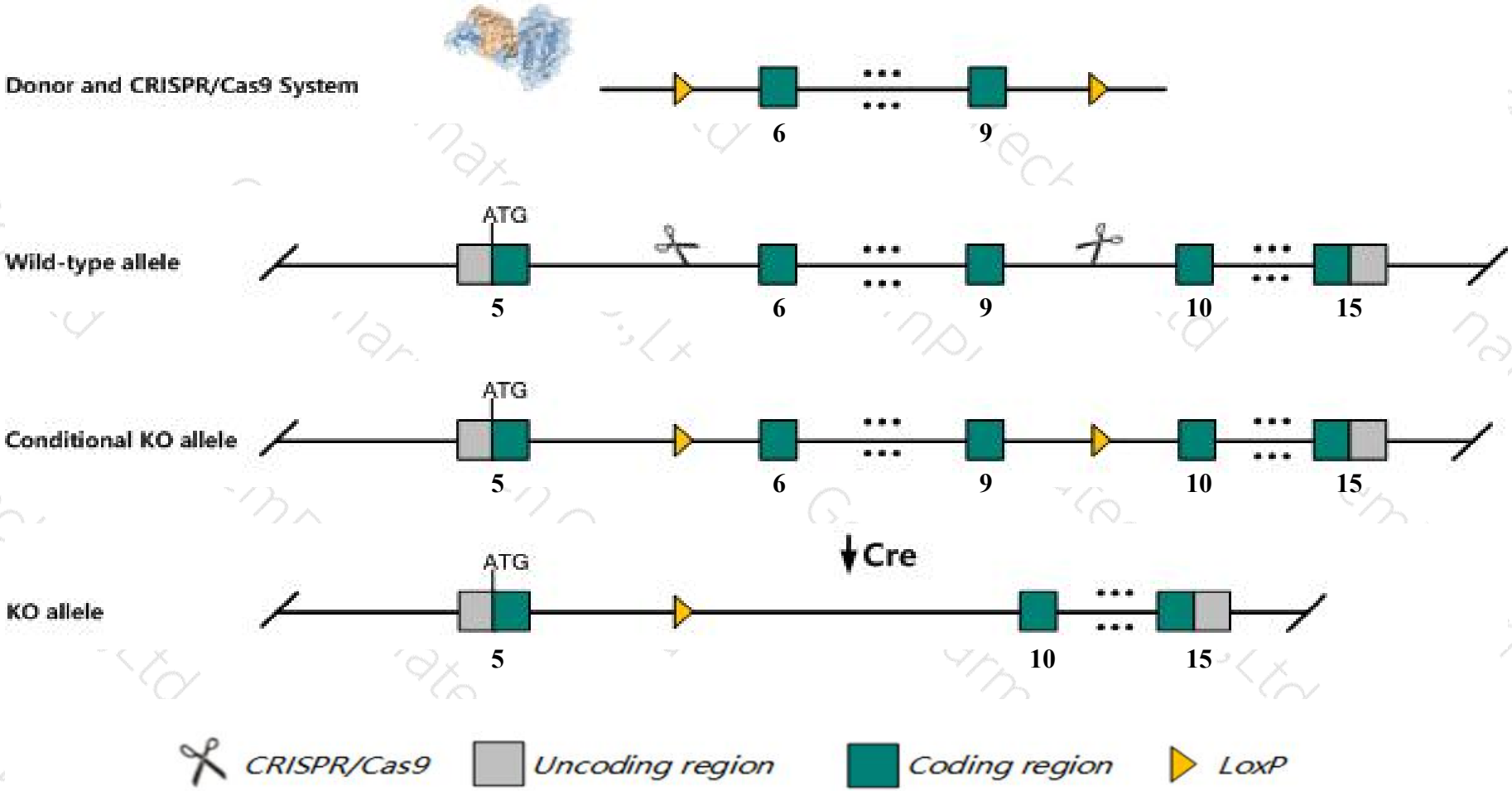
Project Overview

Project Name	***Rbfox3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rbfox3* gene. The schematic diagram is as follows:



Technical routes

- The *Rbfox3* gene has 10 transcripts. According to the structure of *Rbfox3* gene, exon6-exon9 of *Rbfox3-201* (ENSMUST00000017576.10) transcript is recommended as the knockout region.The region contains 346bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Rbfox3* 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.

Notice

- According to the existing MGI data,Mice homozygous for a null allele exhibit reduced brain weight, increased susceptibility kainic acid-induced seizures, decreased anxiety-related behaviors, and deficits in synaptic transmission and plasticity in the dentate gyrus.
- Transcript *Rbfox3*-207&208 may not be affected.
- The *Rbfox3* gene is located on the Chr11. 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）

集萃药康 GemPharmatech

Rbfox3 RNA binding protein, fox-1 homolog (C. elegans) 3 [*Mus musculus* (house mouse)]

Gene ID: 52897, updated on 10-Oct-2019

Summary

Official Symbol	Rbfox3 provided by MGI
Official Full Name	RNA binding protein, fox-1 homolog (C. elegans) 3 provided by MGI
Primary source	MGI:MGI:106368
See related	Ensembl:ENSMUSG00000025576
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	NeuN; Fox-3; Hrnbp3; Neuna60
Expression	Biased expression in cerebellum adult (RPKM 15.0), frontal lobe adult (RPKM 9.4) and 7 other tissues See more
Orthologs	human all

Genomic context

Location: 11 E2; 11 83.22 cM

See Rbfox3 in Genome Data Viewer

Exon count: 21

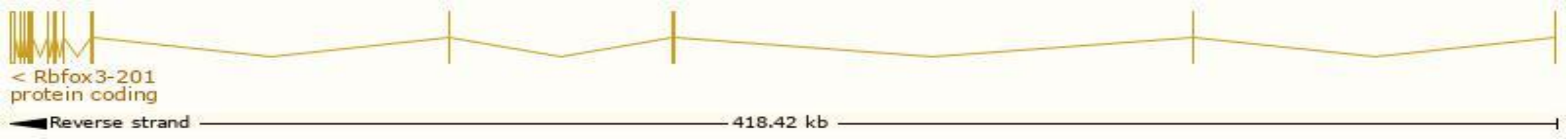
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	11	NC_000077.6 (118489760..118911597, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	11	NC_000077.5 (118351074..118770886, complement)

Transcript information（Ensembl）

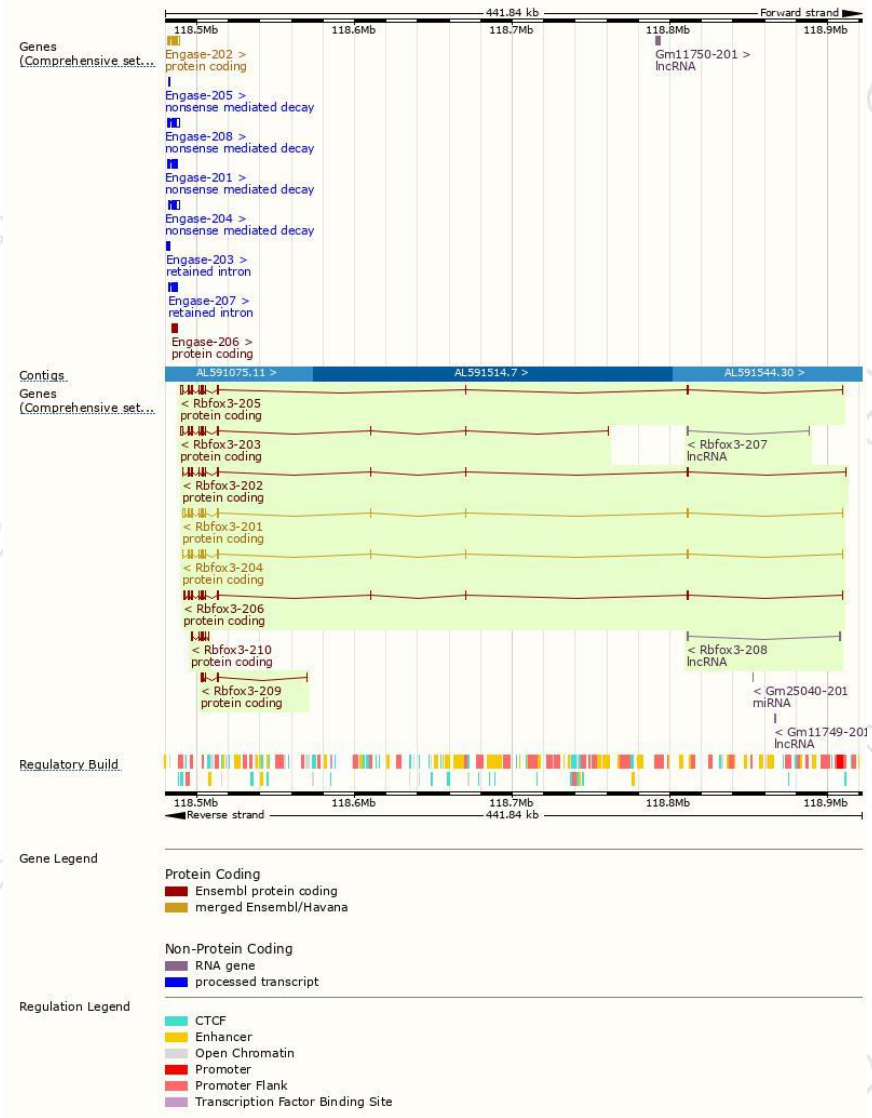
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rbfox3-201	ENSMUST00000017576.10	3150	374aa	Protein coding	CCDS25706	Q8BIF2	TSL:1 GENCODE basic APPRIS P4
Rbfox3-205	ENSMUST00000117731.7	3084	360aa	Protein coding	CCDS70360	Q8BIF2	TSL:1 GENCODE basic APPRIS ALT2
Rbfox3-203	ENSMUST00000103023.7	2809	313aa	Protein coding	CCDS25705	Q8BIF2	TSL:1 GENCODE basic APPRIS ALT2
Rbfox3-202	ENSMUST00000069343.11	1553	329aa	Protein coding	CCDS70358	Q8BIF2	TSL:1 GENCODE basic
Rbfox3-204	ENSMUST00000106278.8	1542	313aa	Protein coding	CCDS25705	Q8BIF2	TSL:1 GENCODE basic APPRIS ALT2
Rbfox3-206	ENSMUST00000120061.7	2150	327aa	Protein coding	-	Q8BIF2	TSL:5 GENCODE basic APPRIS ALT2
Rbfox3-209	ENSMUST00000136551.2	900	169aa	Protein coding	-	A2A4W9	CDS 3' incomplete TSL:5
Rbfox3-210	ENSMUST00000154746.7	710	145aa	Protein coding	-	B7ZC11	CDS 3' incomplete TSL:3
Rbfox3-208	ENSMUST00000134774.1	369	No protein	lncRNA	-	-	TSL:3
Rbfox3-207	ENSMUST00000128863.1	301	No protein	lncRNA	-	-	TSL:5

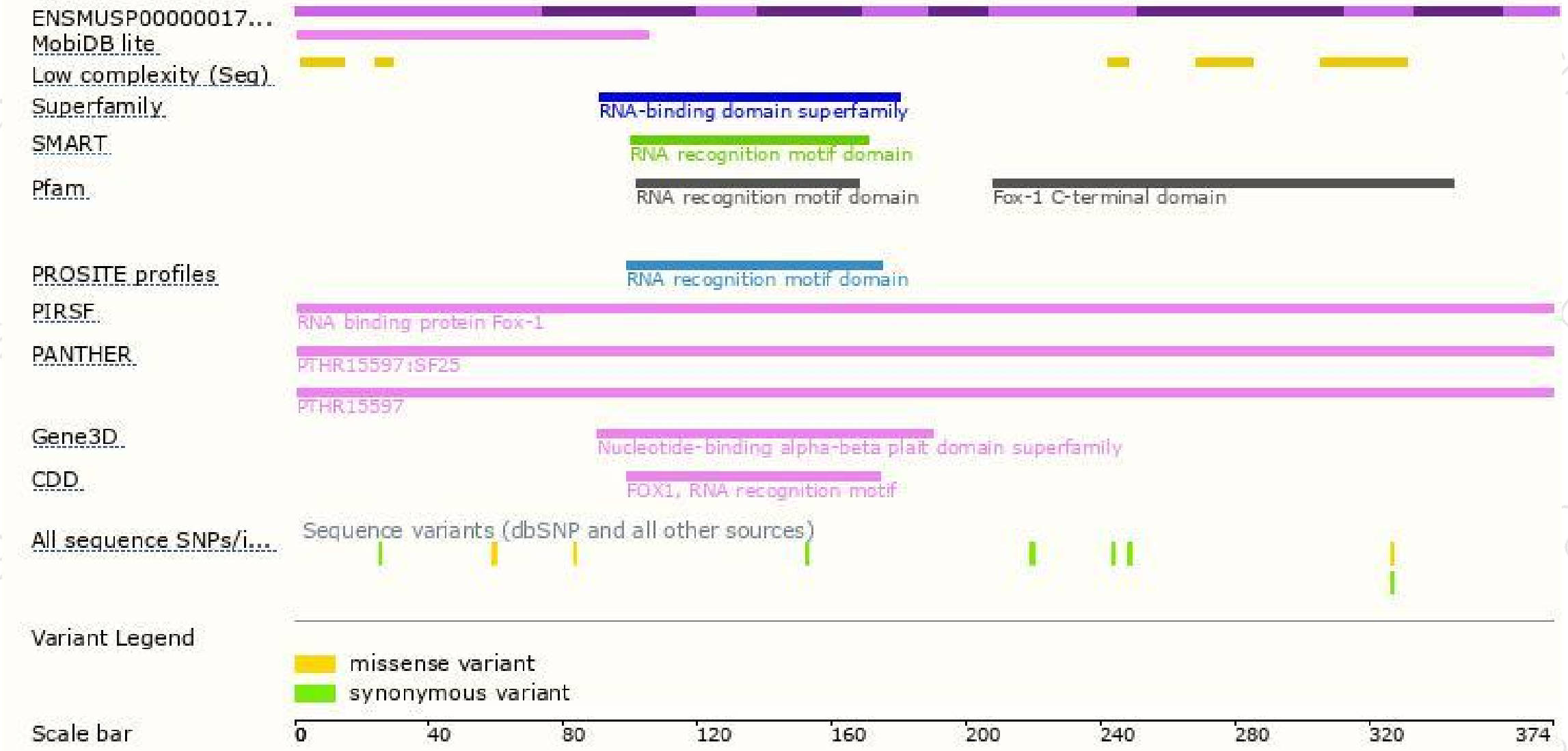
The strategy is based on the design of *Rbfox3-201* transcript,The transcription is shown below



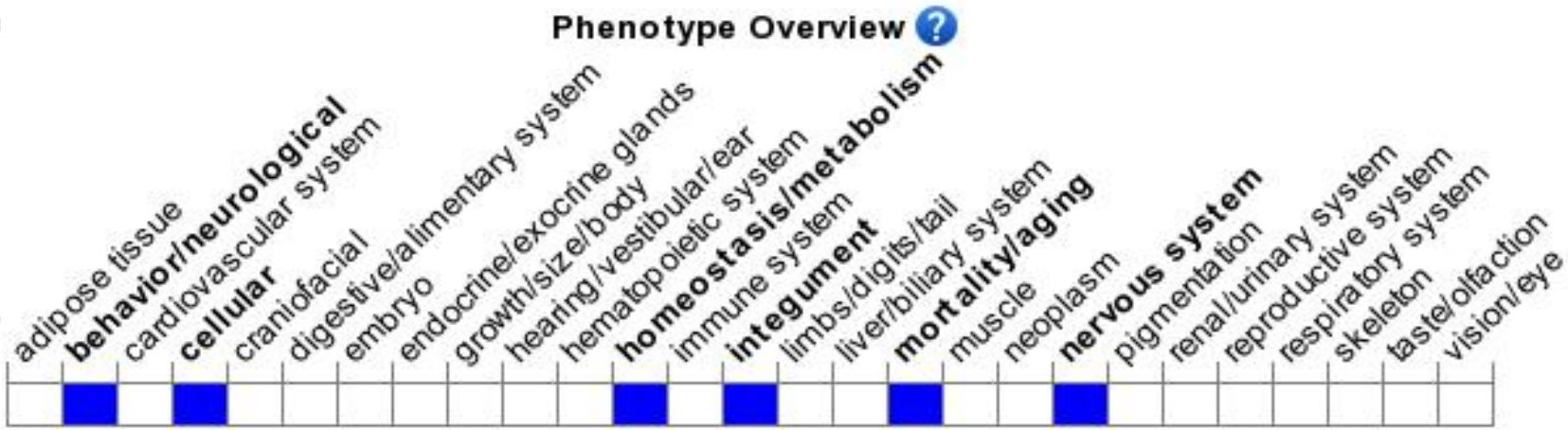
Genomic location distribution



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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