Plxna2 Cas9-CKO *Strategy*

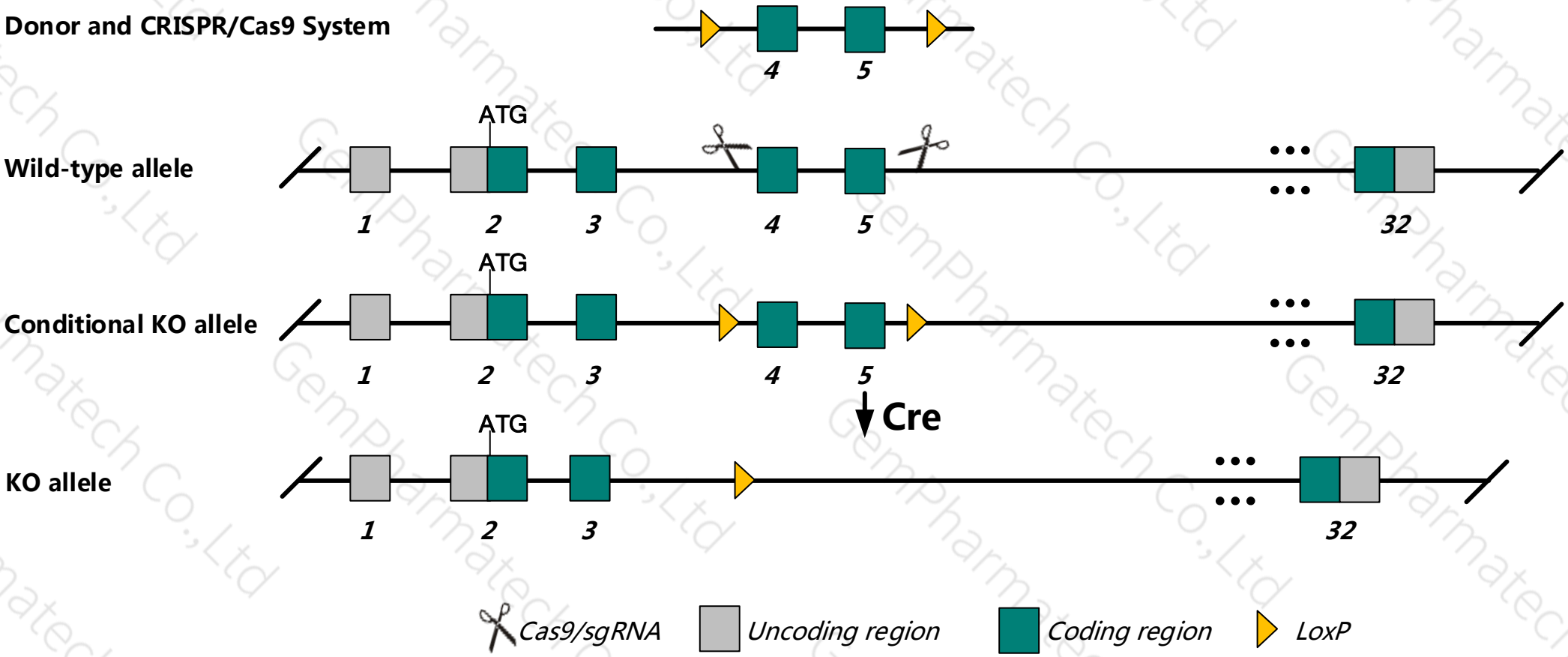
Designer: **Daohua Xu**

Project Overview

Project Name	***Plxna2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Plxna2* gene. The schematic diagram is as follows:



Technical routes

- The *Plxna2* gene has 5 transcripts.According to the structure of *Plxna2* gene, exon4-exon5 of *Plxna2*-201 (
- ENSMUST00000027952.11) transcript is recommended as the knockout region.The region contains 236bp coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Plxna2* 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 or cell types.

Notice

- According to the existing MGI data，Mice homozygous for a knock-out allele show abnormal granule cell migration in the adult cerebellum and aberrant projection of mossy fibers in hippocampal slices. Mice homozygous for an ENU-induced allele are smaller and show granule cell migration defects and mild ataxia with incomplete penetrance.
- The KO region contains functional region of the *2900035J10Rik* gene.Knockout the region may affect the function of *2900035J10Rik* gene.
- The *Plxna2* 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

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Plxna2 plexin A2 [*Mus musculus* (house mouse)]

Gene ID: 18845, updated on 9-Jun-2019

Summary

Official Symbol	Plxna2 provided by MGI
Official Full Name	plexin A2 provided by MGI
Primary source	MGI:MGI:107684
See related	Ensembl:ENSMUSG00000026640
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	OCT; Plxn2; PlexA2; AA589422; AW457381; mKIAA0463; 2810428A13Rik
Expression	Broad expression in CNS E14 (RPKM 20.1), whole brain E14.5 (RPKM 19.4) and 25 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

The gene has 5 transcripts, and all transcripts are shown below:

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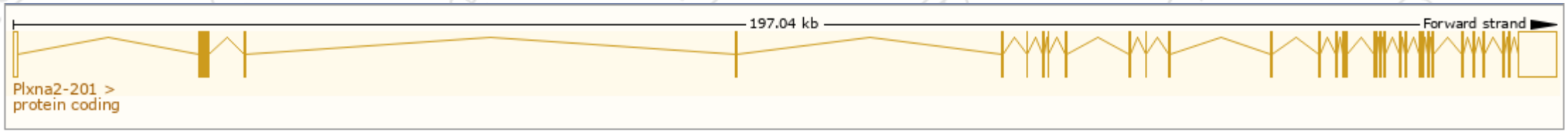
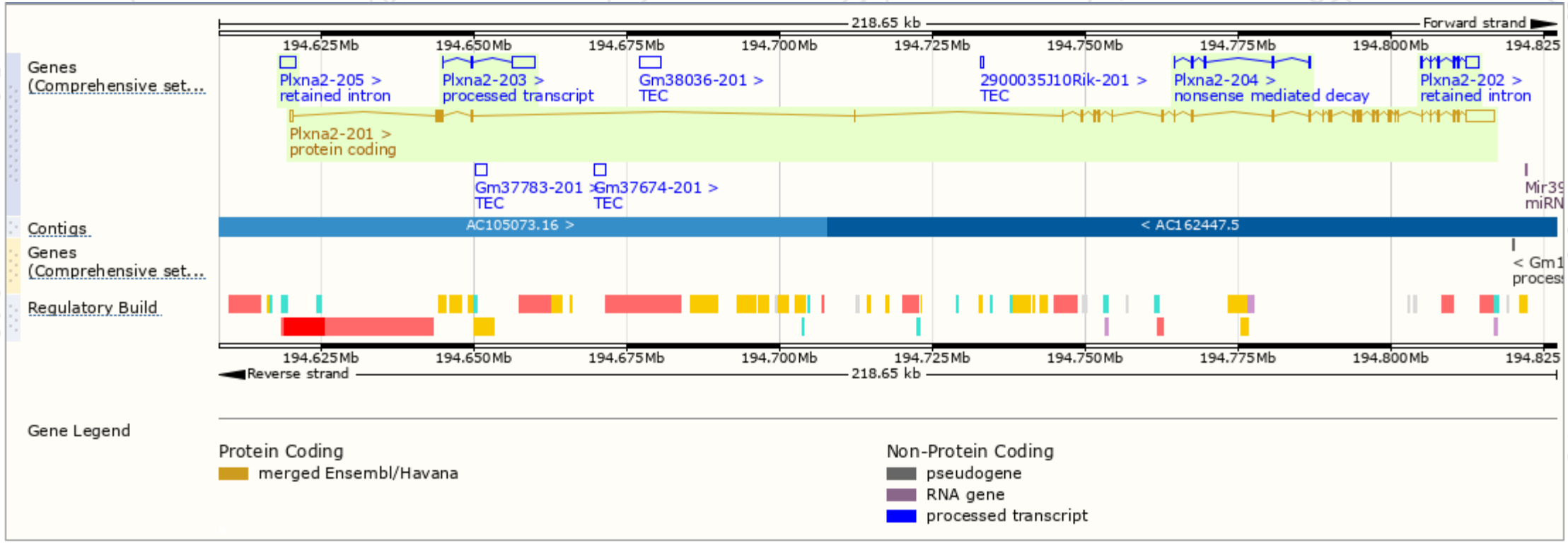
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Plxna2-201	ENSMUST00000027952.11	11040	1894aa	Protein coding	CCDS35827	P70207	TSL:1 GENCODE basic APPRIS P1
Plxna2-204	ENSMUST00000135664.1	605	83aa	Nonsense mediated decay	-	F6VSI0	CDS 5' incomplete TSL:5
Plxna2-203	ENSMUST00000125381.1	3951	No protein	Processed transcript	-	-	TSL:2
Plxna2-202	ENSMUST00000124785.1	3180	No protein	Retained intron	-	-	TSL:1
Plxna2-205	ENSMUST00000194398.1	2650	No protein	Retained intron	-	-	TSL:NA

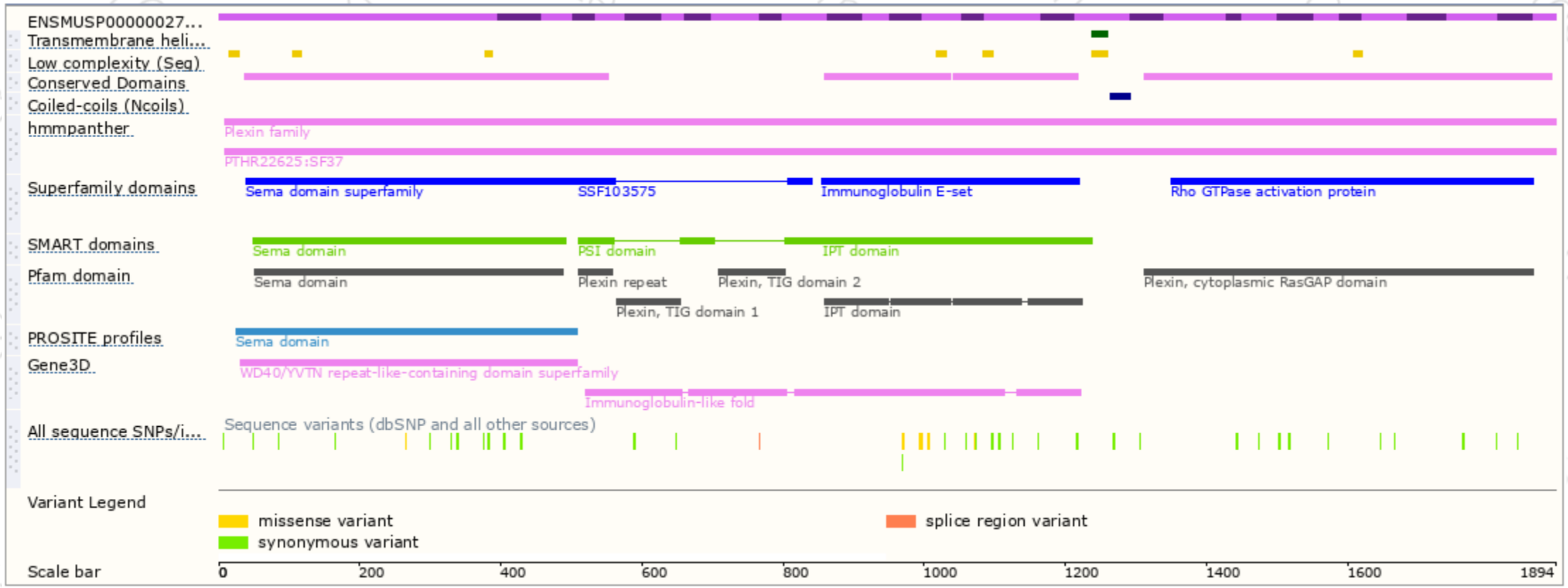
The strategy is based on the design of *Plxna2*-201 transcript,The transcription is shown below



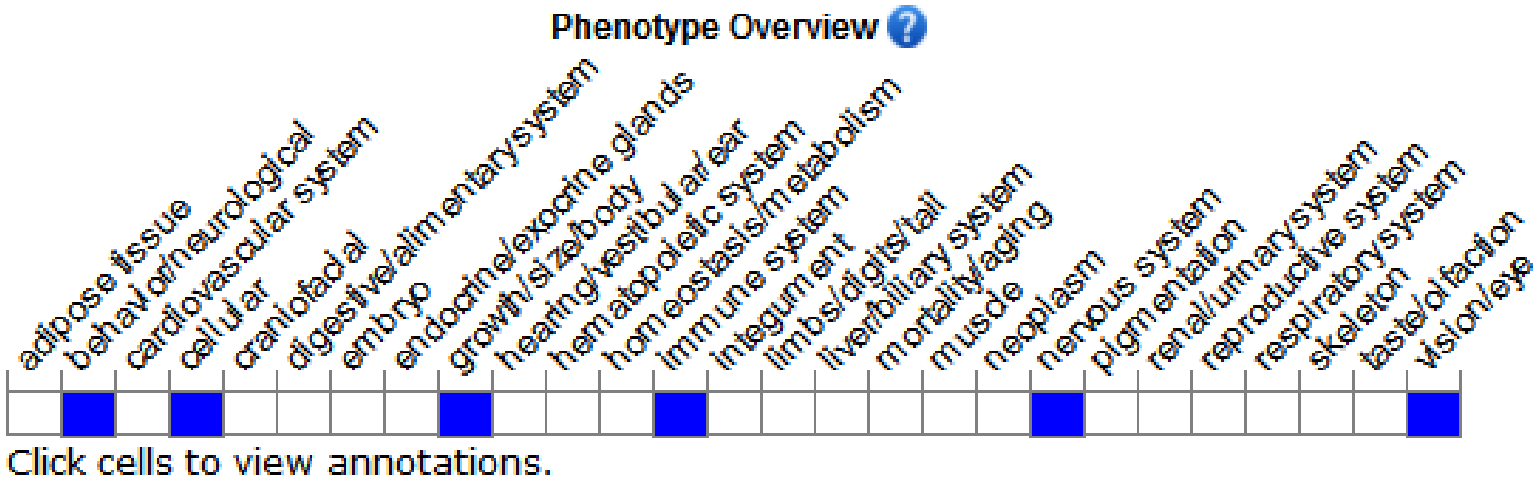
Genomic location distribution



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 a knock-out allele show abnormal granule cell migration in the adult cerebellum and aberrant projection of mossy fibers in hippocampal slices. Mice homozygous for an ENU-induced allele are smaller and show granule cell migration defects and mild ataxia with incomplete penetrance.

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

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