

Cacna2d2 Cas9-CKO Strategy

Designer: Xueting Zhang

Reviewer: Yanhua Shen

Date: 2019-12-23

Project Overview

Project Name

Cacna2d2

Project type

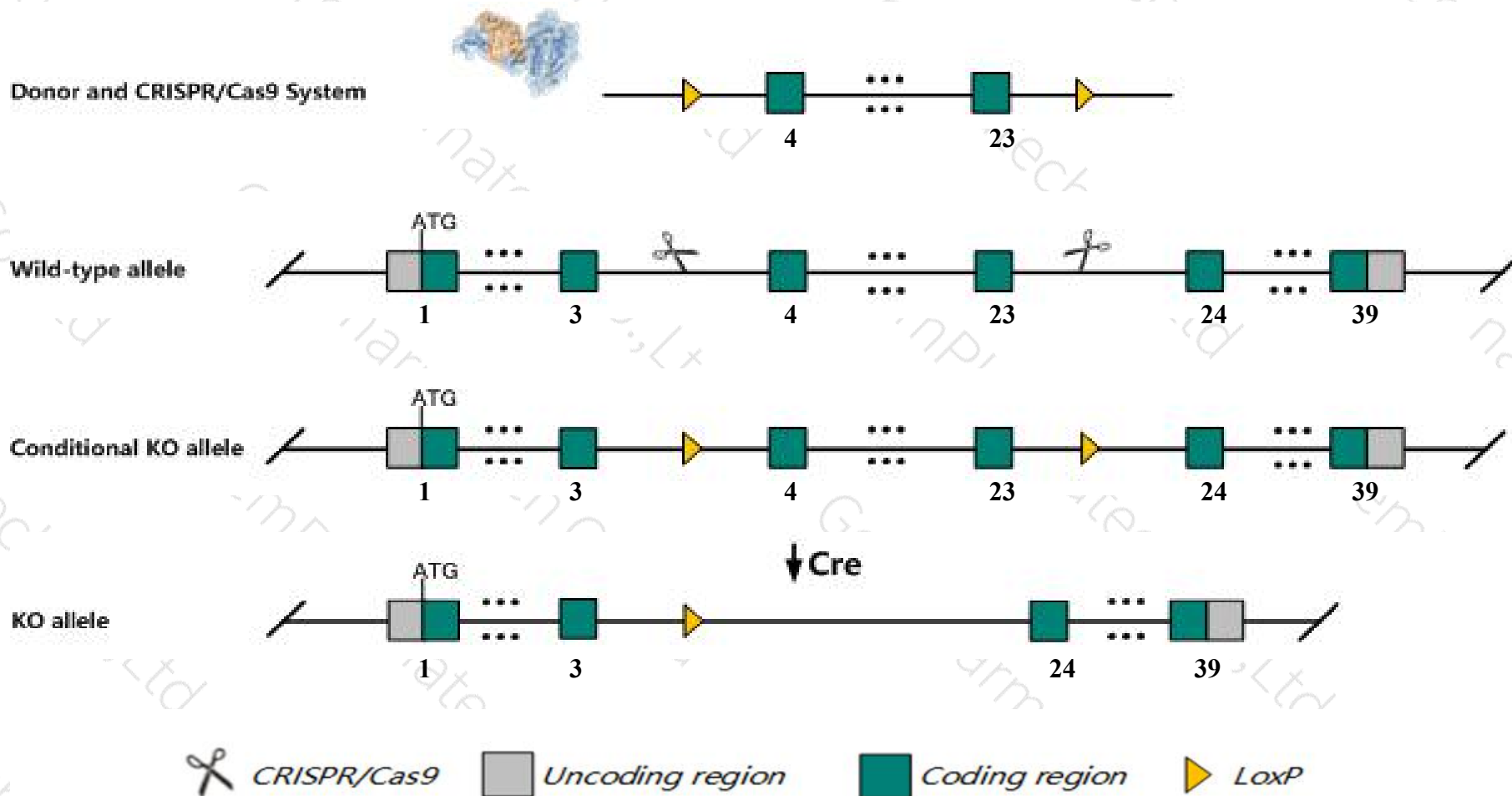
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cacna2d2* gene. The schematic diagram is as follows:



- The *Cacna2d2* gene has 10 transcripts. According to the structure of *Cacna2d2* gene, exon4-exon23 of *Cacna2d2*-205 (ENSMUST00000168532.7) transcript is recommended as the knockout region. The region contains 1600bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cacna2d2* 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.

- According to the existing MGI data, Homozygotes for different mutant alleles show variable movement abnormalities including waddling, reeling or very slow gait, ataxia, and mild spike-wave seizures. While gross CNS abnormalities and demyelination are present in some mutant lines, they are not observed in others.
- Transcript *Cacna2d2*-206&210 may not be affected.
- The *Cacna2d2* gene is located on the Chr9. 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)

Cacna2d2 calcium channel, voltage-dependent, alpha 2/delta subunit 2 [*Mus musculus* (house mouse)]

Gene ID: 56808, updated on 11-Sep-2019

Summary

Official Symbol Cacna2d2 provided by [MGI](#)
Official Full Name calcium channel, voltage-dependent, alpha 2/delta subunit 2 provided by [MGI](#)
Primary source [MGI:MGI:1929813](#)
See related [Ensembl:ENSMUSG00000010066](#)
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 du; td; a2d2; torpid; Cacna2d; mKIAA0558
Expression Biased expression in cerebellum adult (RPKM 24.9), whole brain E14.5 (RPKM 22.0) and 10 other tissues [See more](#)
Orthologs [human](#) [all](#)

Genomic context

Location: 9 F1; 9 58.02 cM

See Cacna2d2 in [Genome Data Viewer](#)

Exon count: 39

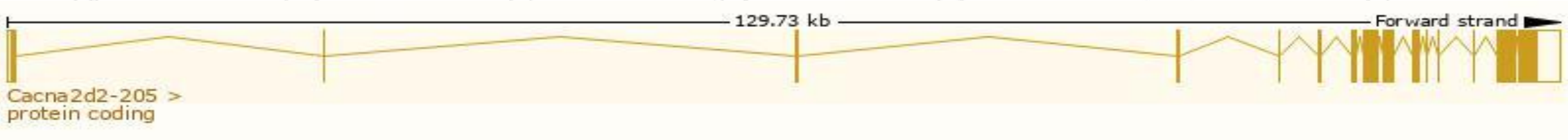
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	9	NC_000075.6 (107399733..107529346)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	9	NC_000075.5 (107302211..107431674)

Transcript information (Ensembl)

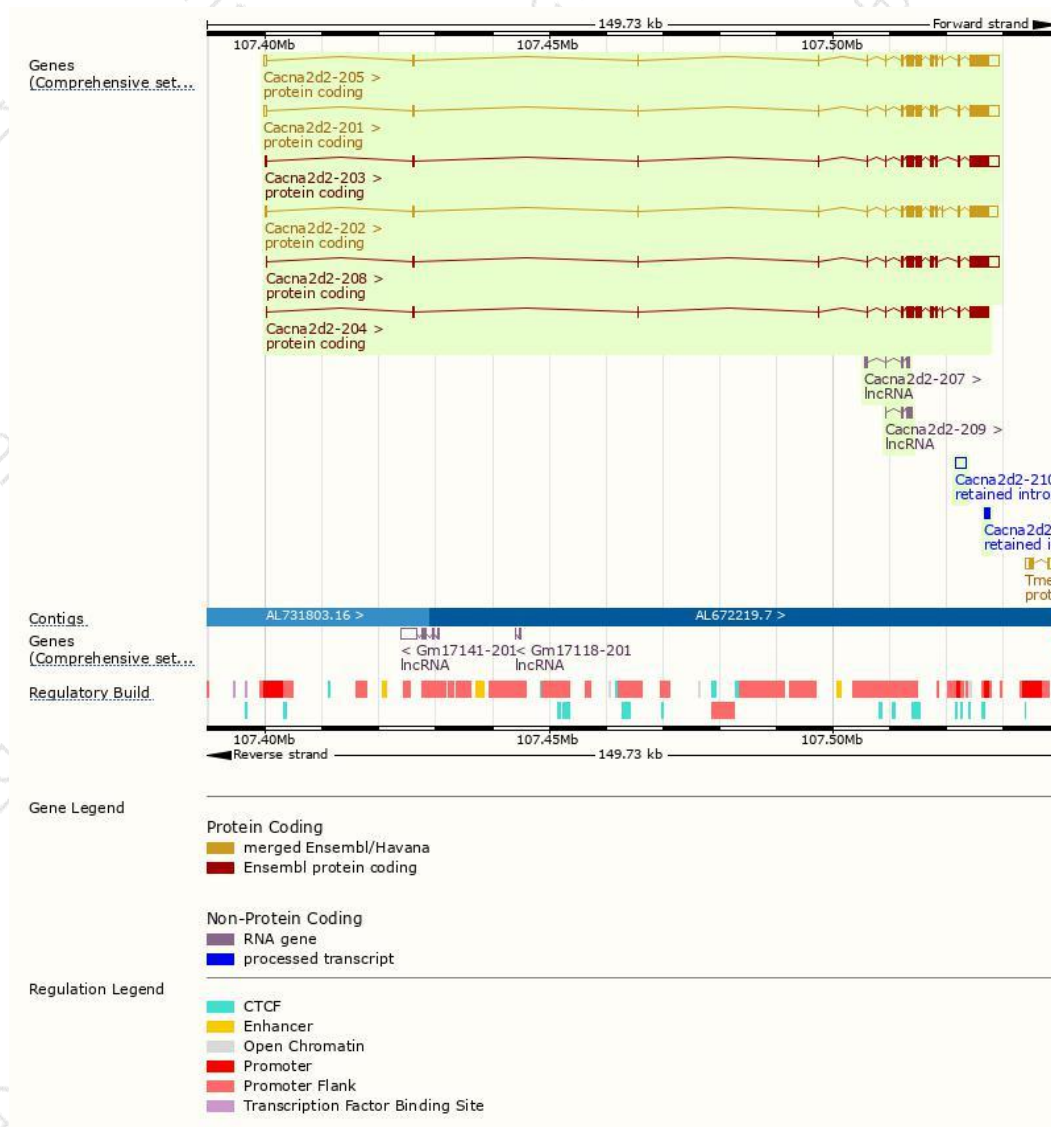
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cacna2d2-205	ENSMUST00000168532.7	5807	1156aa	Protein coding	CCDS72304	Q6PHS9	TSL:1 GENCODE basic APPRIS ALT 2
Cacna2d2-201	ENSMUST0000010210.12	5783	1148aa	Protein coding	CCDS52917	Q6PHS9	TSL:1 GENCODE basic APPRIS ALT 2
Cacna2d2-208	ENSMUST00000170737.2	5332	1150aa	Protein coding	CCDS72305	Q6PHS9	TSL:1 GENCODE basic APPRIS ALT 2
Cacna2d2-202	ENSMUST00000085092.11	5183	1154aa	Protein coding	CCDS52916	Q6PHS9	TSL:5 GENCODE basic APPRIS P4
Cacna2d2-203	ENSMUST00000164988.8	5542	1157aa	Protein coding	-	E9Q683	TSL:5 GENCODE basic APPRIS ALT 2
Cacna2d2-204	ENSMUST00000166799.7	3474	1157aa	Protein coding	-	Q6PHS9	TSL:5 GENCODE basic APPRIS ALT 2
Cacna2d2-210	ENSMUST00000194842.1	1975	No protein	Retained intron	-	-	TSL:NA
Cacna2d2-206	ENSMUST00000168959.1	604	No protein	Retained intron	-	-	TSL:3
Cacna2d2-209	ENSMUST00000171809.1	727	No protein	lncRNA	-	-	TSL:3
Cacna2d2-207	ENSMUST00000169354.7	671	No protein	lncRNA	-	-	TSL:5

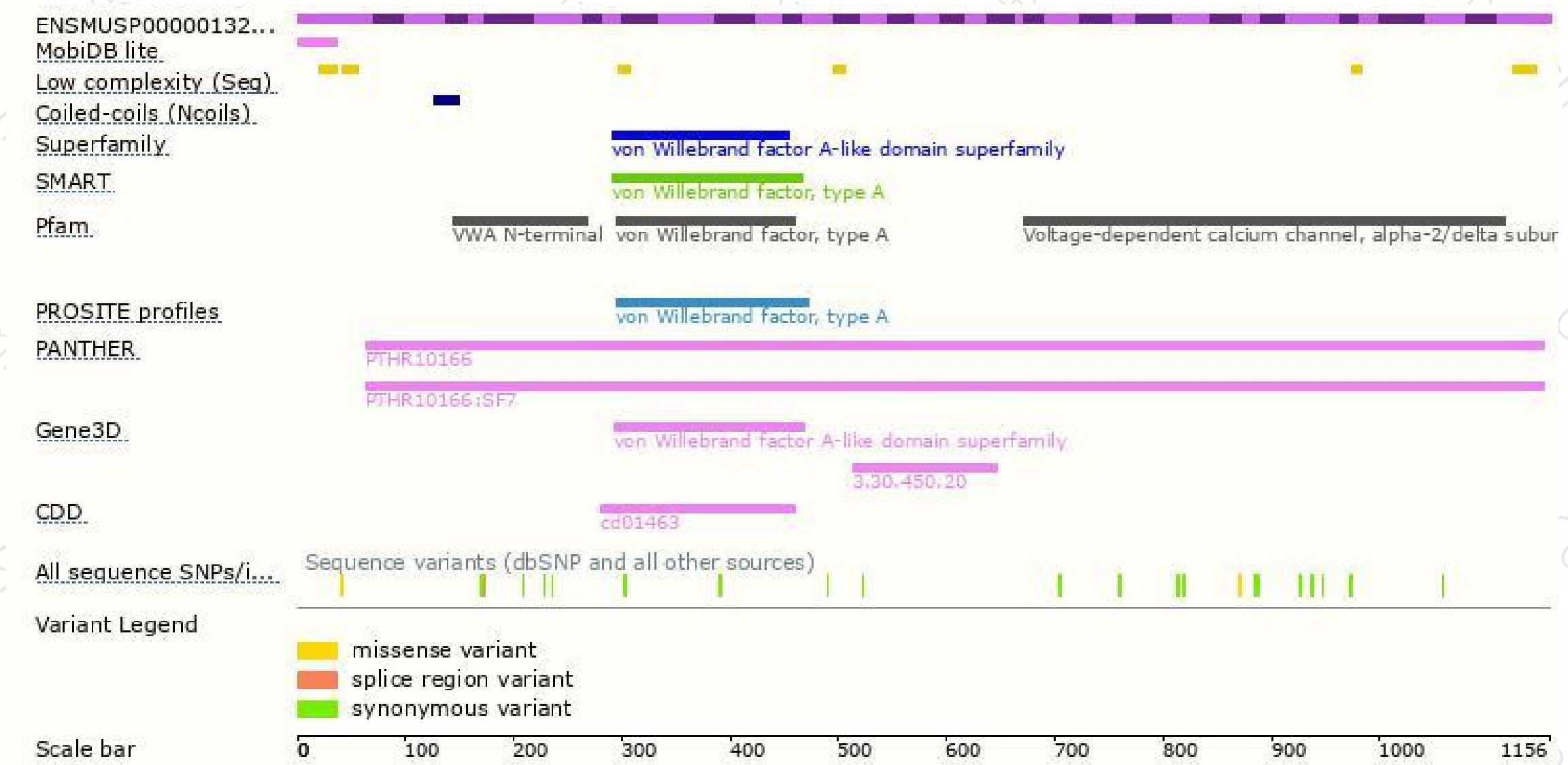
The strategy is based on the design of *Cacna2d2-205* transcript,The transcription is shown below



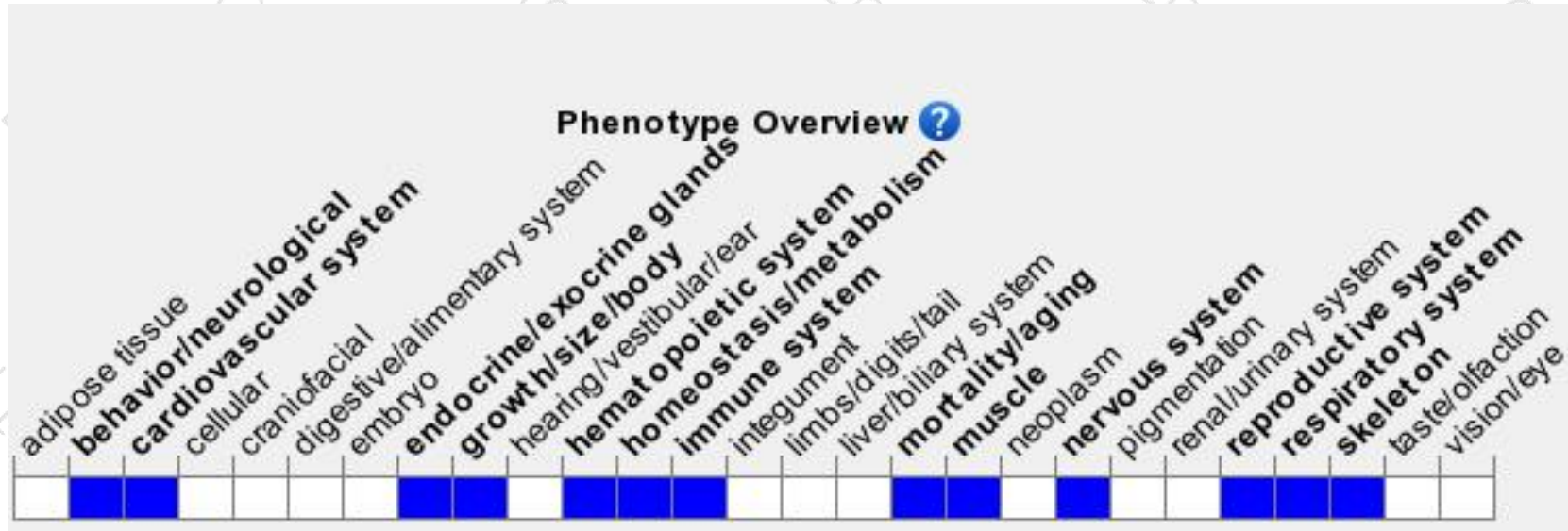
Genomic location distribution



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, Homozygotes for different mutant alleles show variable movement abnormalities including waddling, reeling or very slow gait, ataxia, and mild spike-wave seizures. While gross CNS abnormalities and demyelination are present in some mutant lines, they are not observed in others.

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

Tel: 400-9660890

