Cacna2d4 Cas9-CKO Strategy

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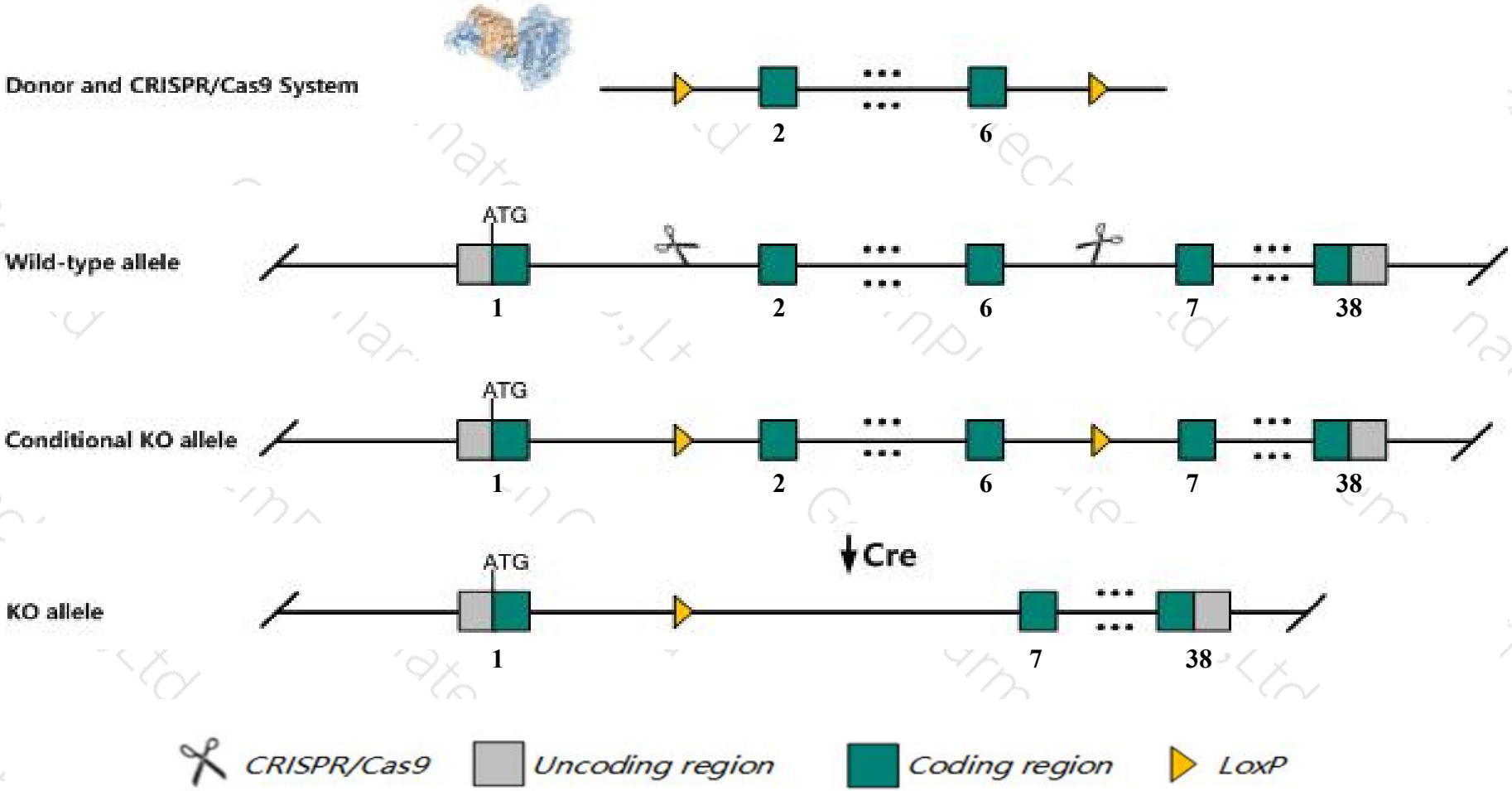
Project Overview

Project Name	***Cacna2d4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cacna2d4* gene. The schematic diagram is as follows:



Technical routes

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- The *Cacna2d4* gene has 10 transcripts. According to the structure of *Cacna2d4* gene, exon2-exon6 of *Cacna2d4-201* (ENSMUST00000037434.12) transcript is recommended as the knockout region.The region contains 554bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Cacna2d4* 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 spontaneous mutation exhibit severe loss of retinal signaling associated with abnormal photoreceptor ribbon synapses and cone-rod dysfunction.
- Transcript *Cacna2d4*-202&203&204&206&207&208&210 may not be affected.
- The *Cacna2d4* gene is located on the Chr6. 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）

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Cacna2d4 calcium channel, voltage-dependent, alpha 2/delta subunit 4 [*Mus musculus* (house mouse)]

Gene ID: 319734, updated on 4-Jan-2020

Summary

Official Symbol	Cacna2d4 provided by MGI
Official Full Name	calcium channel, voltage-dependent, alpha 2/delta subunit 4 provided by MGI
Primary source	MGI:MGI:2442632
See related	Ensembl:ENSMUSG00000041460
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	BE686333; 5730412N02Rik
Expression	Biased expression in thymus adult (RPKM 2.1), lung adult (RPKM 0.6) and 5 other tissues See more
Orthologs	human all

Genomic context

Location: 6; 6 F1

See Cacna2d4 in Genome Data Viewer

Exon count: 40

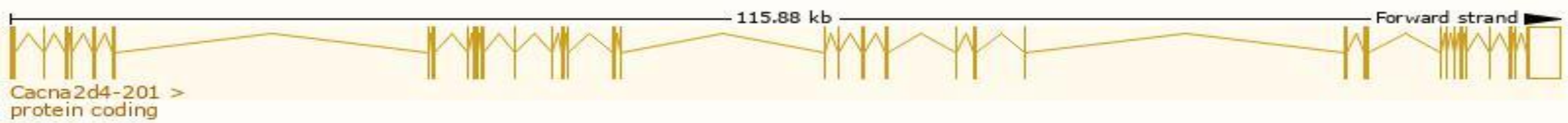
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	6	NC_000072.6 (119236524..119352407)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	6	NC_000072.5 (119186544..119302425)

Transcript information（Ensembl）

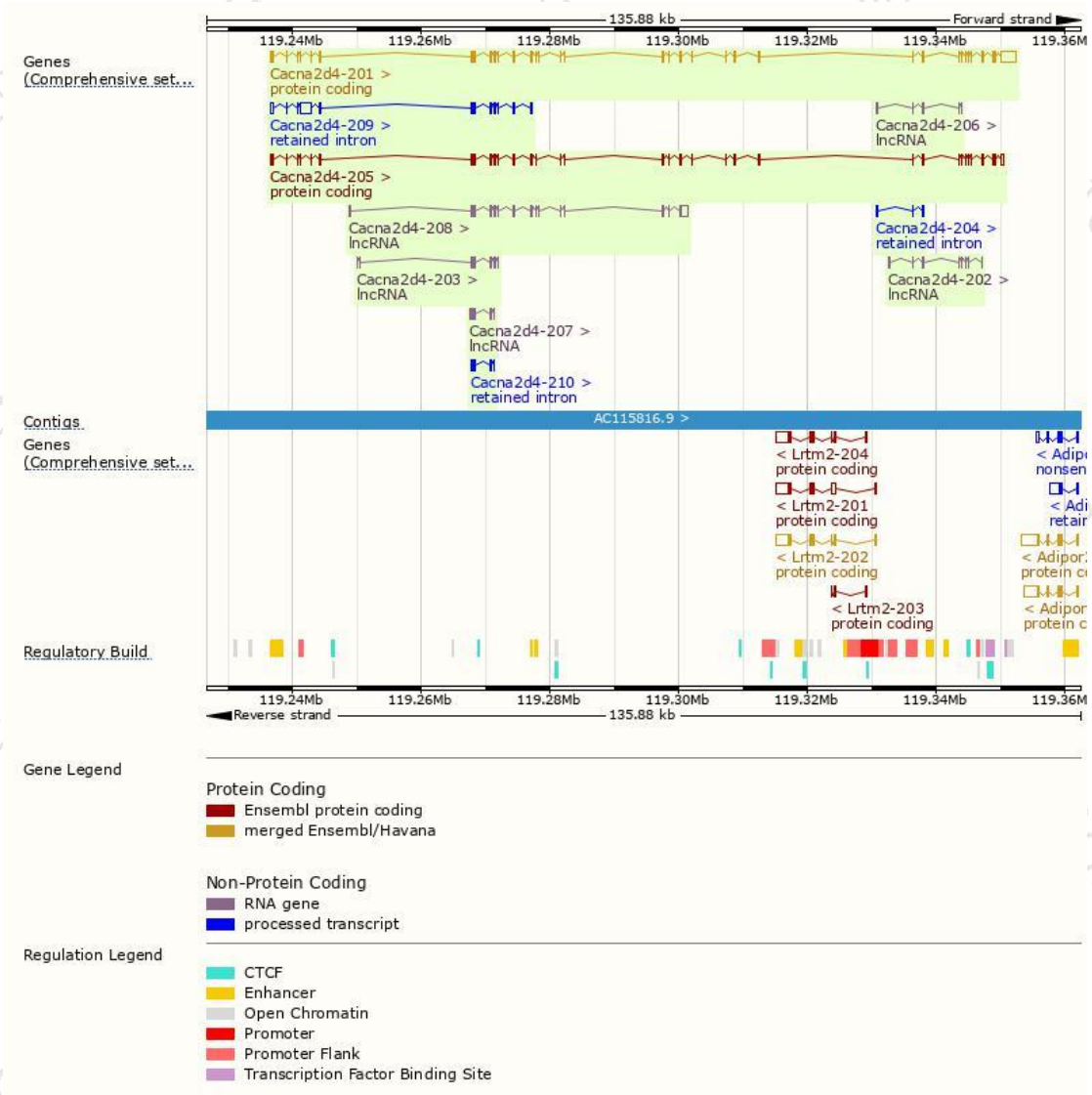
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cacna2d4-201	ENSMUST00000037434.12	5803	1144aa	Protein coding	CCDS51886	F8VPL1	TSL:2 GENCODE basic APPRIS P3
Cacna2d4-205	ENSMUST00000186622.1	3710	1119aa	Protein coding	CCDS85143	A0A087WQH4	TSL:1 GENCODE basic APPRIS ALT2
Cacna2d4-209	ENSMUST00000190015.6	3229	No protein	Retained intron	-	-	TSL:1
Cacna2d4-204	ENSMUST00000186203.6	419	No protein	Retained intron	-	-	TSL:3
Cacna2d4-210	ENSMUST00000191331.1	414	No protein	Retained intron	-	-	TSL:2
Cacna2d4-208	ENSMUST00000188239.6	2578	No protein	lncRNA	-	-	TSL:1
Cacna2d4-202	ENSMUST00000185965.1	761	No protein	lncRNA	-	-	TSL:3
Cacna2d4-203	ENSMUST00000186176.6	748	No protein	lncRNA	-	-	TSL:3
Cacna2d4-206	ENSMUST00000186702.6	448	No protein	lncRNA	-	-	TSL:3
Cacna2d4-207	ENSMUST00000187222.6	404	No protein	lncRNA	-	-	TSL:3

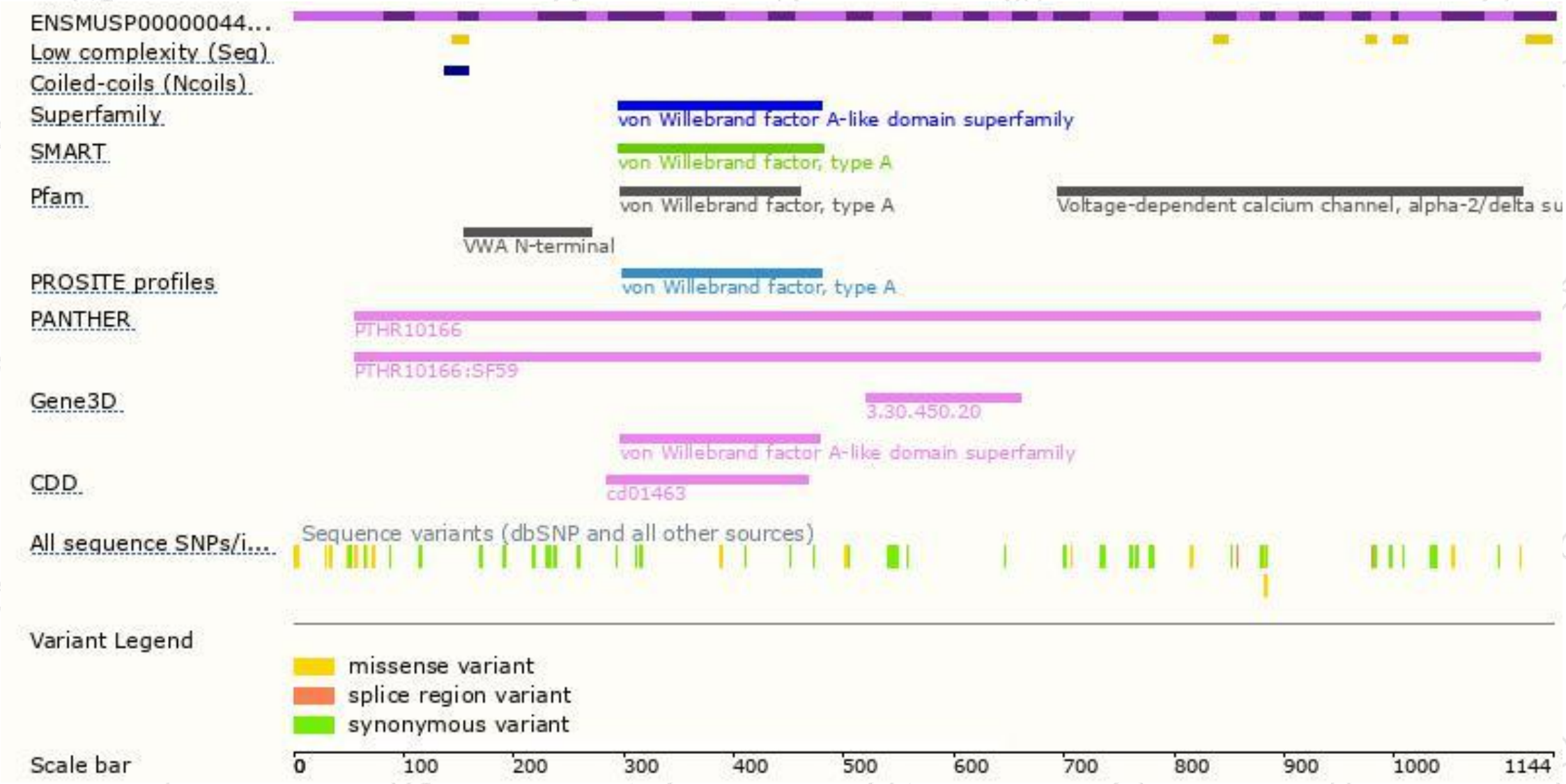
The strategy is based on the design of *Cacna2d4-201* transcript,The transcription is shown below



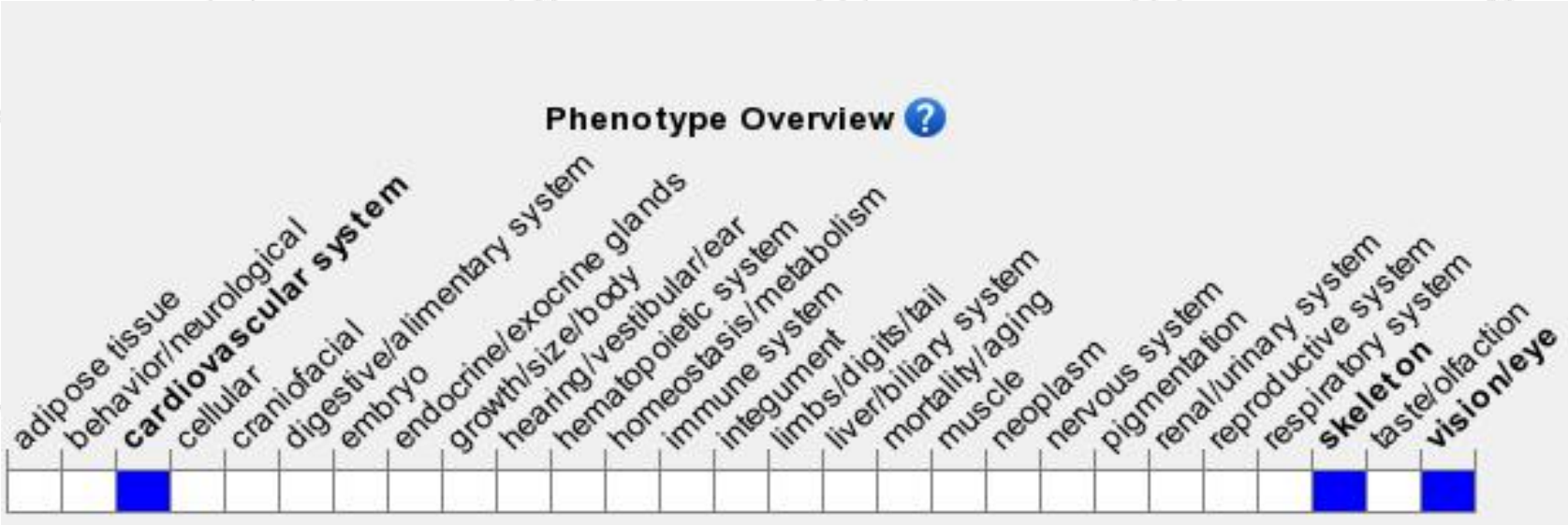
Genomic location distribution



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 spontaneous mutation exhibit severe loss of retinal signaling associated with abnormal photoreceptor ribbon synapses and cone-rod dysfunction.

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

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