Opn4 Cas9-CKO Strategy

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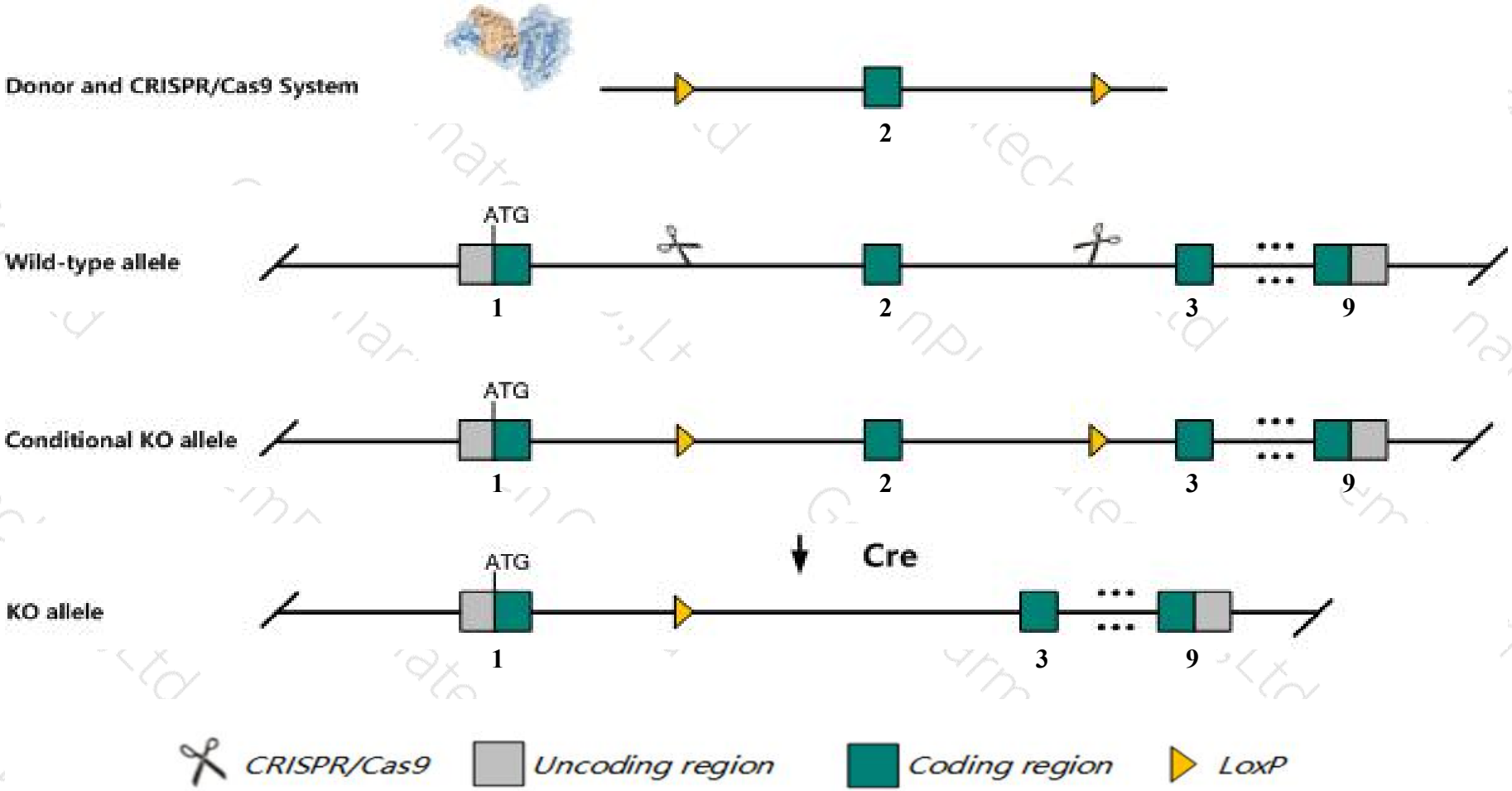
Project Overview

Project Name	***Opn4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Opn4* gene. The schematic diagram is as follows:



Technical routes

- The *Opn4* gene has 3 transcripts. According to the structure of *Opn4* gene, exon2 of *Opn4-201* (ENSMUST00000022331.2) transcript is recommended as the knockout region.The region contains 146bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Opn4* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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,Homozygous inactivation of this gene results in absent intrinsic inner retinal photosensitivity, abnormal pupillary reflex, and abnormal circadian rhythms.

- The *Opn4* gene is located on the Chr14. 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.

- The KO region contains functional region of the *GM49012* gene.Knockout the region may affect the function of *GM49012* gene.

- 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

Opn4 opsin 4 (melanopsin) [Mus musculus (house mouse)]

Gene ID: 30044, updated on 26-Mar-2019

Summary

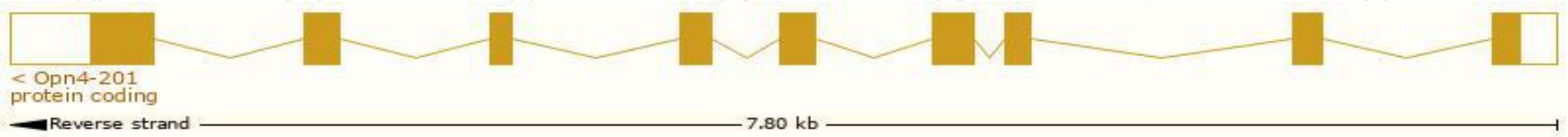
Official Symbol	Opn4 provided by MGI
Official Full Name	opsin 4 (melanopsin) provided byMGI
Primary source	MGI:MGI:1353425
See related	Ensembl:ENSMUSG00000021799
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1110007J02Rik, Gm533
Expression	Biased expression in heart adult (RPKM 1.8), thymus adult (RPKM 0.2) and 3 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

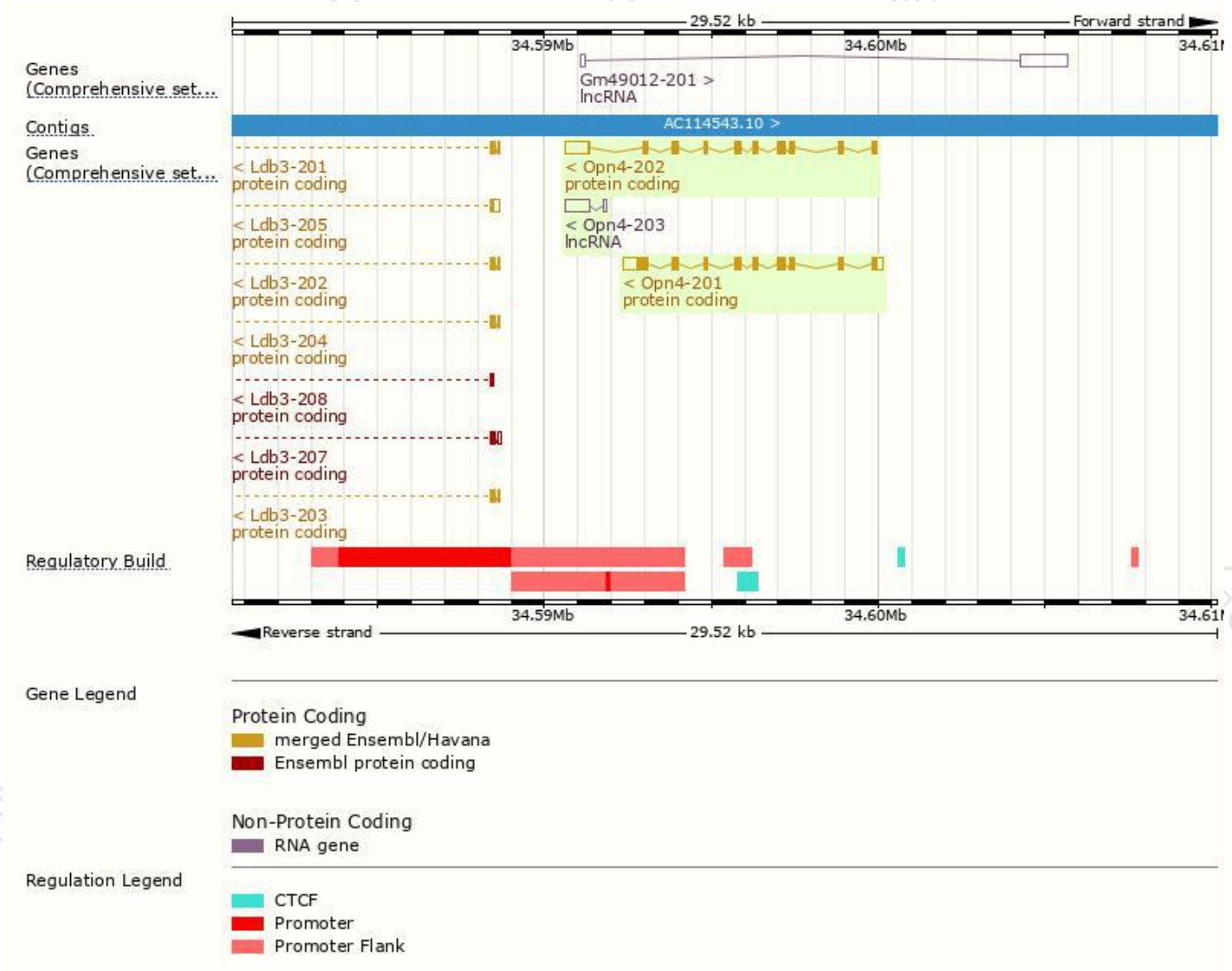
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Opn4-201	ENSMUST00000022331.2	2156	521aa	Protein coding	CCDS26943	Q9QXZ9	TSL:1 GENCODE basic APPRIS P3
Opn4-202	ENSMUST00000168444.8	2075	466aa	Protein coding	CCDS49446	Q9QXZ9	TSL:1 GENCODE basic APPRIS ALT2
Opn4-203	ENSMUST00000226806.1	795	No protein	lncRNA	-	-	

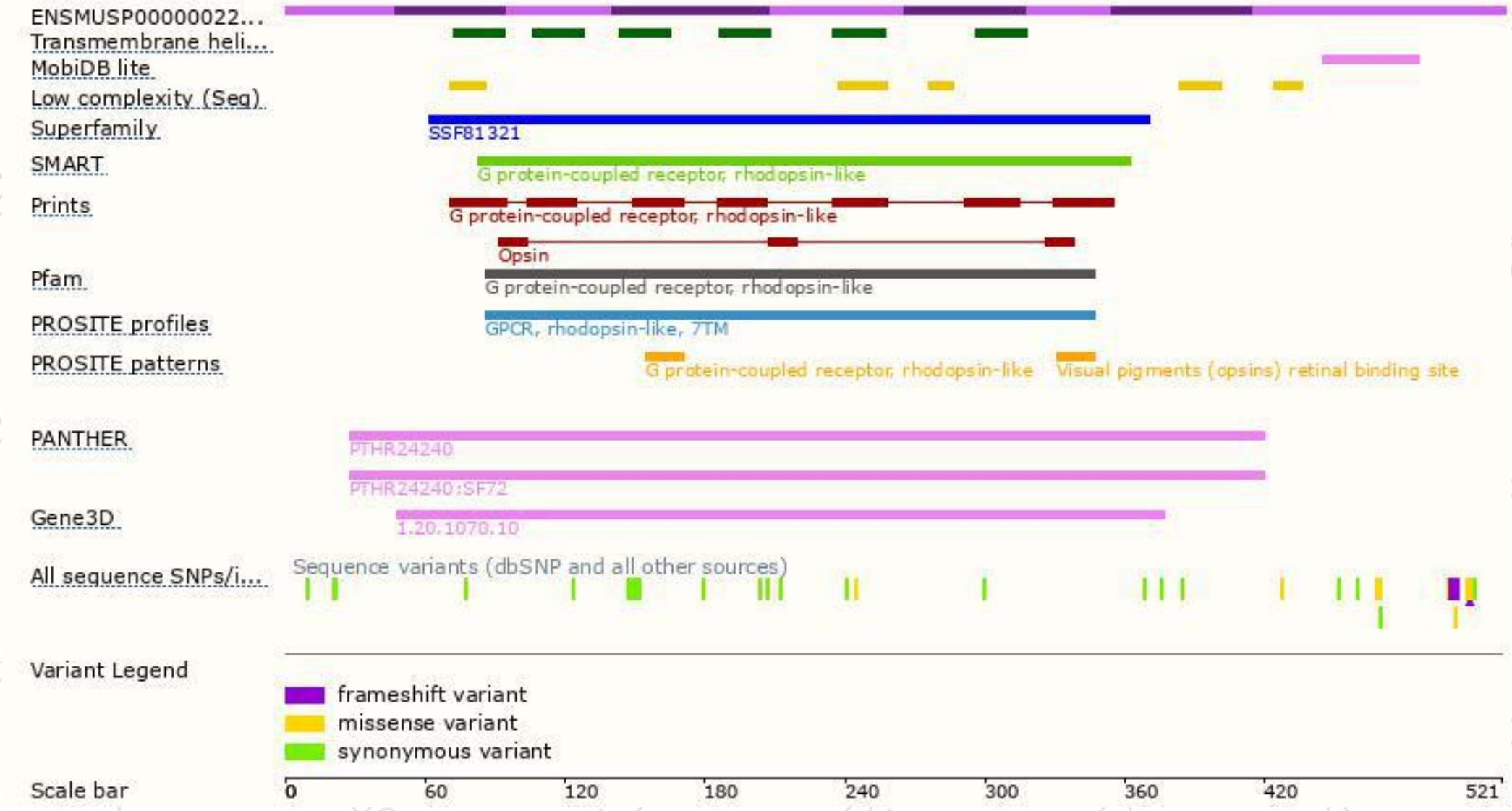
The strategy is based on the design of *Opn4-201* transcript,The transcription is shown below



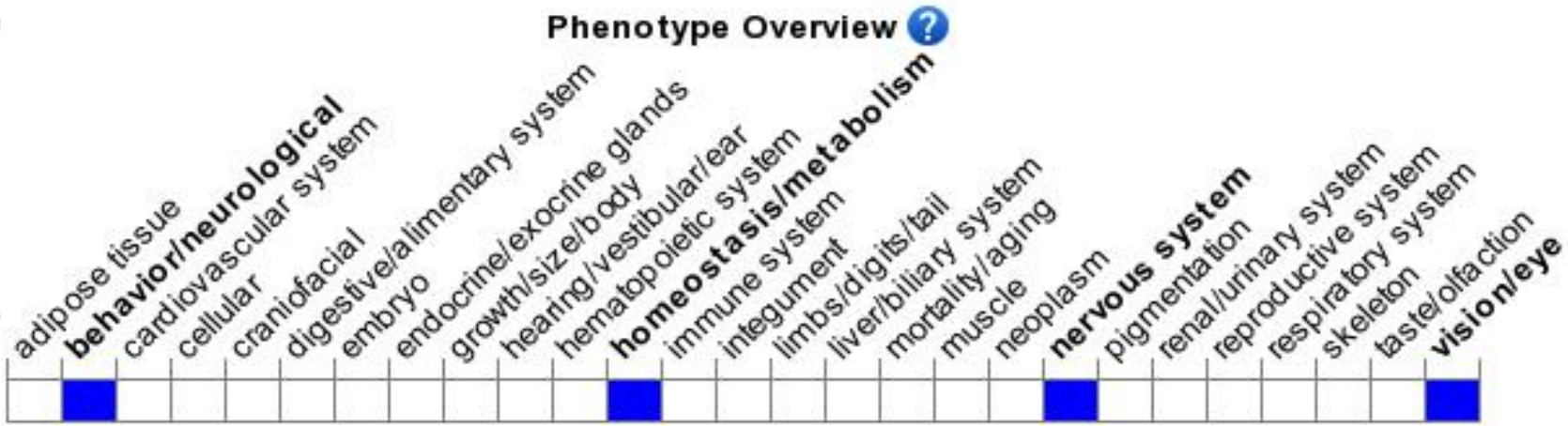
Genomic location distribution



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 inactivation of this gene results in absent intrinsic inner retinal photosensitivity, abnormal pupillary reflex, and abnormal circadian rhythms.

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

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