Rgr Cas9-CKO Strategy

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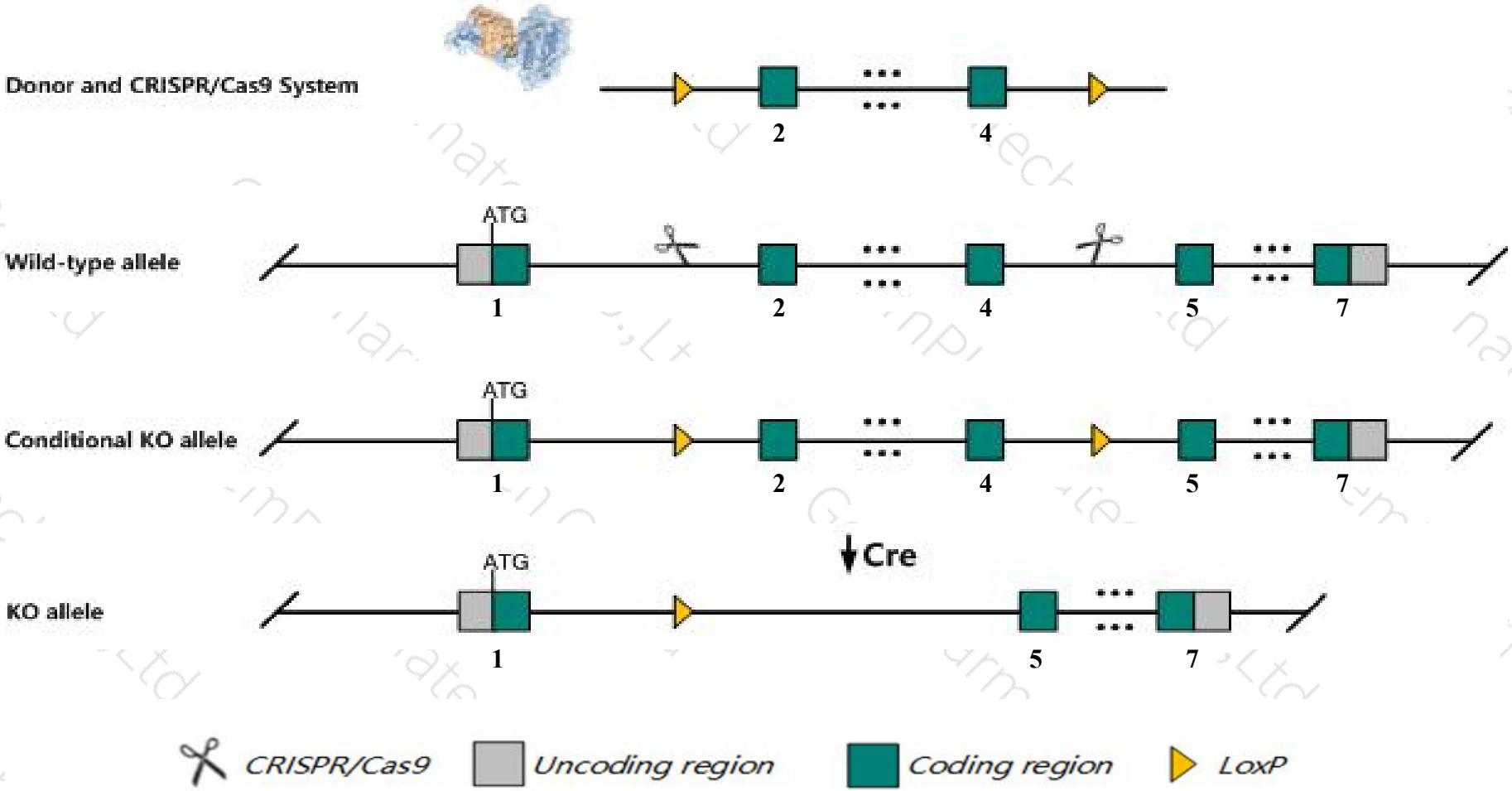
Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Rgr* gene has 6 transcripts. According to the structure of *Rgr* gene, exon2-exon4 of *Rgr-201* (ENSMUST00000022338.6) transcript is recommended as the knockout region.The region contains 433bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Rgr* 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,Homozygotes for a targeted null mutation, following 8 hours of light, exhibit reductions in both total retinal (mostly 11-cis-retinal) and rhodopsin levels, and over-accumulate all-trans-retinal indicating an impaired visual cycle.

- The *Rgr* 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.

- 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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Rgr retinal G protein coupled receptor [Mus musculus (house mouse)]

Gene ID: 57811, updated on 31-Jan-2019

Summary

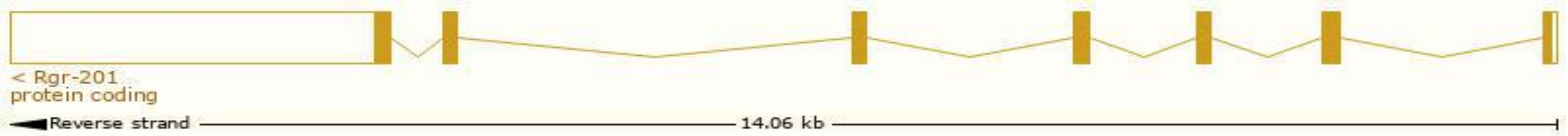
Official Symbol	Rgr provided by MGI
Official Full Name	retinal G protein coupled receptor provided byMGI
Primary source	MGI:MGI:1929473
See related	Ensembl:ENSMUSG00000021804
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Summary	The gene is a member of the opsin family of G-protein coupled receptors. The encoded protein is expressed in the retina, and acts as a photoisomerase to catalyze the conversion of all-trans-retinal to 11-cis-retinal. Disruption of a similar gene in human is associated with autosomal recessive (arRP) and autosomal dominant retinitis pigmentosa (adRP). Alternative splicing results in multiple transcript variants. [provided by RefSeq, Sep 2014]
Expression	Low expression observed in reference datasetSee more
Orthologs	human all

Transcript information（Ensembl）

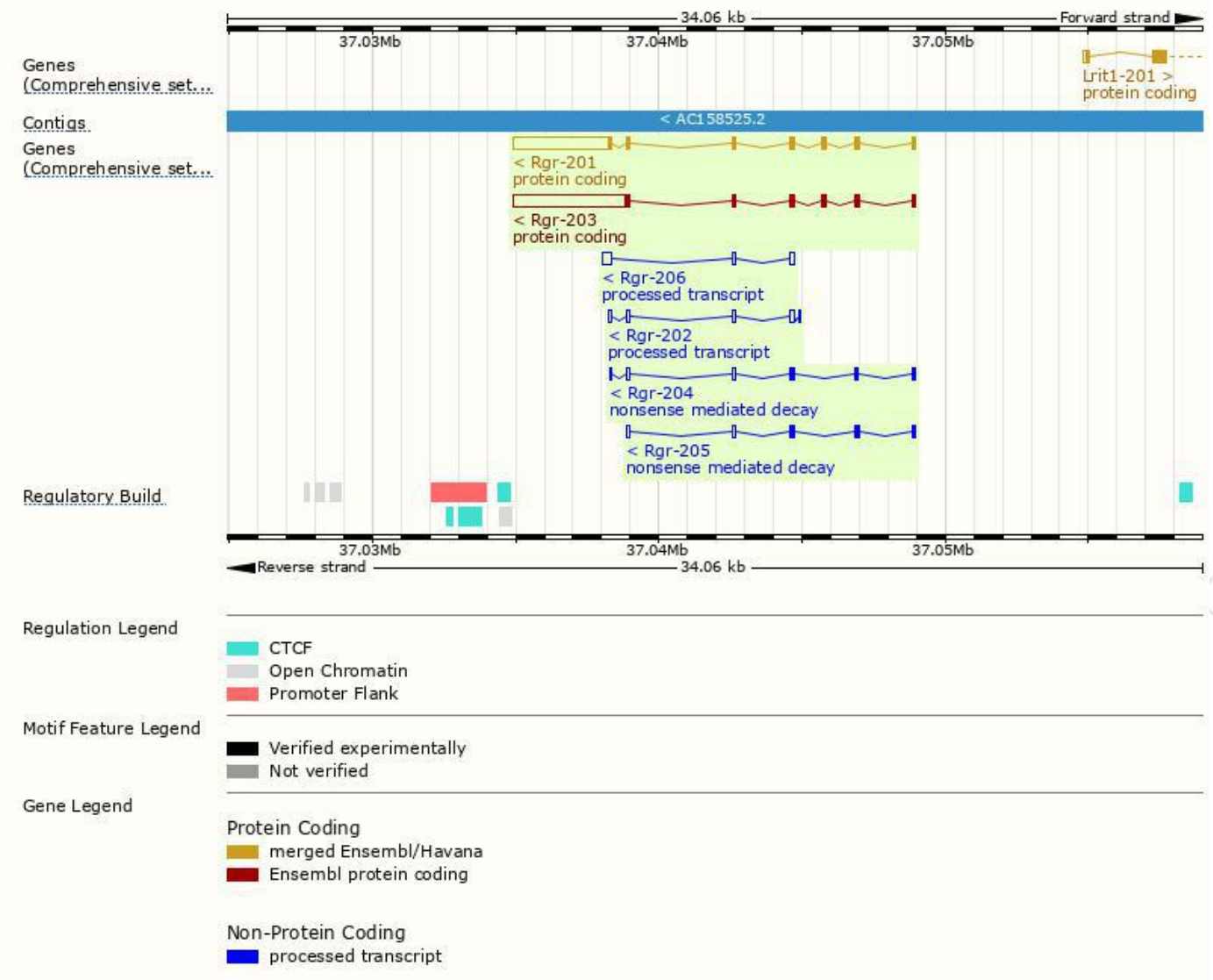
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rgr-201	ENSMUST00000022338.6	4240	291aa	Protein coding	CCDS26949	Q543X1 Q9Z2B3	TSL:1 GENCODE basic APPRIS P1
Rgr-203	ENSMUST00000225070.1	4704	261aa	Protein coding	-	A0A286YCJ5	GENCODE basic
Rgr-204	ENSMUST00000225229.1	648	89aa	Nonsense mediated decay	-	A0A286YDT4	
Rgr-205	ENSMUST00000225403.1	633	112aa	Nonsense mediated decay	-	A0A286YCJ0	
Rgr-202	ENSMUST00000224506.1	584	No protein	Processed transcript	-	-	
Rgr-206	ENSMUST00000225634.1	572	No protein	Processed transcript	-	-	

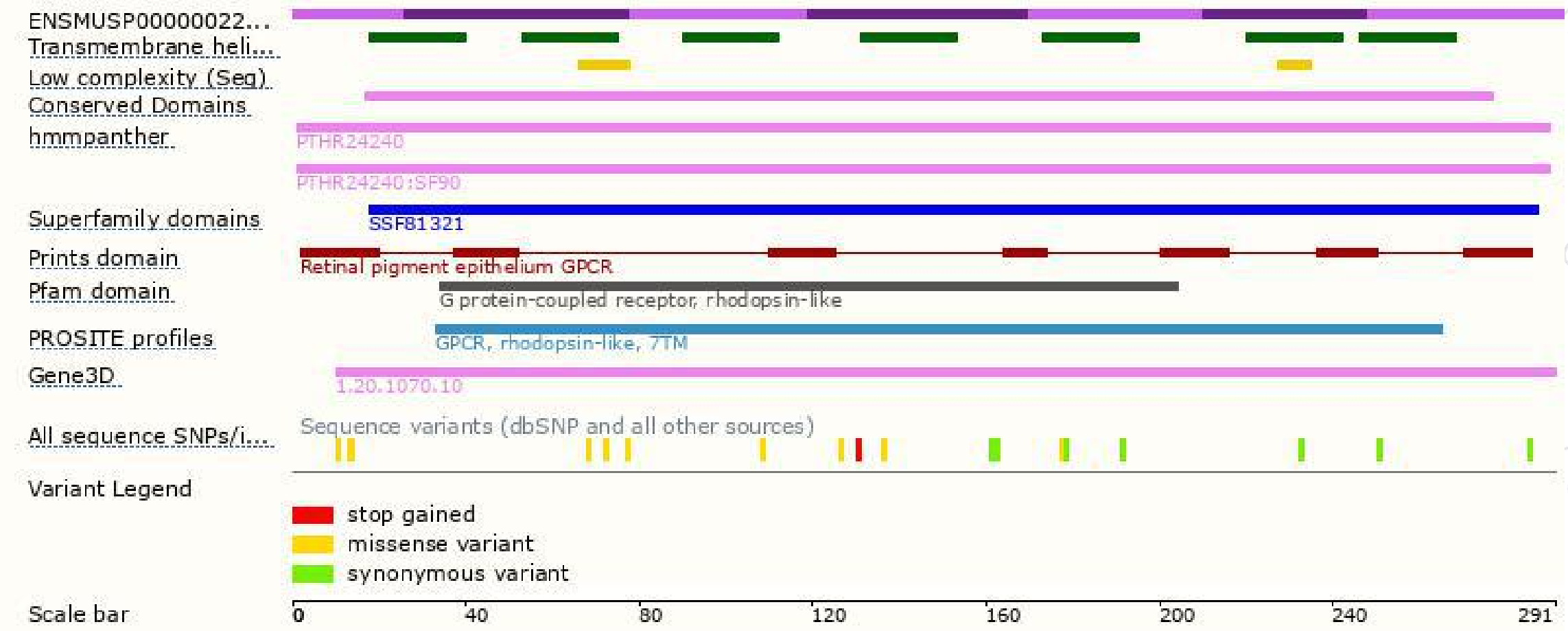
The strategy is based on the design of *Rgr-201* transcript,The transcription is shown below



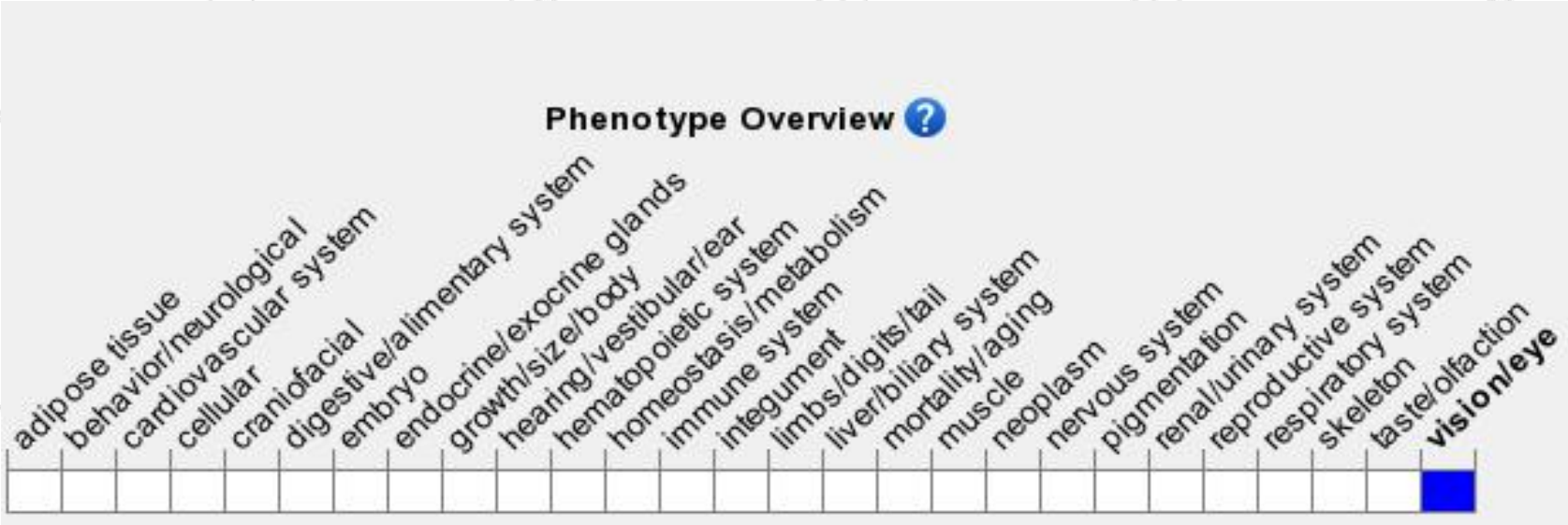
Genomic location distribution



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,Homozygotes for a targeted null mutation, following 8 hours of light, exhibit reductions in both total retinal (mostly 11-cis-retinal) and rhodopsin levels, and over-accumulate all-trans-retinal indicating an impaired visual cycle.

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

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