Abca4 Cas9-CKO Strategy

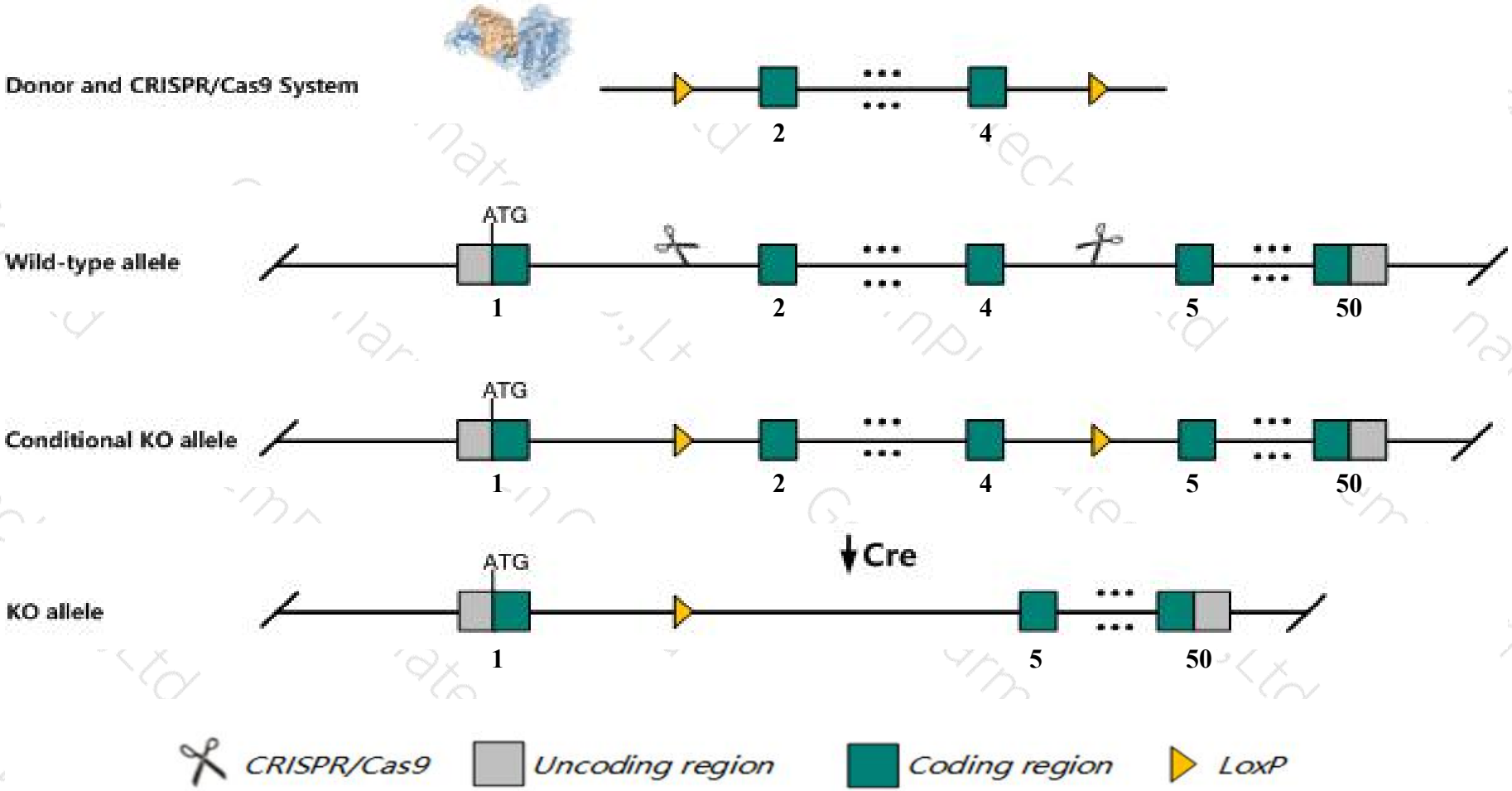
Designer: Xiaojing Li
Design Date: 2019-9-16
Reviewer: JiaYu

Project Overview

Project Name	***Abca4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Abca4* gene. The schematic diagram is as follows:



Technical routes

集萃药康 GemPharmatech

- The *Abca4* gene has 10 transcripts. According to the structure of *Abca4* gene, exon2-exon4 of *Abca4-201* (ENSMUST00000013995.12) transcript is recommended as the knockout region.The region contains 376bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Abca4* 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 targeted mutations that inactivate the gene display delayed rod dark adaptation and are a model for juvenile macular degeneration.

- The *Abca4* gene is located on the Chr3. 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）

集萃药康 GemPharmatech

Abca4 ATP-binding cassette, sub-family A (ABC1), member 4 [Mus musculus (house mouse)]

Gene ID: 11304, updated on 31-Jan-2019

Summary

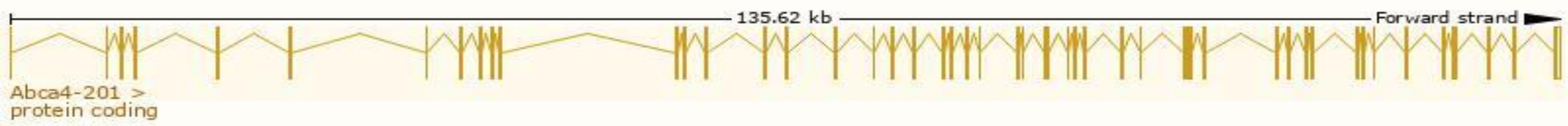
Official Symbol	Abca4 provided by MGI
Official Full Name	ATP-binding cassette, sub-family A (ABC1), member 4 provided by MGI
Primary source	MGI:MGI:109424
See related	Ensembl:ENSMUSG00000028125
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AW050280, Abc10, Abcr, D430003I15Rik, RmP
Summary	The membrane-associated protein encoded by this gene is a member of the superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extra- and intracellular membranes. ABC genes are divided into seven distinct subfamilies (ABC1, MDR/TAP, MRP, ALD, OABP, GCN20, White). This protein is a member of the ABC1 subfamily. Members of the ABC1 subfamily comprise the only major ABC subfamily found exclusively in multicellular eukaryotes. This protein was the first of the ABC transporters to be observed in photoreceptors and may play a role in the photoresponse. Mutations in the human gene are found in patients diagnosed with Stargardt disease and are associated with retinitis pigmentosa-19 and macular degeneration age-related 2. [provided by RefSeq, Jul 2008]
Expression	Broad expression in kidney adult (RPKM 1.4), ovary adult (RPKM 1.3) and 20 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

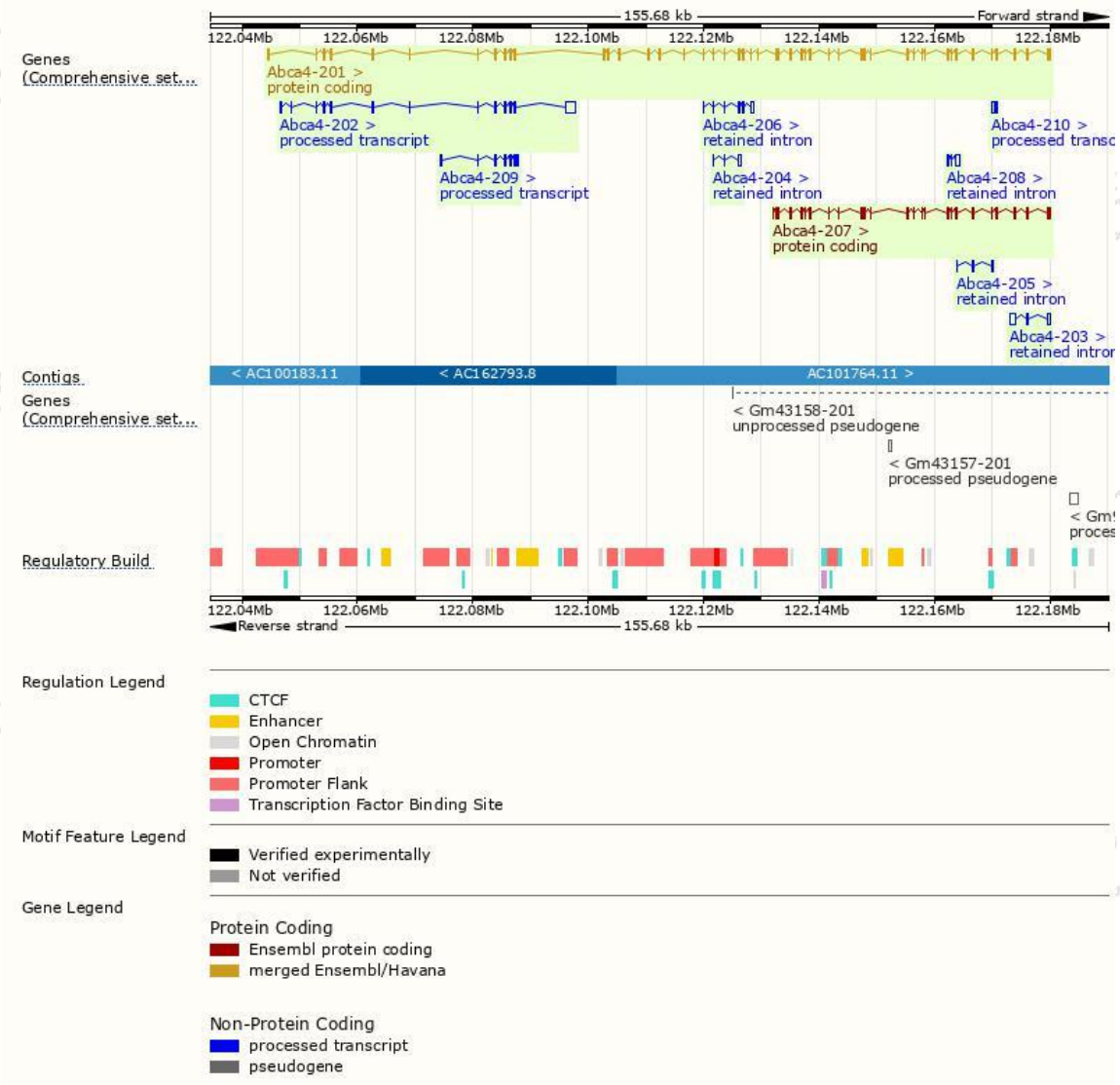
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Abca4-201	ENSMUST00000013995.12	7263	2310aa	Protein coding	CCDS38617	O35600	TSL:1 GENCODE basic APPRIS P1
Abca4-207	ENSMUST00000141135.4	3606	1102aa	Protein coding	-	A0A0G2JGG8	TSL:1 GENCODE basic
Abca4-202	ENSMUST00000132199.7	3511	No protein	Processed transcript	-	-	TSL:1
Abca4-209	ENSMUST00000150738.2	1128	No protein	Processed transcript	-	-	TSL:1
Abca4-210	ENSMUST00000197728.1	765	No protein	Processed transcript	-	-	TSL:3
Abca4-203	ENSMUST00000136358.2	1520	No protein	Retained intron	-	-	TSL:5
Abca4-206	ENSMUST00000140913.7	1198	No protein	Retained intron	-	-	TSL:1
Abca4-208	ENSMUST00000144949.1	700	No protein	Retained intron	-	-	TSL:3
Abca4-205	ENSMUST00000137616.2	652	No protein	Retained intron	-	-	TSL:3
Abca4-204	ENSMUST00000136624.1	531	No protein	Retained intron	-	-	TSL:3

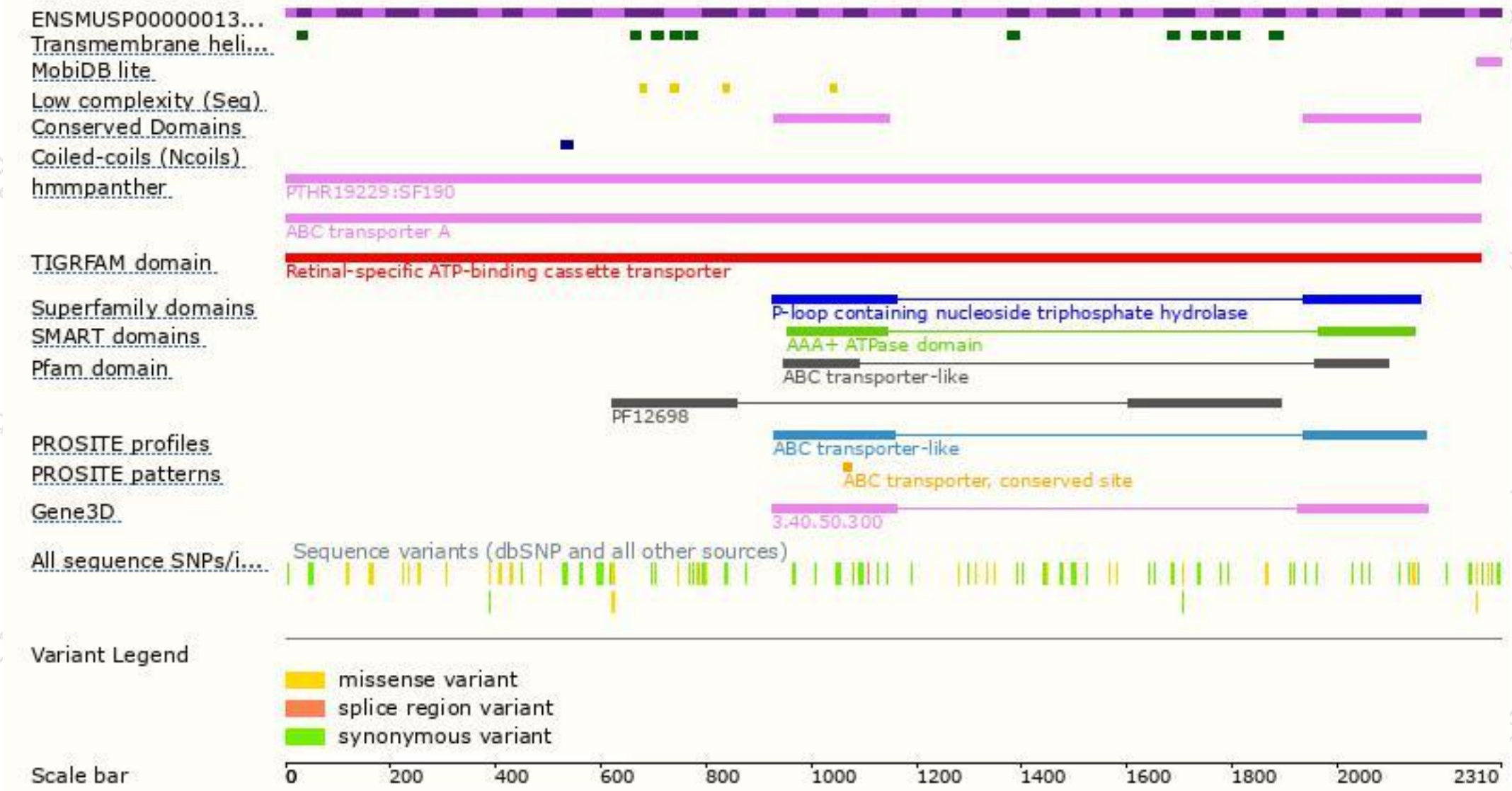
The strategy is based on the design of *Abca4-201* transcript,The transcription is shown below



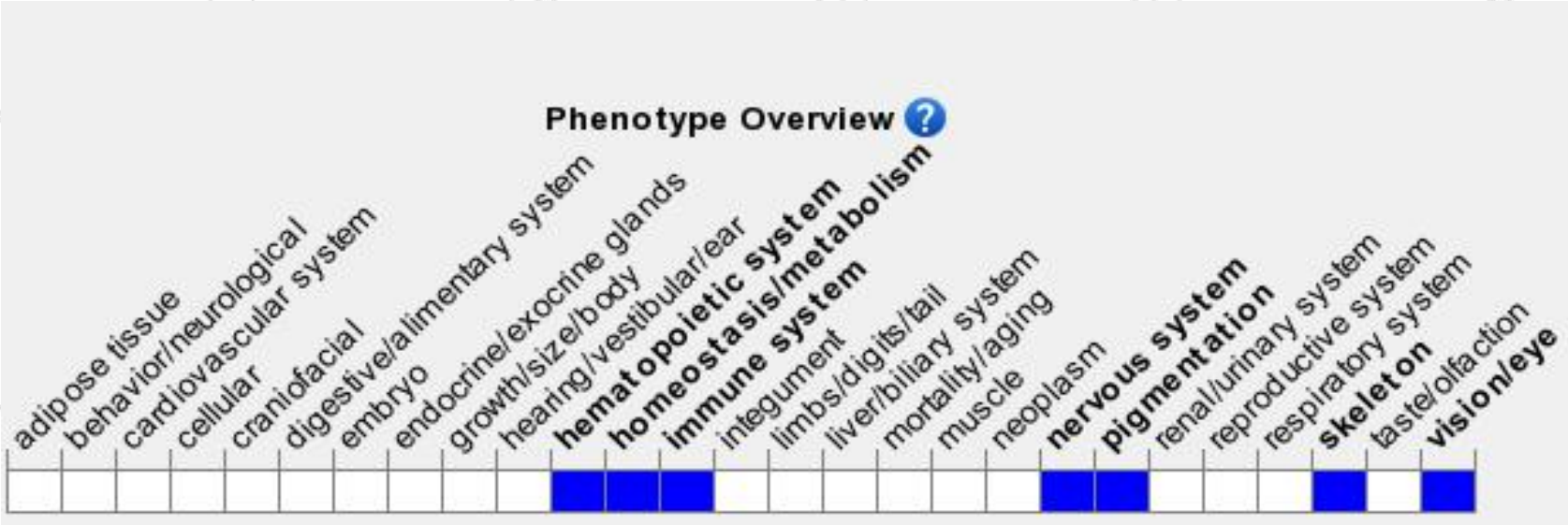
Genomic location distribution



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 targeted mutations that inactivate the gene display delayed rod dark adaptation and are a model for juvenile macular degeneration.

集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd