C1qtnf5 Cas9-CKO Strategy

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Reviewer: Xueting Zhang

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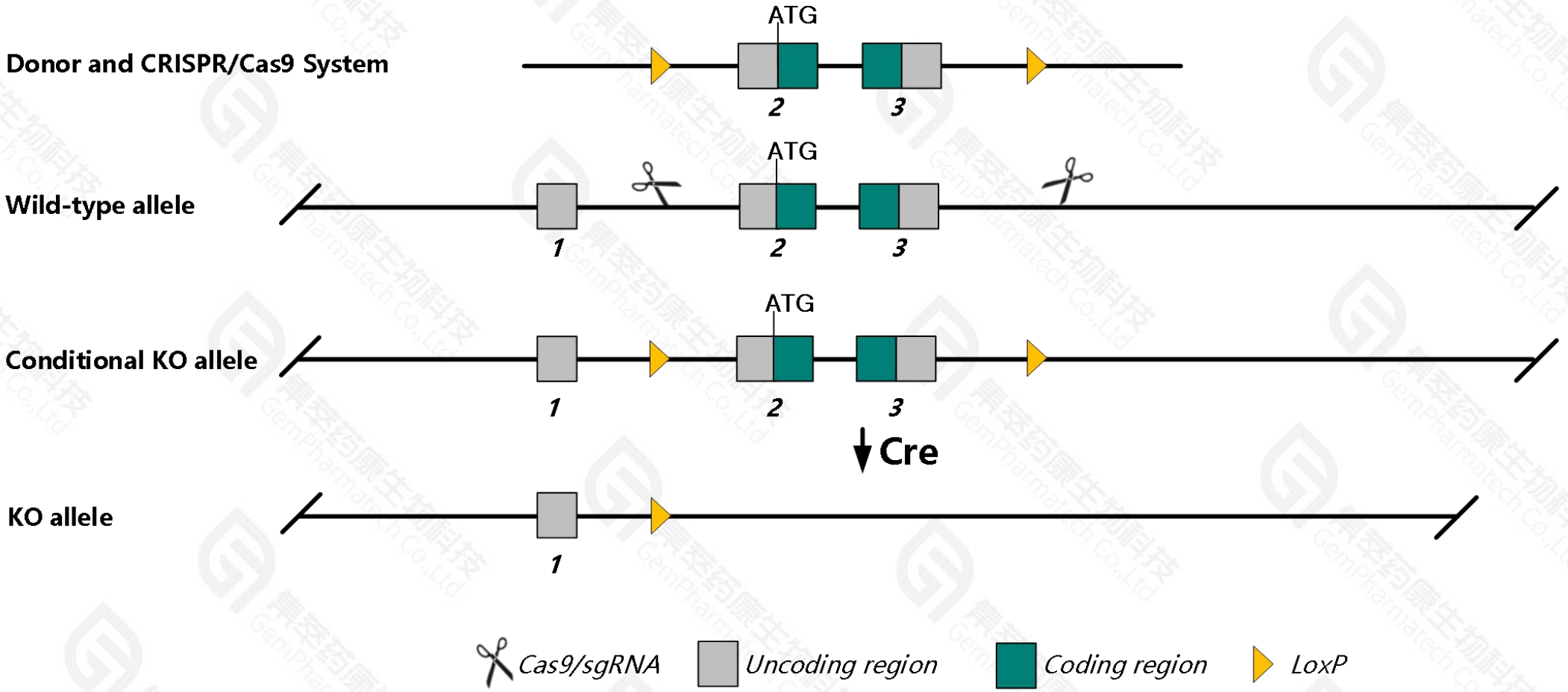
Project Overview

Project Name	***C1qtnf5***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *C1qtnf5* gene. The schematic diagram is as follows:



Technical routes

➢ The *C1qtnf5* gene has 8 transcripts. According to the structure of *C1qtnf5* gene, exon2-exon3 of *C1qtnf5-202*(ENSMUST00000114816.8) transcript is recommended as the knockout region.The region contains all of the coding sequence.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *C1qtnf5* 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,heterozygotes for a knock-in mutation show features of late-onset retinal degeneration, whereas hetero- or homozygotes for the same knock-in generated by a different group lack retinal defects. Homozygous null mice exhibit reduced hepatic steatosis and improved insulin action on a high-fat diet.

➢ The KO region contains functional region of the *Rnf26,Mfrp,Gm20444,Gm47327,Gm26737,Gm49380* gene.Knockout the region may affect the function of *Rnf26,Mfrp,Gm20444,Gm47327,Gm26737,Gm49380* gene.

➢ The Intron1 is only 361bp,loxp insertion may affect mRNA splicing.

➢ The *C1qtnf5* 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）

集萃药康 GemPharmatech

C1qtnf5 C1q and tumor necrosis factor related protein 5 [Mus musculus (house mouse)]

Gene ID: 235312, updated on 25-Sep-2020

Summary

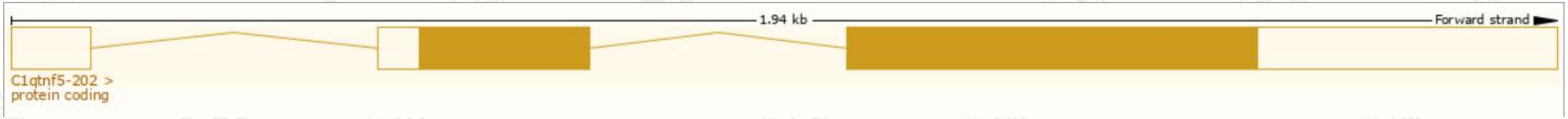
Official Symbol	C1qtnf5 provided by MGI
Official Full Name	C1q and tumor necrosis factor related protein 5 provided by MGI
Primary source	MGI:MGI:2385958
See related	Ensembl:ENSMUSG00000079592
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Adie, CTR, Ctrp5, Mfrp
Summary	The protein encoded by this gene is a member of the C1q/tumor necrosis factor superfamily. This family member is a secretory protein that functions in eye development. Mutations in this gene are thought to underlie the pathophysiology of late-onset retinal degeneration (L-ORD) and early-onset long anterior zonules (LAZ). Bicistronic transcripts composed of the coding sequences for this gene (C1qtnf5) and the membrane-type frizzled-related protein gene (Mfrp) have been identified, and the resulting products can interact with each other. Co-transcription of C1qtnf5 and Mfrp has been observed in both human and mouse. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Jun 2010]
Expression	Biased expression in genital fat pad adult (RPKM 22.4), adrenal adult (RPKM 3.8) and 12 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

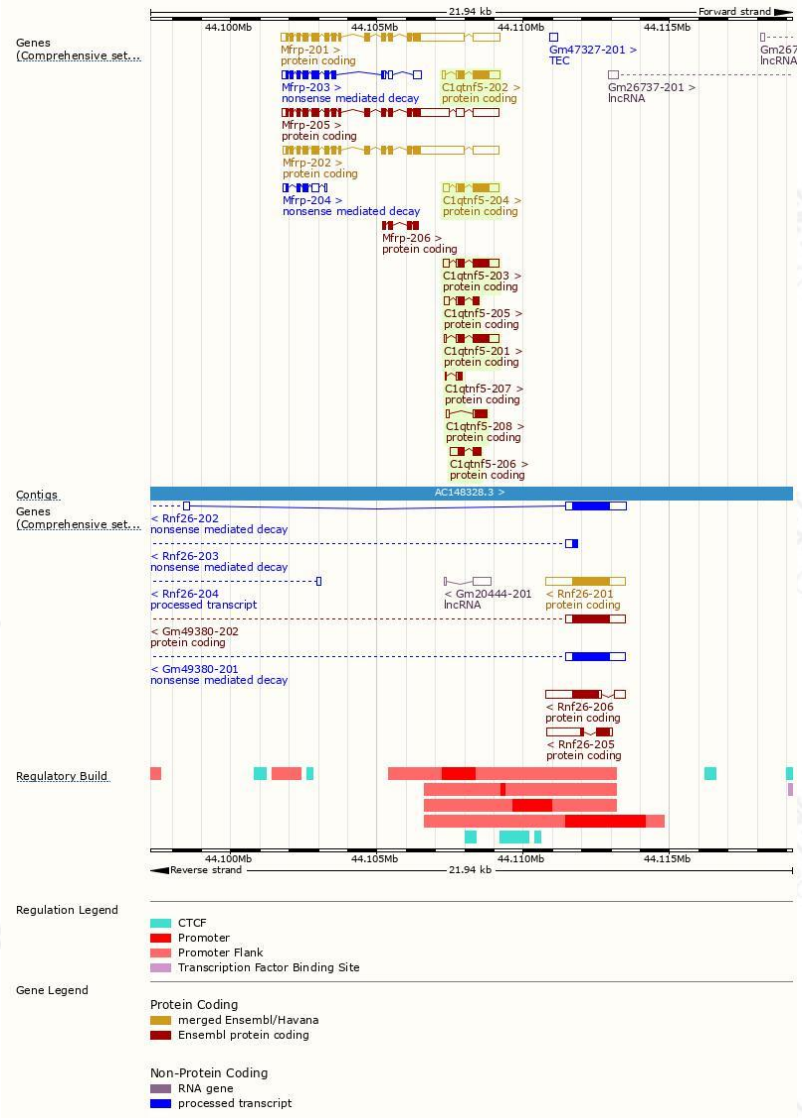
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
C1qtnf5-204	ENSMUST00000114821.9	1365	243aa	Protein coding	CCDS52777		TSL:2 , GENCODE basic , APPRIS P1 ,
C1qtnf5-203	ENSMUST00000114818.9	1337	243aa	Protein coding	CCDS52777		TSL:5 , GENCODE basic , APPRIS P1 ,
C1qtnf5-202	ENSMUST00000114816.8	1259	243aa	Protein coding	CCDS52777		TSL:1 , GENCODE basic , APPRIS P1 ,
C1qtnf5-201	ENSMUST00000114815.3	1215	243aa	Protein coding	CCDS52777		TSL:1 , GENCODE basic , APPRIS P1 ,
C1qtnf5-206	ENSMUST00000205500.2	707	154aa	Protein coding	-		CDS 3' incomplete , TSL:2 ,
C1qtnf5-205	ENSMUST00000152956.8	640	137aa	Protein coding	-		CDS 3' incomplete , TSL:2 ,
C1qtnf5-208	ENSMUST00000206769.2	565	142aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
C1qtnf5-207	ENSMUST00000206295.2	260	50aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,

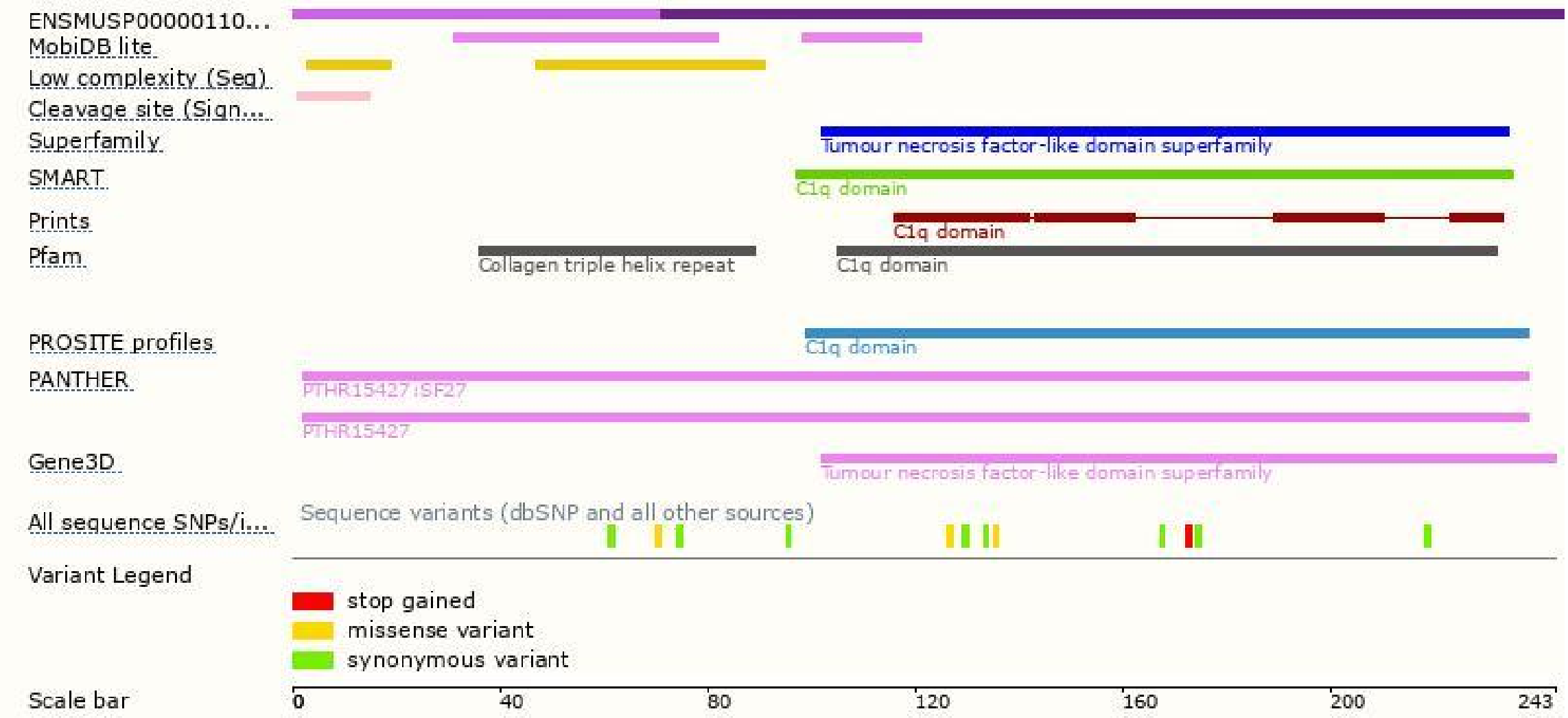
The strategy is based on the design of *C1qtnf5-202* transcript,the transcription is shown below:



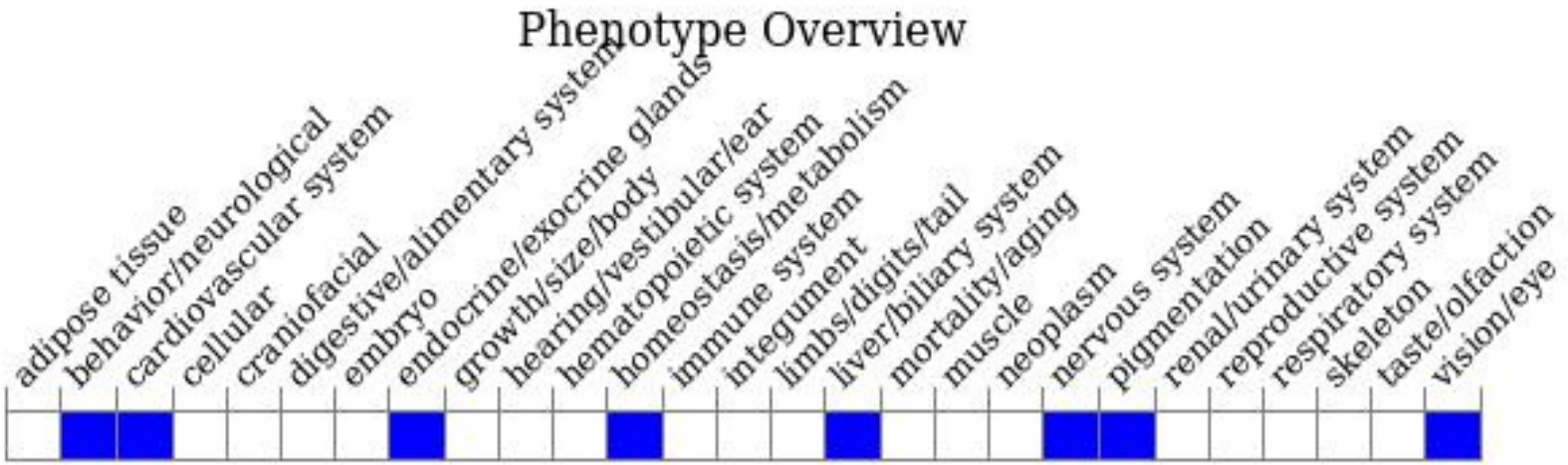
Genomic location distribution



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,heterozygotes for a knock-in mutation show features of late-onset retinal degeneration, whereas hetero- or homozygotes for the same knock-in generated by a different group lack retinal defects. Homozygous null mice exhibit reduced hepatic steatosis and improved insulin action on a high-fat diet.

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