

Aqp9 Cas9-CKO Strategy

Designer:	Huan Wang
Reviewer:	Huan Fan
Design Date:	2020-5-26

Project Overview

Project Name

Aqp9

Project type

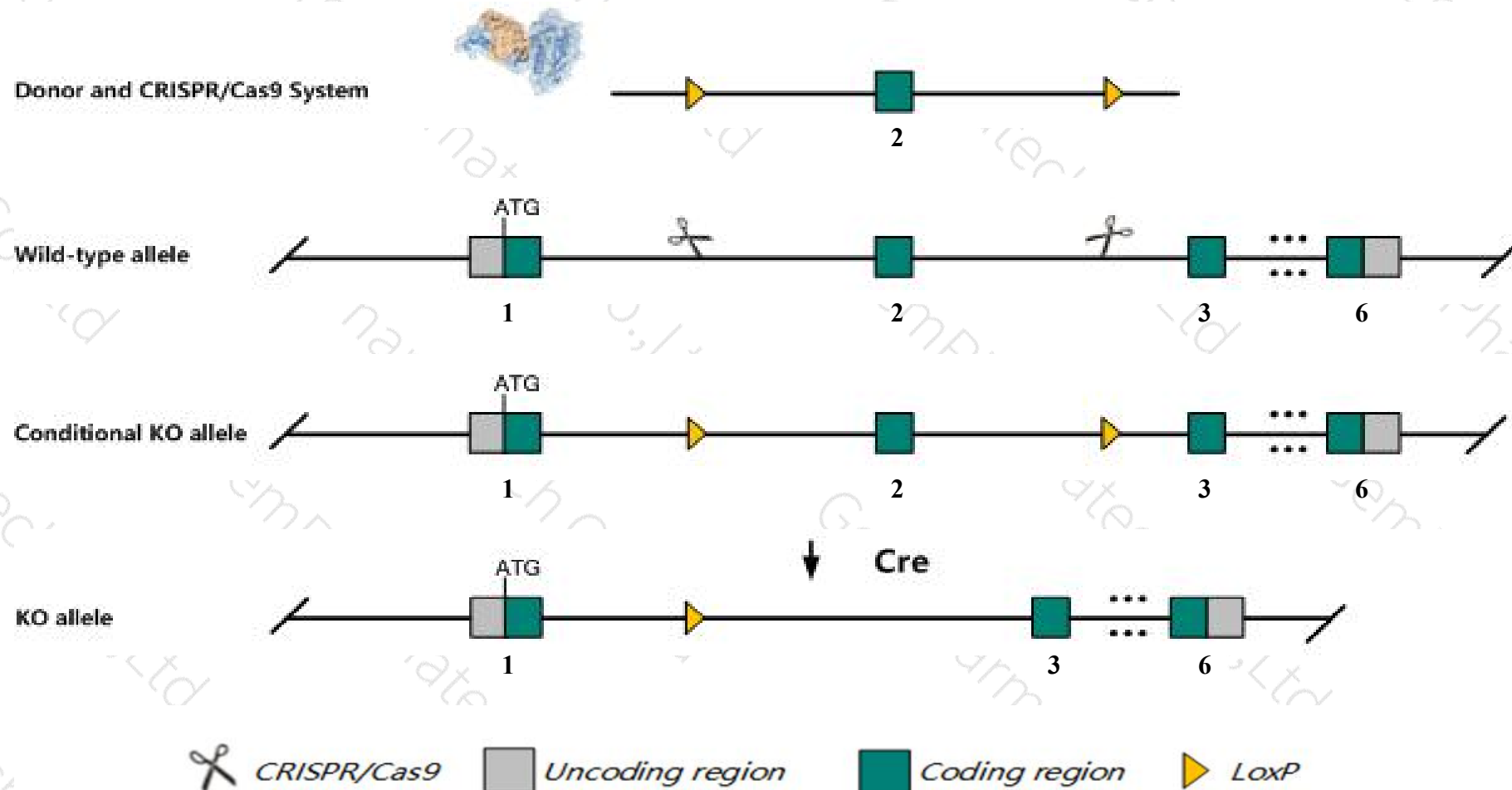
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Aqp9* gene. The schematic diagram is as follows:



- The *Aqp9* gene has 10 transcripts. According to the structure of *Aqp9* gene, exon2 of *Aqp9-201* (ENSMUST00000060917.11) transcript is recommended as the knockout region. The region contains 127bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Aqp9* 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.

- According to the existing MGI data, mice homozygous for a null allele have increased glycerol and triglyceride levels, increased resistance to glycerol-induced lysis, decreased glycerol permeability, and decreased susceptibility to the early stages of parasitic infection.
- The *Aqp9* 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)

Aqp9 aquaporin 9 [Mus musculus (house mouse)]

Gene ID: 64008, updated on 13-Mar-2020

Summary



Official Symbol Aqp9 provided by [MGI](#)

Official Full Name aquaporin 9 provided by [MGI](#)

Primary source [MGI:MGI:1891066](#)

See related [Ensembl:ENSMUSG00000032204](#)

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 1700020I22Rik, AI266899, AQP-9

Summary This gene encodes a member of the aquaglyceroporin subfamily of aquaporins. This protein transports water, glycerol, urea, purines and pyrimidines and plays a role in glycerol metabolism and osteoclast differentiation. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Dec 2012]

Expression Biased expression in liver adult (RPKM 30.0), liver E18 (RPKM 8.4) and 4 other tissues [See more](#)

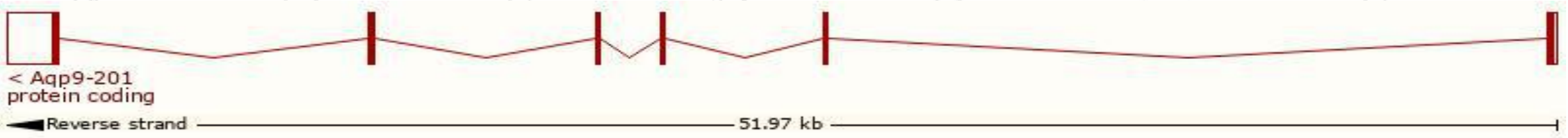
Orthologs [human](#) [all](#)

Transcript information (Ensembl)

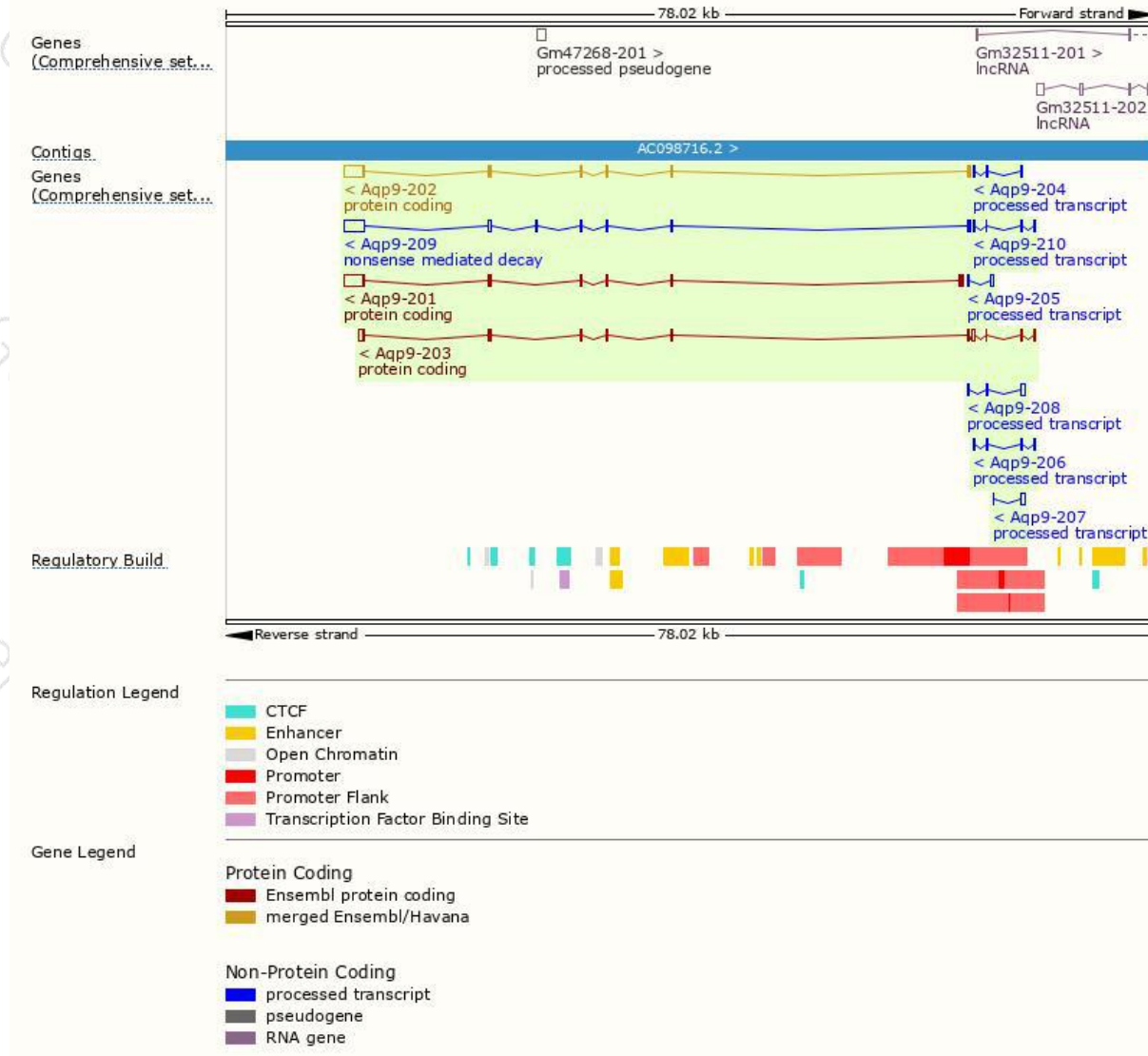
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Aqp9-202	ENSMUST00000074465.8	2646	295aa	Protein coding	CCDS23325	Q4FK77 Q9JJJ3	TSL:1 GENCODE basic APPRIS P1
Aqp9-201	ENSMUST00000060917.11	2643	321aa	Protein coding	CCDS72270	D3YYD6	TSL:1 GENCODE basic
Aqp9-203	ENSMUST00000113570.7	1712	295aa	Protein coding	CCDS23325	Q4FK77 Q9JJJ3	TSL:5 GENCODE basic APPRIS P1
Aqp9-209	ENSMUST00000144618.7	2765	192aa	Nonsense mediated decay	-	E9Q8P3	TSL:1
Aqp9-208	ENSMUST00000139495.7	619	No protein	Processed transcript	-	-	TSL:3
Aqp9-207	ENSMUST00000138364.1	472	No protein	Processed transcript	-	-	TSL:5
Aqp9-205	ENSMUST00000135892.1	428	No protein	Processed transcript	-	-	TSL:2
Aqp9-210	ENSMUST00000153050.7	422	No protein	Processed transcript	-	-	TSL:3
Aqp9-206	ENSMUST00000136798.1	399	No protein	Processed transcript	-	-	TSL:3
Aqp9-204	ENSMUST00000133239.7	379	No protein	Processed transcript	-	-	TSL:1

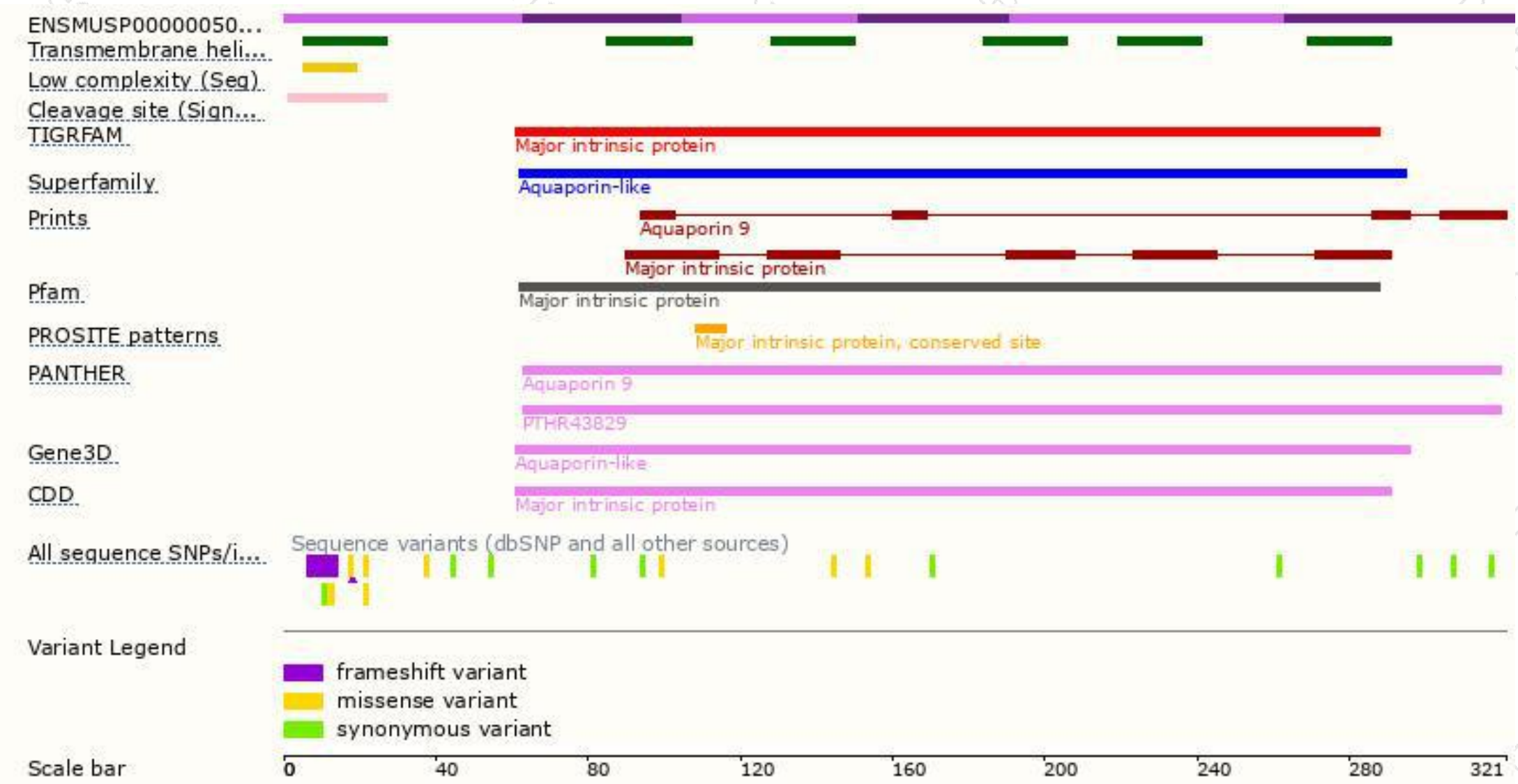
The strategy is based on the design of *Aqp9-201* transcript,the transcription is shown below:



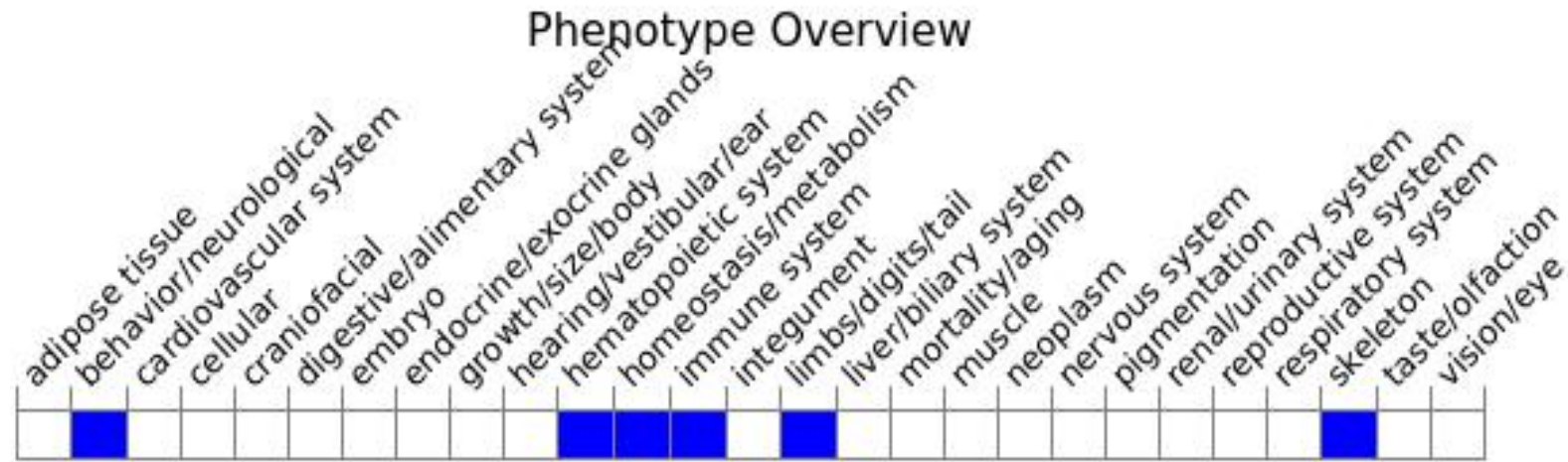
Genomic location distribution



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 null allele have increased glycerol and triglyceride levels, increased resistance to glycerol-induced lysis, decreased glycerol permeability, and decreased susceptibility to the early stages of parasitic infection.

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

Tel: 400-9660890

