Aqp2 Cas9-CKO Strategy

Designer: Longyun Hu

Reviewer: Yun Li

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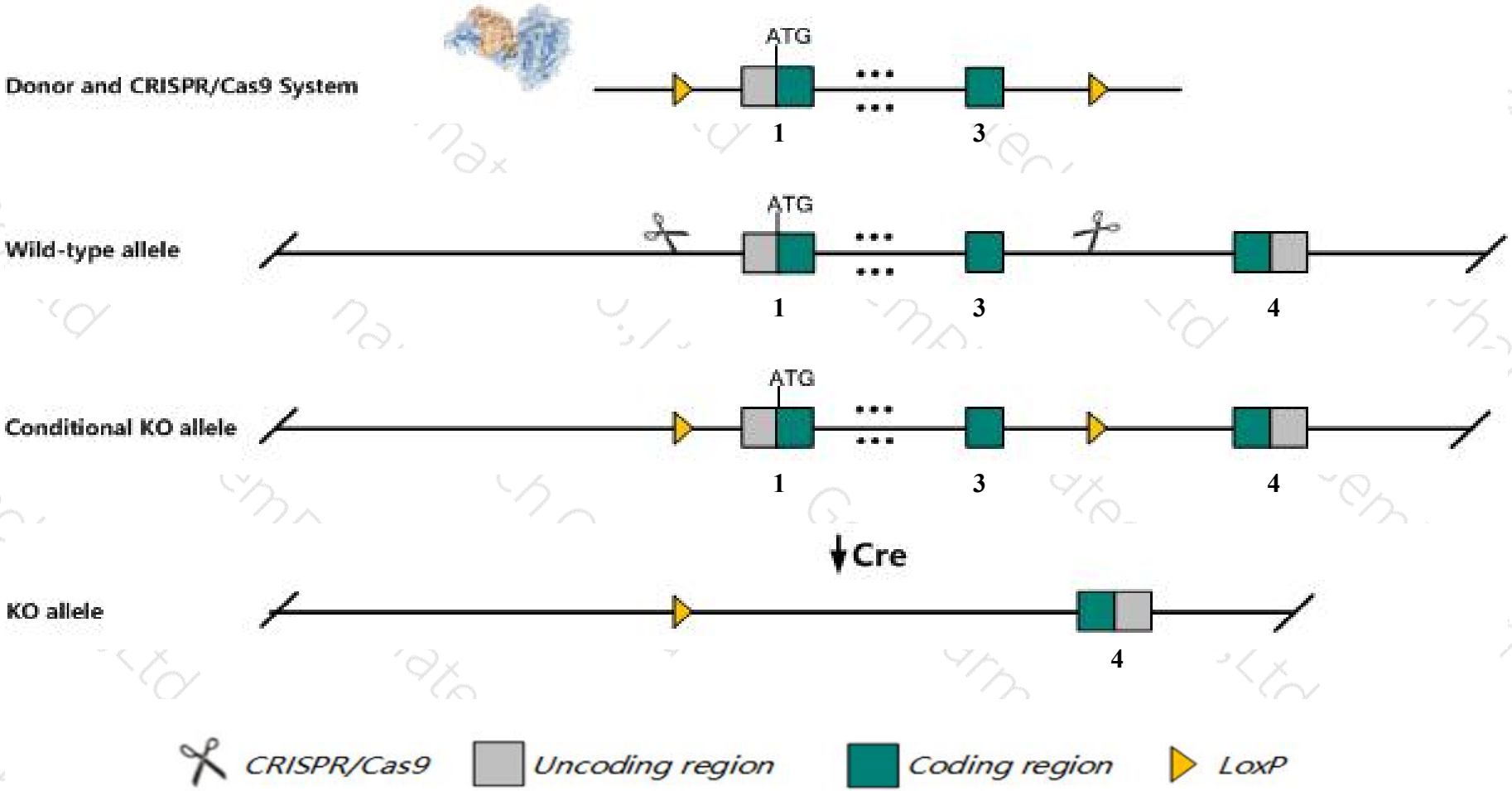
Project Overview

Project Name	***Aqp2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Aqp2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Aqp2* gene has 1 transcript. According to the structure of *Aqp2* gene, exon1-exon3 of *Aqp2-201*(ENSMUST00000023752.5) transcript is recommended as the knockout region.The region contains start codon ATG.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Aqp2* 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 either a null or knock-in allele fail to thrive and die within days of birth due to severe urinary concentration defects and hydronephrosis. Other knock-in, spontaneous, ENU-induced, and tissue-specific knock-out mutants are growth retarded and polyuric but survive to adulthood.

➢ The *Aqp2* gene is located on the Chr15. 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

Aqp2 aquaporin 2 [Mus musculus (house mouse)]

Gene ID: 11827, updated on 13-Mar-2020

Summary

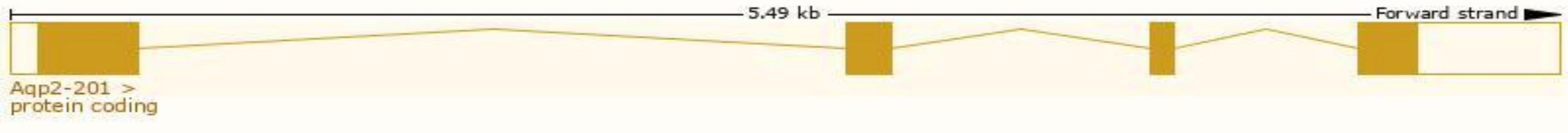
Official Symbol	Aqp2 provided by MGI
Official Full Name	aquaporin 2 provided byMGI
Primary source	MGI:MGI:1096865
See related	Ensembl:ENSMUSG00000023013
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AQP-CD, WCH-CD, cph, jpk
Expression	Restricted expression toward kidney adult (RPKM 289.8)See more
Orthologs	human all

Transcript information（Ensembl）

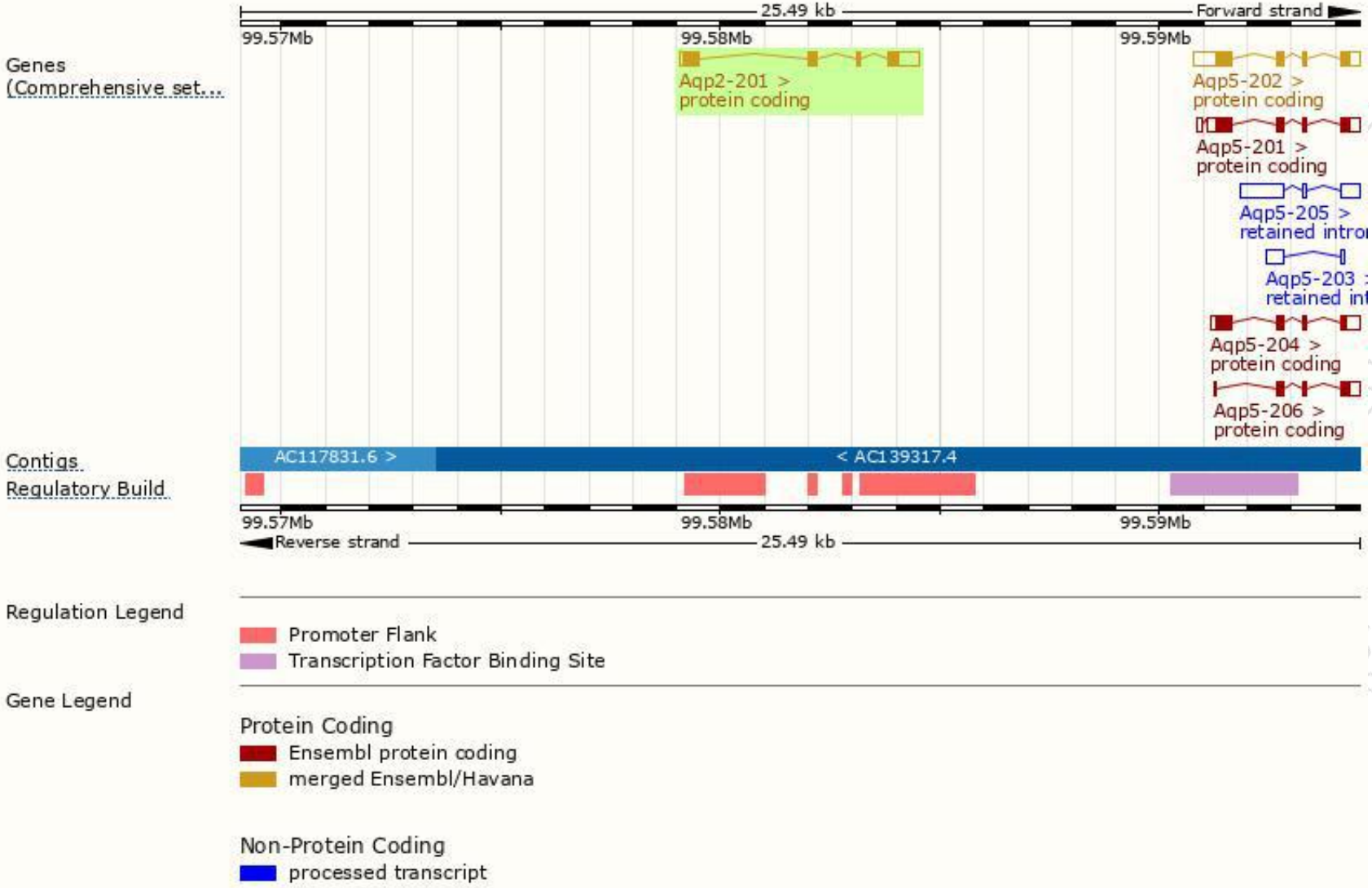
The gene has 1 transcript,and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Aqp2-201	ENSMUST00000023752.5	1419	271aa	Protein coding	CCDS27822	P56402 Q3UQD4	TSL:1 GENCODE basic APPRIS P1

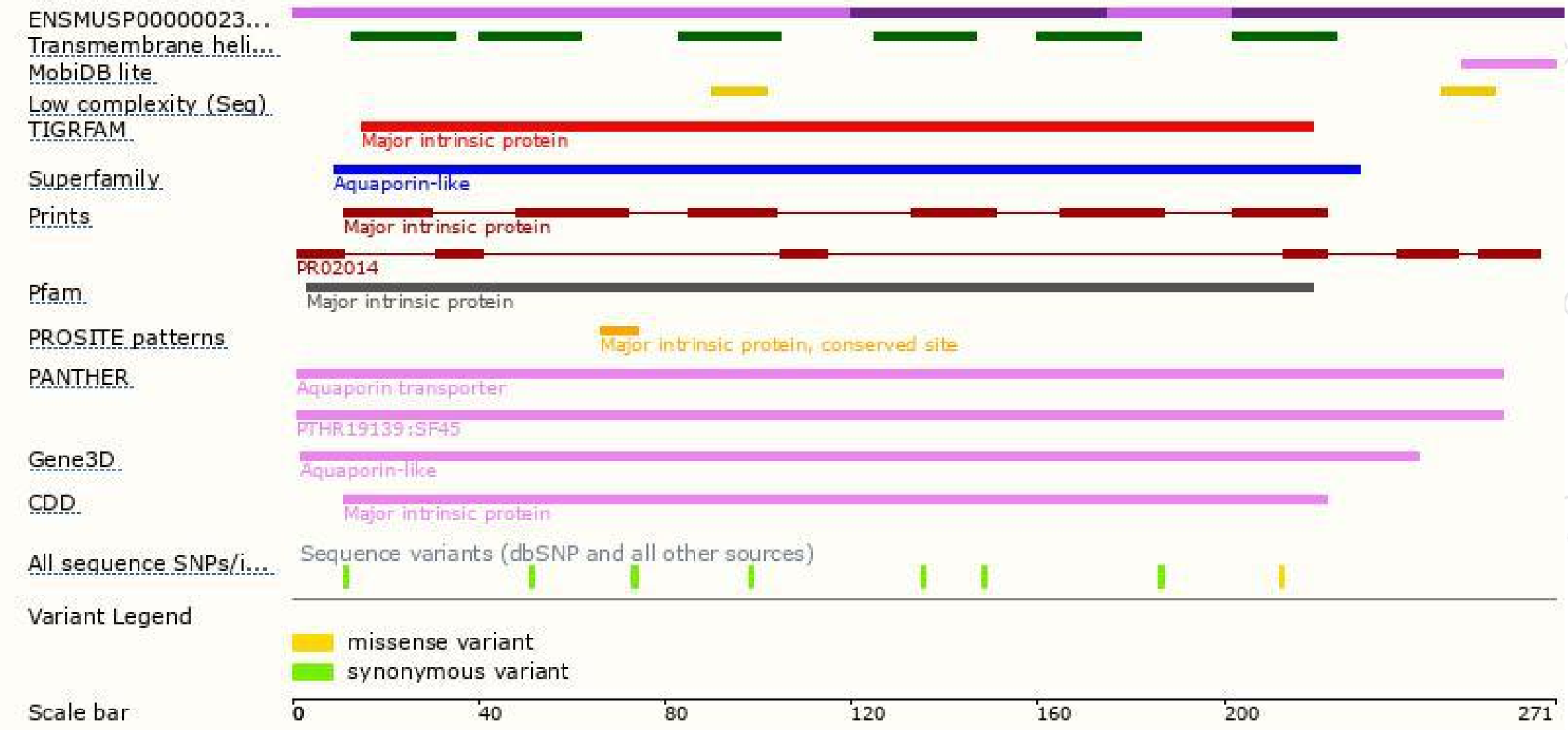
The strategy is based on the design of *Aqp2-201* transcript,the transcription is shown below:



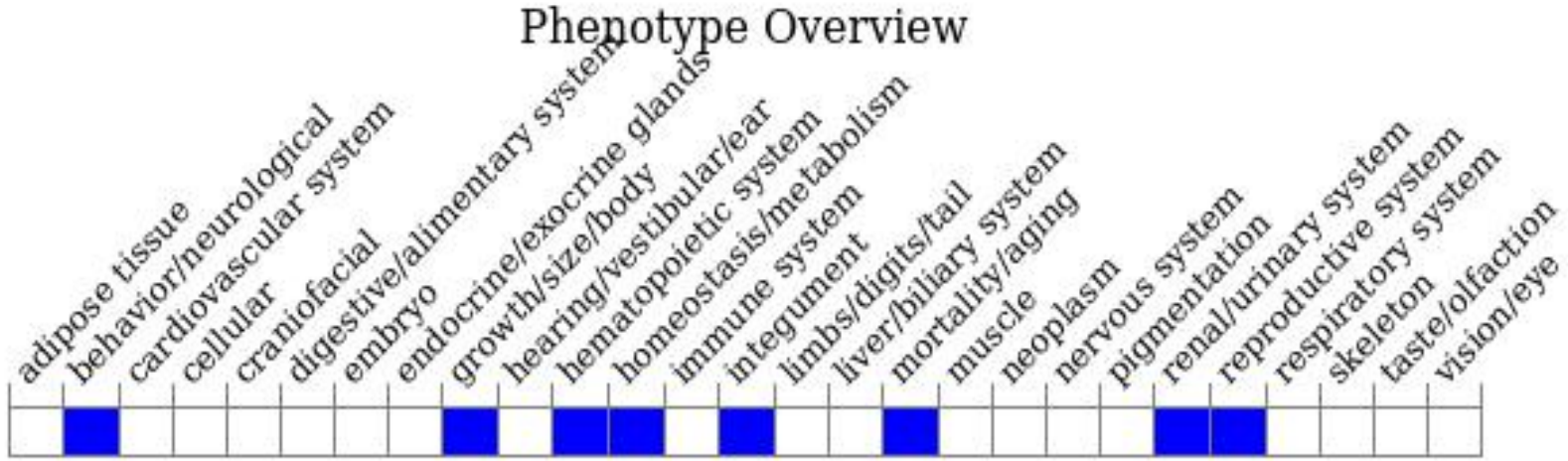
Genomic location distribution



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 either a null or knock-in allele fail to thrive and die within days of birth due to severe urinary concentration defects and hydronephrosis. Other knock-in, spontaneous, ENU-induced, and tissue-specific knock-out mutants are growth retarded and polyuric but survive to adulthood.

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd