

Wnk3 Cas9-CKO Strategy

Designer:

Huimin Su

Reviewer:

Ruirui Zhang

Design Date:

2020-2-21

Project Overview

Project Name

Wnk3

Project type

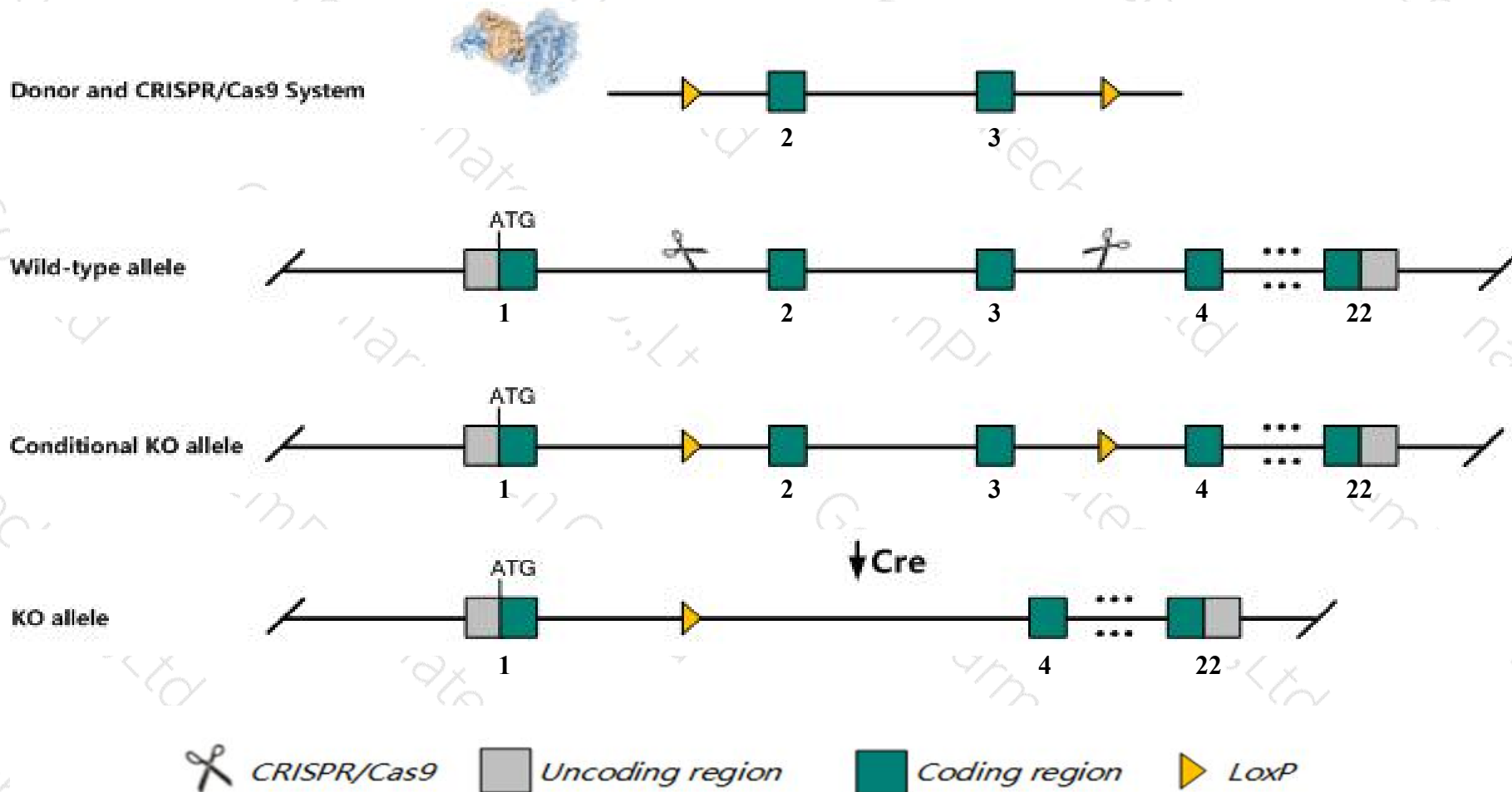
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Wnk3* gene. The schematic diagram is as follows:



Technical routes

- The *Wnk3* gene has 6 transcripts. According to the structure of *Wnk3* gene, exon2-exon3 of *Wnk3*-205 (ENSMUST00000184392.7) transcript is recommended as the knockout region. The region contains 394bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Wnk3* 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.

- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit lower blood pressure when fed a low-salt diet.
- The *Wnk3* gene is located on the ChrX. 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)

Wnk3 WNK lysine deficient protein kinase 3 [*Mus musculus* (house mouse)]

Gene ID: 279561, updated on 14-Sep-2019

Summary

Official Symbol Wnk3 provided by MGI
Official Full Name WNK lysine deficient protein kinase 3 provided by MGI
Primary source MGI:MGI:2652875
See related Ensembl:ENSMUSG00000041245
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 Prkwnk3; Wnk3-ps
Expression Biased expression in whole brain E14.5 (RPKM 3.2), CNS E18 (RPKM 3.1) and 8 other tissues [See more](#)
Orthologs [human](#) [all](#)

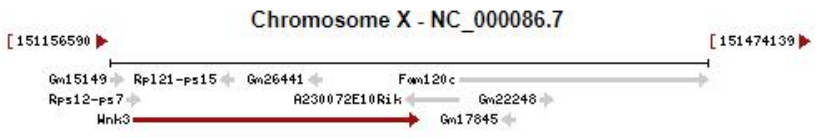
Genomic context

Location: X; X F3

[See Wnk3 in Genome Data Viewer](#)

Exon count: 27

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	X	NC_000086.7 (151168777..151320194)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	X	NC_000086.6 (147632683..147754695)

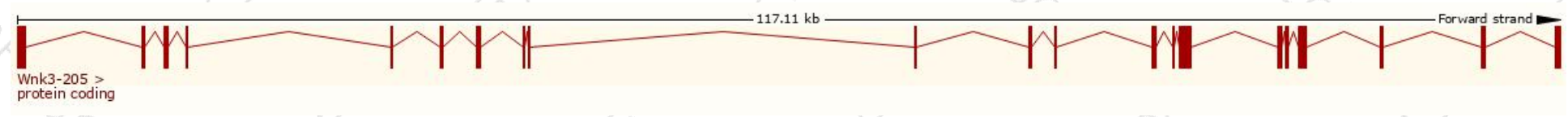


Transcript information (Ensembl)

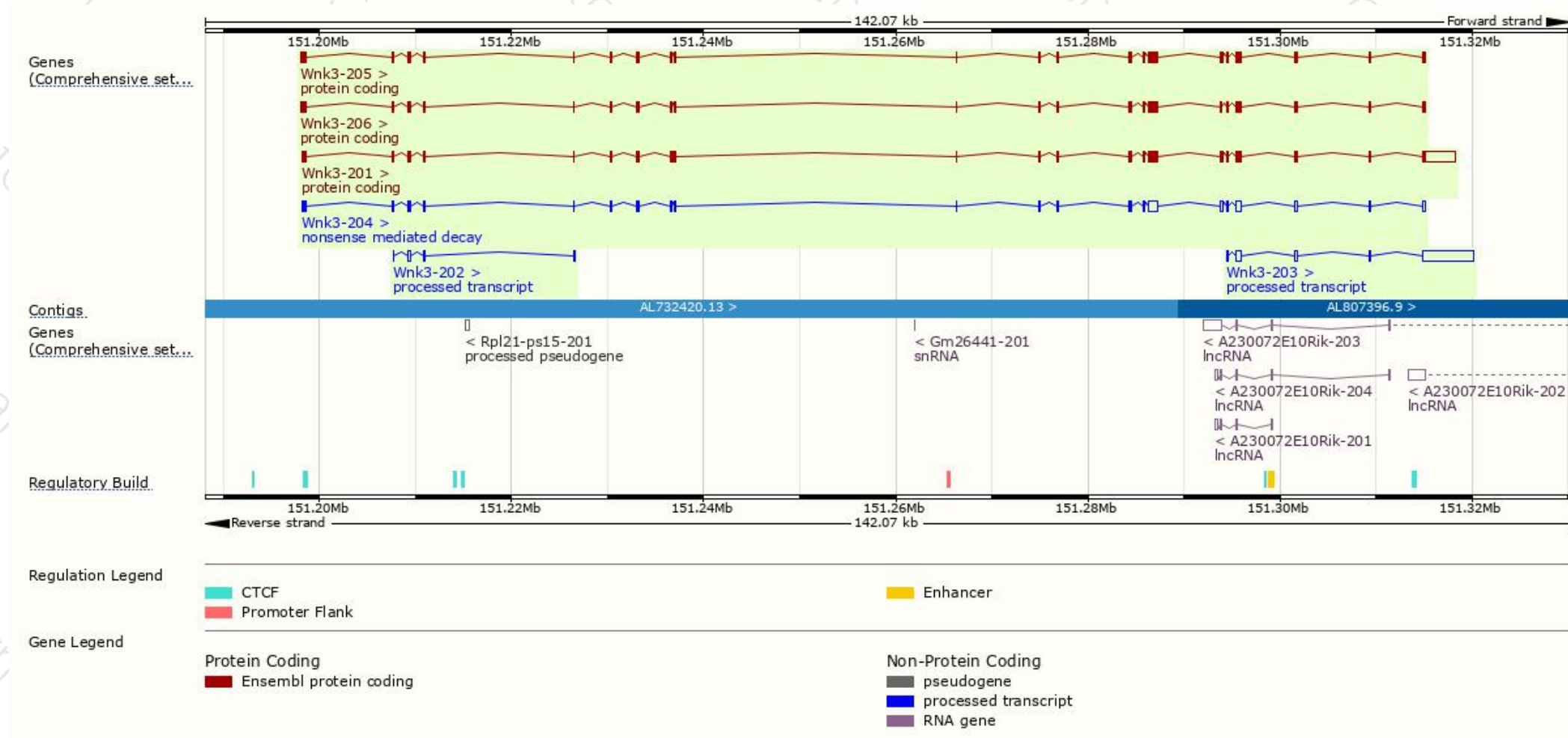
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Wnk3-205	ENSMUST00000184392.7	5394	1757aa	Protein coding	CCDS72451	Q80XP9	TSL:1 GENCODE basic APPRIS P4
Wnk3-206	ENSMUST00000184730.7	5253	1710aa	Protein coding	CCDS72450	Q80XP9	TSL:1 GENCODE basic APPRIS ALT2
Wnk3-201	ENSMUST00000096285.4	8551	1789aa	Protein coding	-	V9GWU7	TSL:5 GENCODE basic APPRIS ALT2
Wnk3-204	ENSMUST00000149140.7	5367	623aa	Nonsense mediated decay	-	V9GXP7	TSL:5
Wnk3-203	ENSMUST00000133852.1	6410	No protein	Processed transcript	-	-	TSL:1
Wnk3-202	ENSMUST00000131844.1	609	No protein	Processed transcript	-	-	TSL:5

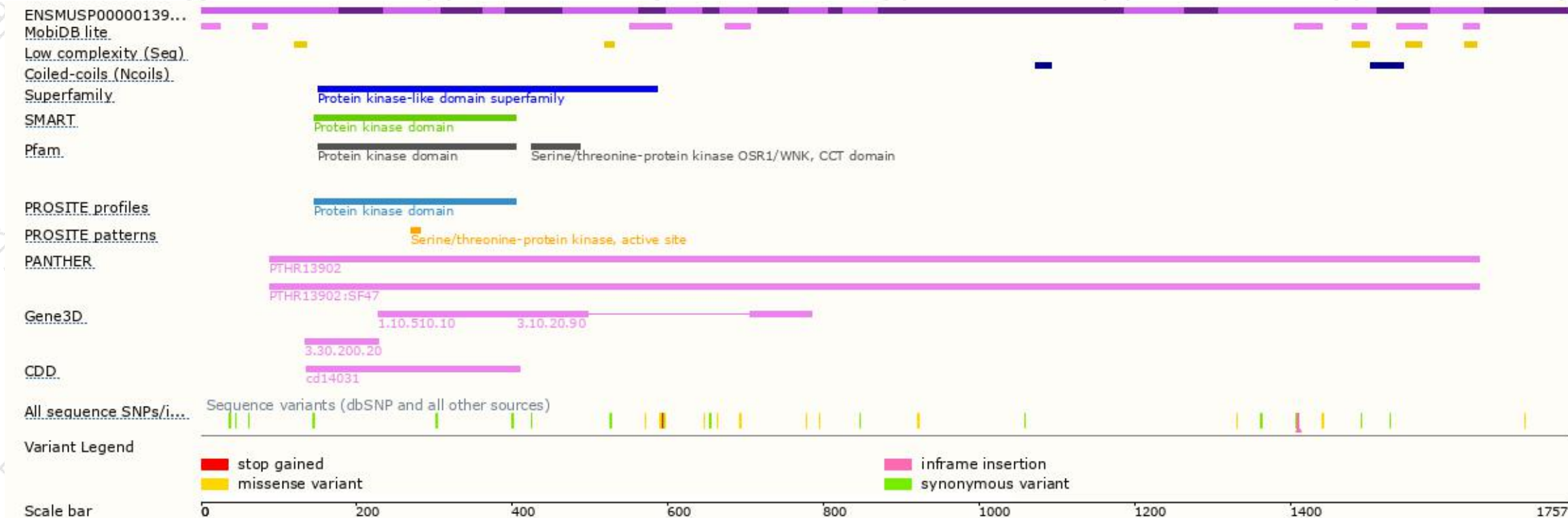
The strategy is based on the design of *Wnk3-205* transcript,The transcription is shown below



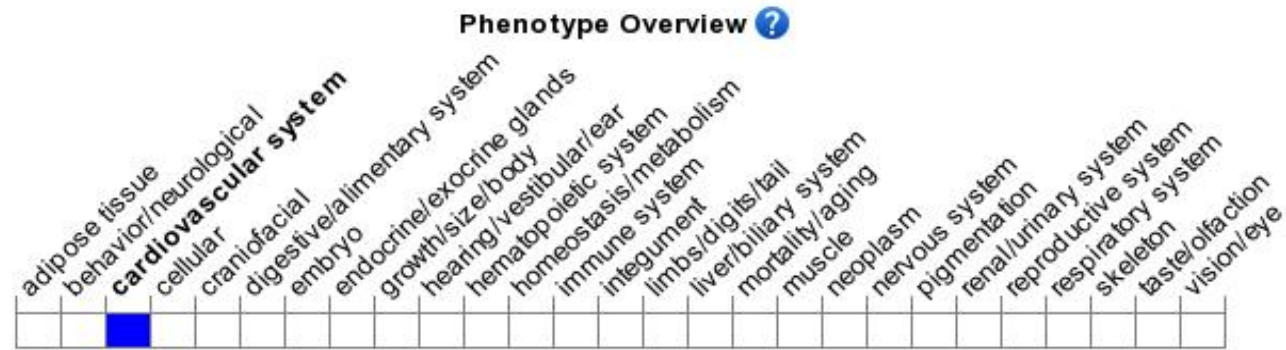
Genomic location distribution



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 knock-out allele exhibit lower blood pressure when fed a low-salt diet.

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

Tel: 400-9660890

