

Itsn2 Cas9-CKO Strategy

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Design Date:

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Project Overview

Project Name

Itsn2

Project type

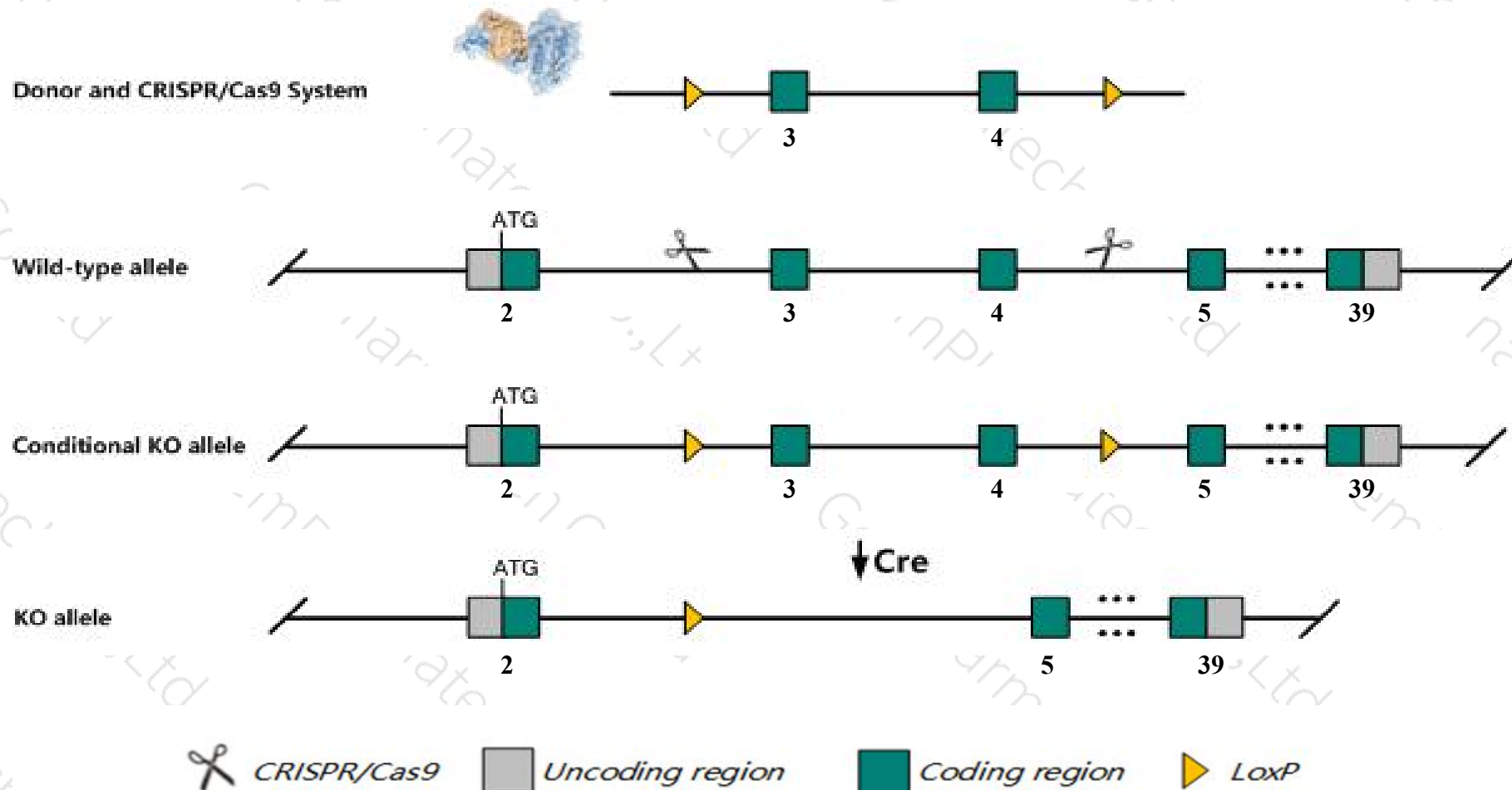
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Itsn2* gene. The schematic diagram is as follows:



- The *Itsn2* gene has 15 transcripts. According to the structure of *Itsn2* gene, exon3-exon4 of *Itsn2-201* (ENSMUST00000062580.7) transcript is recommended as the knockout region. The region contains 157bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Itsn2* 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 normal brain morphology and function and behavior. Mice lacking the long isoform exhibit delayed recovery from LPS-induced kidney injury.
- The knockout region contains part intron1-2 of *Gm48678-201* gene.
- The transcript *Itsn2-202* is incomplete, so the effect on it is unknown.
- The *Itsn2* gene is located on the Chr12. 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)

Itsn2 intersectin 2 [*Mus musculus* (house mouse)]

Gene ID: 20403, updated on 24-Oct-2019

Summary

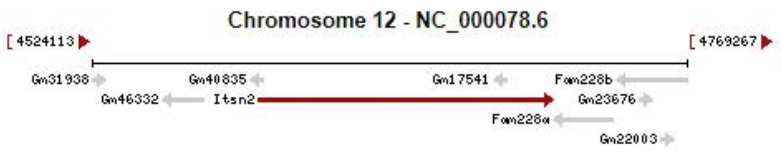
Official Symbol Itsn2 provided by [MGI](#)
Official Full Name intersectin 2 provided by [MGI](#)
Primary source [MGI:MGI:1338049](#)
See related [Ensembl:ENSMUSG00000020640](#)
Gene type protein coding
RefSeq status VALIDATED
Organism [Mus musculus](#)
Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as Ese2; Sh3d1B; Sh3p18; AI327390; mKIAA1256
Expression Ubiquitous expression in thymus adult (RPKM 7.2), spleen adult (RPKM 6.5) and 28 other tissues [See more](#)
Orthologs [human](#) [all](#)

Genomic context

Location: 12; 12 A1.1 [See Itsn2 in Genome Data Viewer](#)

Exon count: 42

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	12	NC_000078.6 (4592643..4713952)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	12	NC_000078.5 (4599814..4720758)

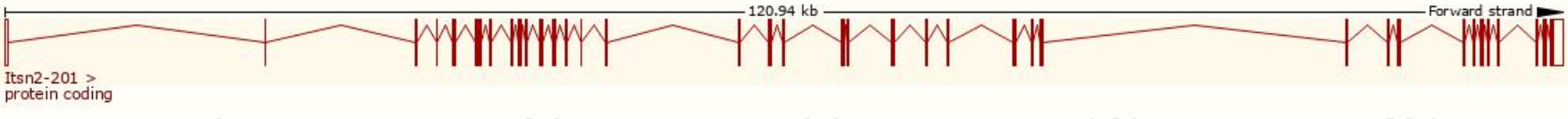


Transcript information (Ensembl)

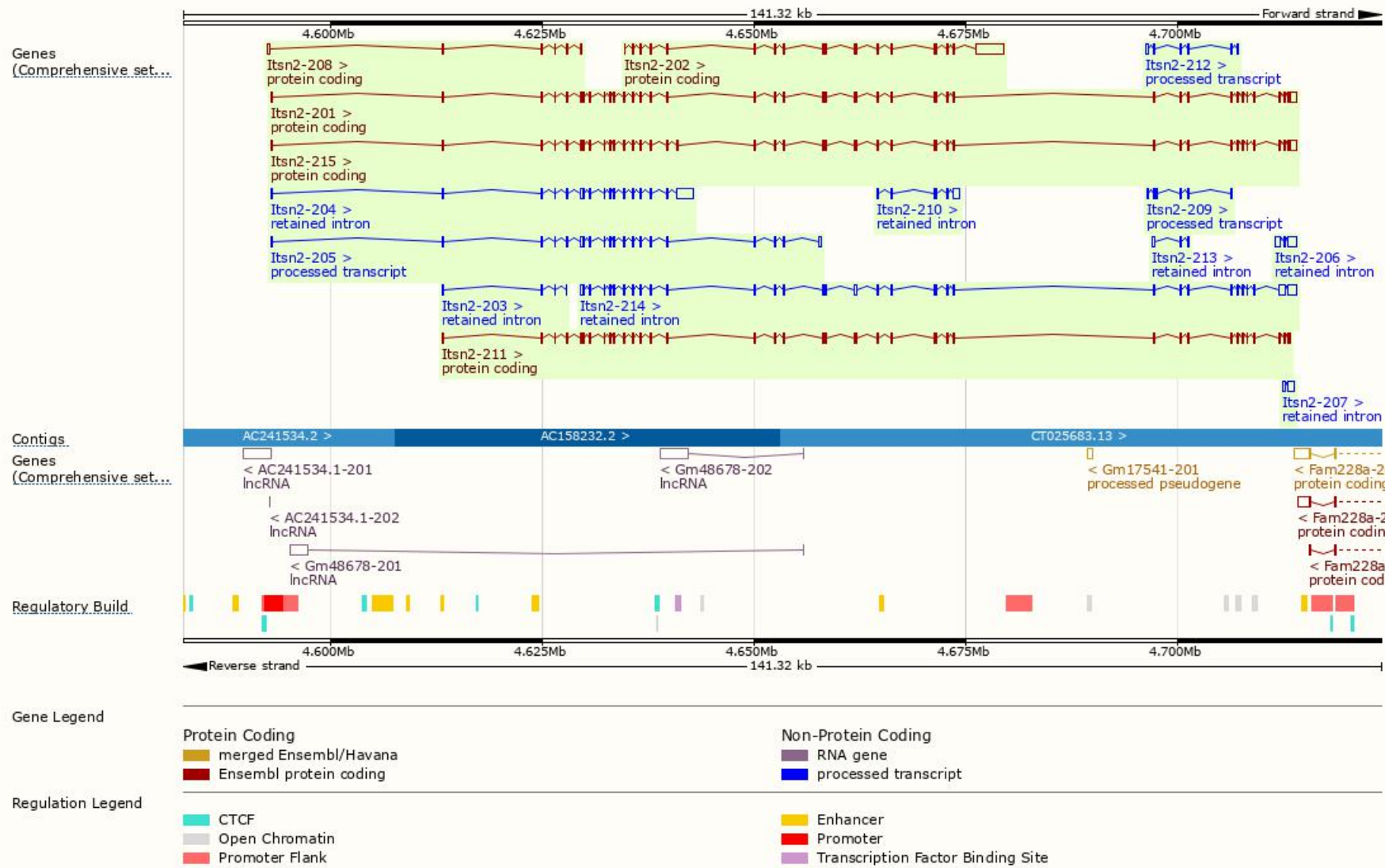
The gene has 15 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Itsn2-201	ENSMUST00000062580.7	5987	1658aa	Protein coding	CCDS36398	E9QNG1	TSL:5 GENCODE basic APPRIS P2
Itsn2-211	ENSMUST00000219007.1	4977	1658aa	Protein coding	CCDS36398	E9QNG1	TSL:1 GENCODE basic APPRIS P2
Itsn2-215	ENSMUST000002220311.1	6080	1685aa	Protein coding	-	B2RR82	TSL:1 GENCODE basic APPRIS ALT2
Itsn2-202	ENSMUST00000217672.1	5431	712aa	Protein coding	-	A0A1W2P775	CDS 5' incomplete TSL:1
Itsn2-208	ENSMUST00000218402.1	851	175aa	Protein coding	-	A0A1W2P7G8	CDS 3' incomplete TSL:2
Itsn2-205	ENSMUST00000218072.1	2640	No protein	Processed transcript	-	-	TSL:1
Itsn2-212	ENSMUST00000219182.1	832	No protein	Processed transcript	-	-	TSL:3
Itsn2-209	ENSMUST00000218923.1	751	No protein	Processed transcript	-	-	TSL:3
Itsn2-214	ENSMUST00000219832.1	5804	No protein	Retained intron	-	-	TSL:1
Itsn2-204	ENSMUST00000217981.1	3968	No protein	Retained intron	-	-	TSL:1
Itsn2-206	ENSMUST00000218084.1	1606	No protein	Retained intron	-	-	TSL:1
Itsn2-210	ENSMUST00000218985.1	1272	No protein	Retained intron	-	-	TSL:2
Itsn2-207	ENSMUST00000218211.1	942	No protein	Retained intron	-	-	TSL:1
Itsn2-213	ENSMUST00000219308.1	616	No protein	Retained intron	-	-	TSL:3
Itsn2-203	ENSMUST00000217942.1	319	No protein	Retained intron	-	-	TSL:3

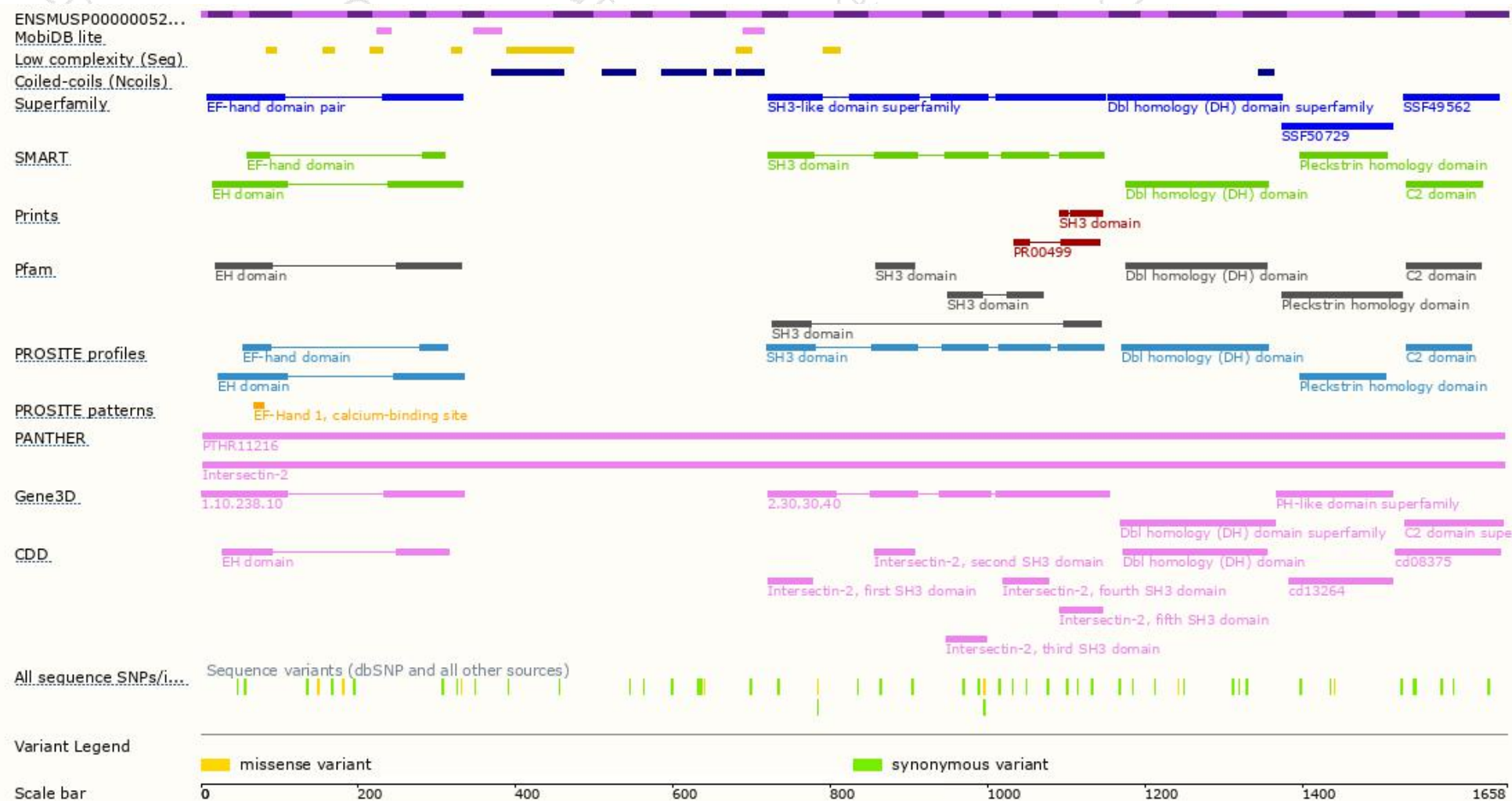
The strategy is based on the design of *Itsn2-201* transcript, the transcription is shown below:



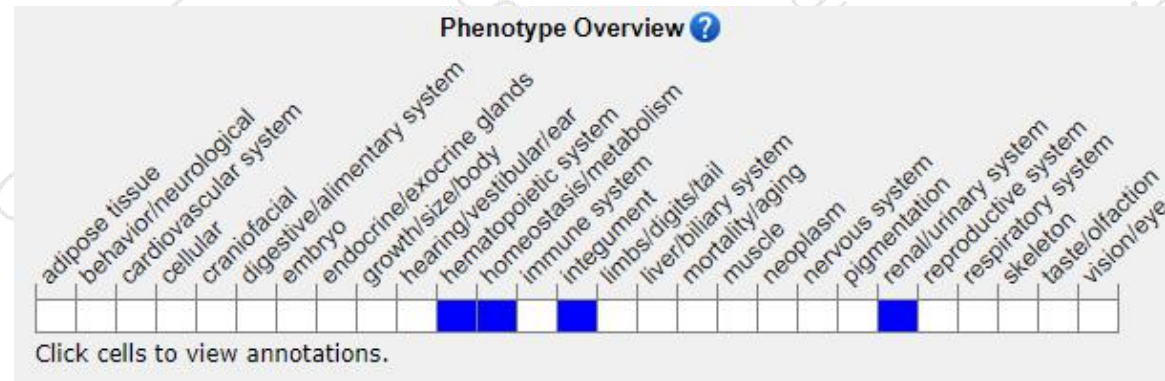
Genomic location distribution



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 knock-out allele exhibit normal brain morphology and function and behavior. Mice lacking the long isoform exhibit delayed recovery from LPS-induced kidney injury.

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

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