

# Tjp2 Cas9-CKO Strategy

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Reviewer: Miaomiao Cui

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## **Project Overview**



Project Name Tjp2

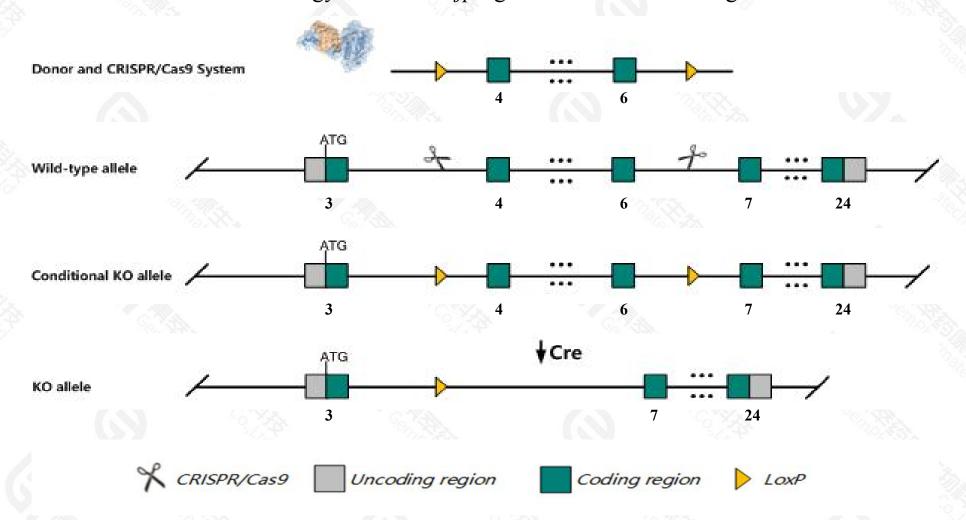
Project type Cas9-CKO

Strain background C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Tjp2* gene. The schematic diagram is as follows:



### **Technical routes**



- ➤ The *Tjp2* gene has 7 transcripts. According to the structure of *Tjp2* gene, exon4-exon6 of *Tjp2*204(ENSMUST00000233658.3) transcript is recommended as the knockout region. The region contains 847bp coding sequence.

  Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Tjp2* 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, homozygous mutation of this gene results in lethality shortly after implantation due to arrest in early gastrulation. Structure and permeability barrier of the apical junctional complex are altered in cells.
- > The *Tjp2* gene is located on the Chr19. 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)



#### Tjp2 tight junction protein 2 [Mus musculus (house mouse)]

Gene ID: 21873, updated on 13-Mar-2020

#### Summary

☆ ?

Official Symbol Tjp2 provided by MGI

Official Full Name tight junction protein 2 provided by MGI

Primary source MGI:MGI:1341872

See related Ensembl: ENSMUSG00000024812

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 ZO-2

Expression Ubiquitous expression in lung adult (RPKM 24.4), small intestine adult (RPKM 19.3) and 27 other tissuesSee more

Orthologs <u>human all</u>

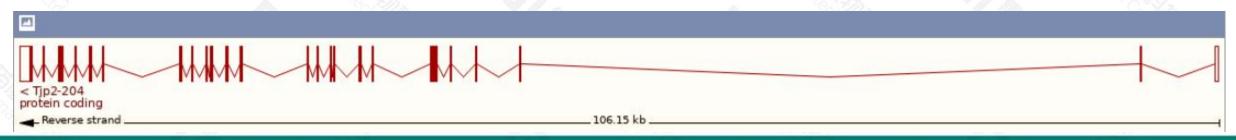
## Transcript information (Ensembl)



#### The gene has 7 transcripts, all transcripts are shown below:

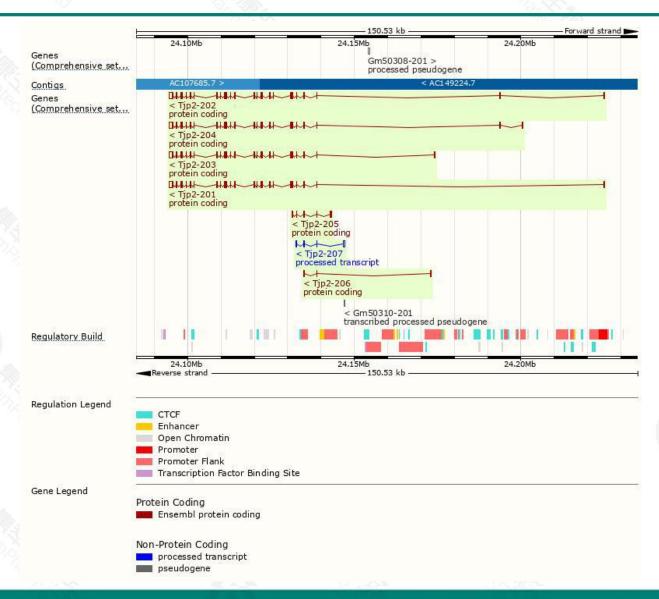
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tjp2-204	ENSMUST00000233658.2	4920	<u>1167aa</u>	Protein coding	CCDS37939	A0A3B2W7V8 Q9Z0U1	GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P2
Tjp2-201	ENSMUST00000099558.4	4591	<u>1167aa</u>	Protein coding	CCDS37939	<u>Q9Z0U1</u>	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P2
Tjp2-202	ENSMUST00000232956.1	4728	1165aa	Protein coding	-	A0A3B2WBH9	GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Tjp2-203	ENSMUST00000233027.1	4668	<u>1190aa</u>	Protein coding	2	A0A3B2WCN9	GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Tjp2-205	ENSMUST00000236570.1	741	<u>171aa</u>	Protein coding	-	A0A494B9E6	CDS 3' incomplete
Tjp2-206	ENSMUST00000237550.1	400	<u>53aa</u>	Protein coding	-	A0A494BAA0	CDS 3' incomplete
Tjp2-207	ENSMUST00000237937.1	615	No protein	Processed transcript	-	-	

The strategy is based on the design of *Tjp2-204* transcript, the transcription is shown below:



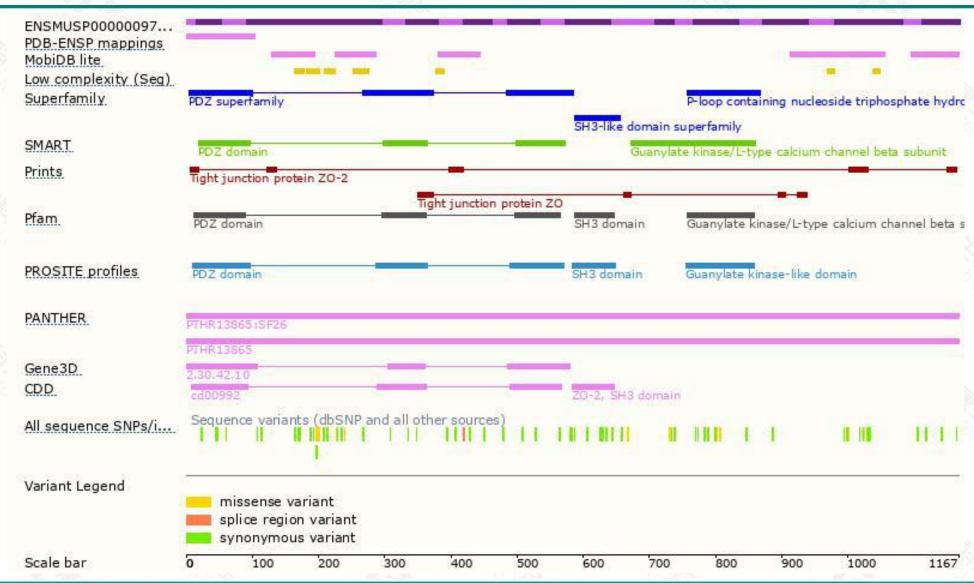
### Genomic location distribution





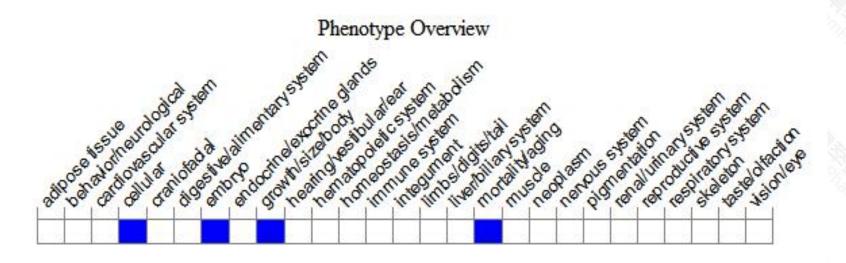
### Protein domain





## 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, homozygous mutation of this gene results in lethality shortly after implantation due to arrest in early gastrulation. Structure and permeability barrier of the apical junctional complex are altered in cells.



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

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