

# Coro2b Cas9-CKO Strategy

Designer: Miaomiao Cui

Reviewer: Lingyan Wu

**Design Date: 2021-7-9** 

# **Project Overview**

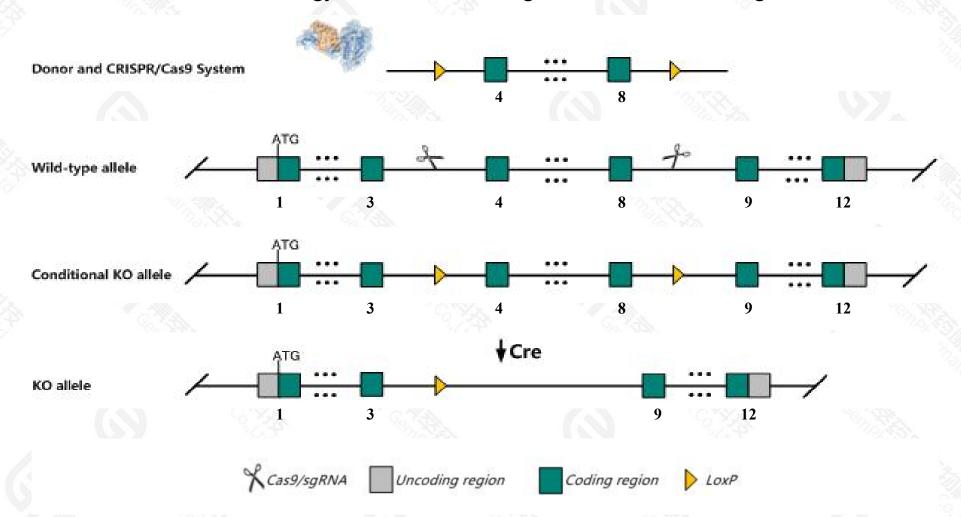


Project Name	Coro2b			
Project type	Cas9-CKO			
Strain background	C57BL/6JGpt			

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Coro2b* gene. The schematic diagram is as follows:



### **Technical routes**



- ➤ The *Coro2b* gene has 7 transcripts. According to the structure of *Coro2b* gene, exon4-exon8 of *Coro2b*-201(ENSMUST00000048043.12) transcript is recommended as the knockout region. The region contains 634bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Coro2b* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor was constructed.Cas9, sgRNA 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 was 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 knockout reduces susceptibility to Doxorubicin-induced focal segmental glomerulosclerosis with lower levels of proteinuria and less renal glomerulus damage.
- > The *Coro2b* 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)



#### Coro2b coronin, actin binding protein, 2B [Mus musculus (house mouse)]

Gene ID: 235431, updated on 17-Dec-2020

#### Summary

☆ ?

Official Symbol Coro2b provided by MGI

Official Full Name coronin, actin binding protein, 2B provided by MGI

Primary source MGI:MGI:2444283

See related Ensembl:ENSMUSG00000041729

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 CLIPINC, E130012P22Rik

Expression Broad expression in cerebellum adult (RPKM 45.9), cortex adult (RPKM 26.6) and 16 other tissuesSee more

Orthologs <u>human all</u>

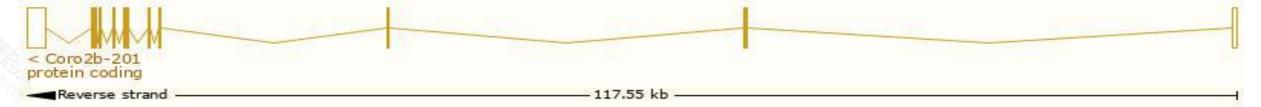
## Transcript information (Ensembl)



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

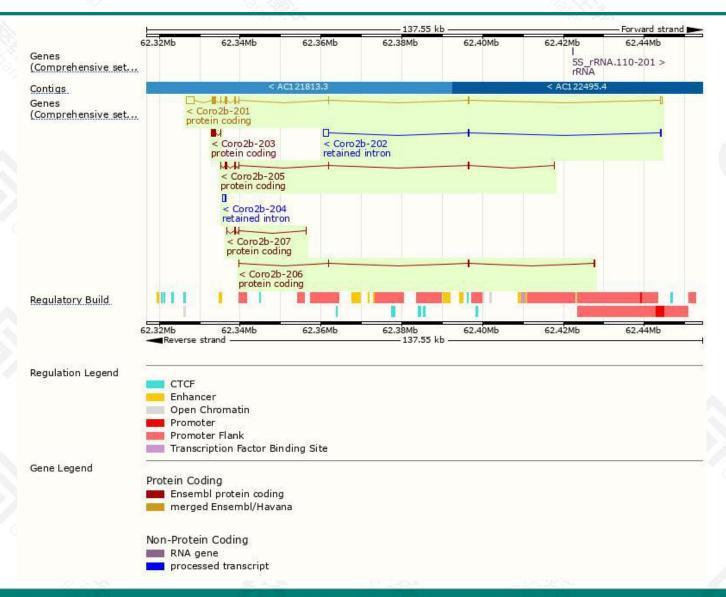
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Coro2b-201	ENSMUST00000048043.12	3610	480aa	Protein coding	CCDS23264		TSL:1 , GENCODE basic , APPRIS P1 ,
Coro2b-205	ENSMUST00000164246.9	987	<u>317aa</u>	Protein coding	9		CDS 3' incomplete , TSL:5 ,
Coro2b-203	ENSMUST00000131981.2	618	<u>181aa</u>	Protein coding	Đ.		CDS 5' incomplete , TSL:3 ,
Coro2b-206	ENSMUST00000173171.3	396	<u>121aa</u>	Protein coding	.T.		CDS 3' incomplete , TSL:5 ,
Coro2b-207	ENSMUST00000174439.2	393	<u>94aa</u>	Protein coding	22		CDS 3' incomplete , TSL:5 ,
Coro2b-202	ENSMUST00000123379.2	1872	No protein	Retained intron	aī.		TSL:1,
Coro2b-204	ENSMUST00000151604.2	816	No protein	Retained intron	-		TSL:3,

The strategy is based on the design of *Coro2b-201* transcript, the transcription is shown below:



### Genomic location distribution





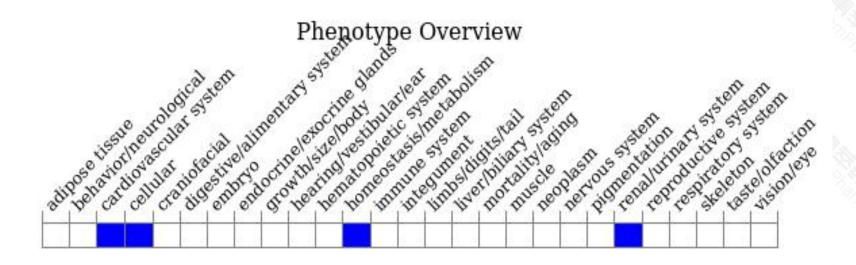
### Protein domain





## 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, homozygous knockout reduces susceptibility to Doxorubicin-induced focal segmental glomerulosclerosis with lower levels of proteinuria and less renal glomerulus damage.



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

Tel: 025-5864 1534





