

# Cdca2 Cas9-CKO Strategy

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Reviewer: Daohua Xu

**Design Date: 2020-8-13** 

# **Project Overview**



Project Name Cdca2

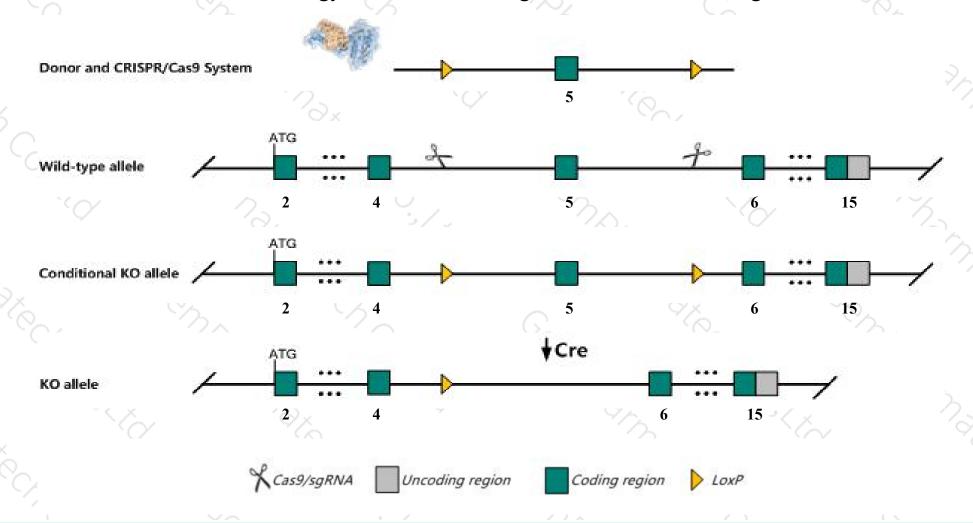
Project type Cas9-CKO

Strain background C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Cdca2 gene. The schematic diagram is as follows:



### Technical routes



- ➤ The *Cdca2* gene has 7 transcripts. According to the structure of *Cdca2* gene, exon5 of *Cdca2*207(ENSMUST00000163100.7) transcript is recommended as the knockout region. The region contains 148bp coding sequence.

  Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Cdca2* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector 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**



- > The *Cdca2* gene is located on the Chr14. 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.
- ➤ Transcript *Cdca2*-202 may not be affected.
- ➤ The effect on transcript *Cdca2*-203 is unknown.
- 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)



#### Cdca2 cell division cycle associated 2 [Mus musculus (house mouse)]

Gene ID: 108912, updated on 13-Mar-2020

#### Summary

☆ ?

Official Symbol Cdca2 provided by MGI

Official Full Name cell division cycle associated 2 provided by MGI

Primary source MGI:MGI:1919787

See related Ensembl: ENSMUSG00000048922

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 2610311M19Rik, Al586158

Expression Biased expression in testis adult (RPKM 12.3), CNS E11.5 (RPKM 7.2) and 12 other tissuesSee more

Orthologs <u>human all</u>

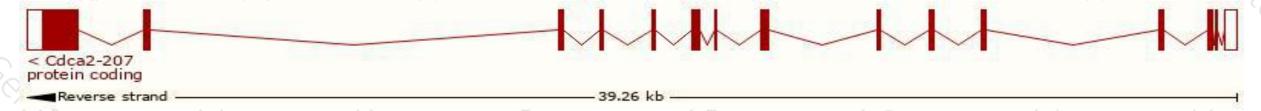
# Transcript information (Ensembl)



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

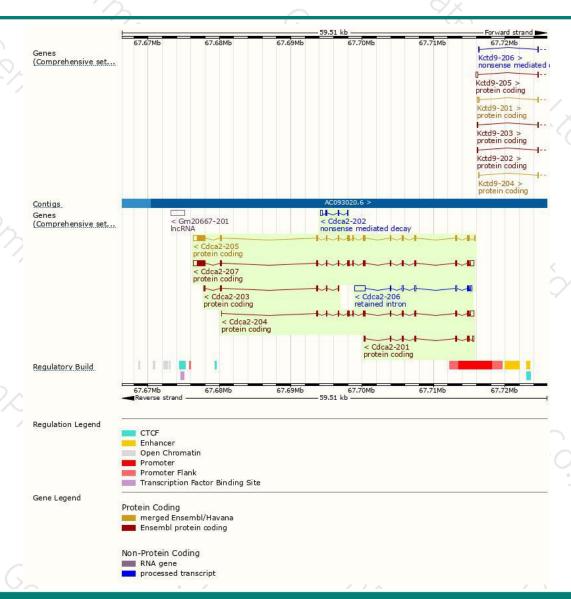
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cdca2-207	ENSMUST00000163100.7	3841	982aa	Protein coding	CCD536962	Q14B71	TSL:5 GENCODE basic APPRIS P
Cdca2-205	ENSMUST00000150006.8	3568	982aa	Protein coding	CCDS36962	Q14B71	TSL:1 GENCODE basic APPRIS P.
Cdca2-204	ENSMUST00000132705.7	2034	549aa	Protein coding	-	D3YZE8	CDS 3' incomplete TSL:5
Cdca2-201	ENSMUST00000124045.2	1124	310aa	Protein coding	-	D3Z5P7	CDS 3' incomplete TSL:3
Cdca2-203	ENSMUST00000131179.7	676	<u>226aa</u>	Protein coding	-	<u>F6VP65</u>	CDS 5' and 3' incomplete TSL:3
Cdca2-202	ENSMUST00000130922.1	647	<u>87aa</u>	Nonsense mediated decay		F6RTJ7	CDS 5' incomplete TSL:5
Cdca2-206	ENSMUST00000155312.1	2419	No protein	Retained intron	-	-:	TSL:2

The strategy is based on the design of *Cdca2-207* transcript, the transcription is shown below:



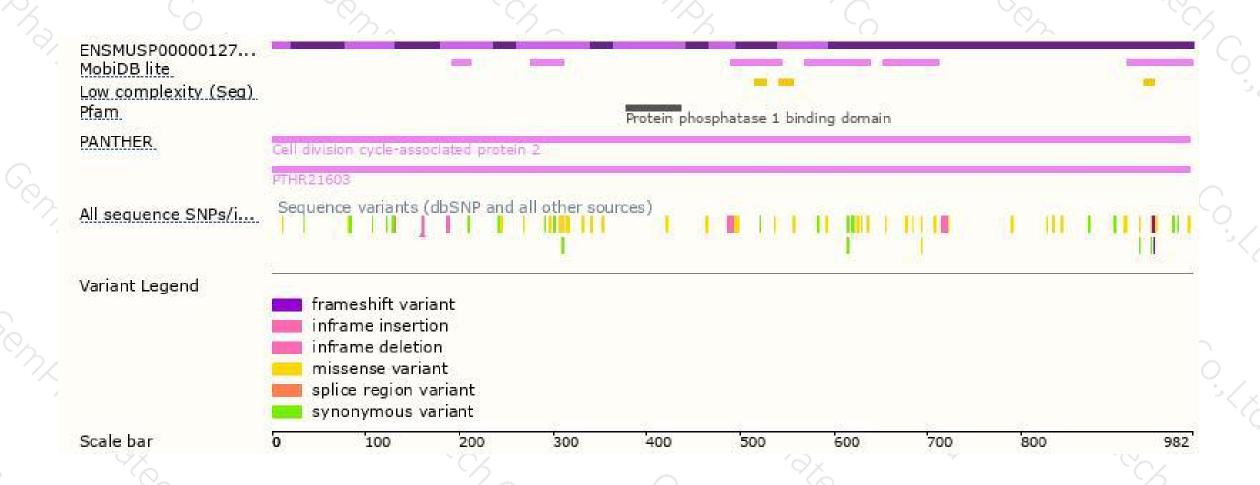
### Genomic location distribution





### Protein domain







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

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