

Prkca Cas9-CKO Strategy

Designer:

Huimin Su

Reviewer:

Ruirui Zhang

Design Date:

2019/8/29

Project Overview

Project Name

Prkca

Project type

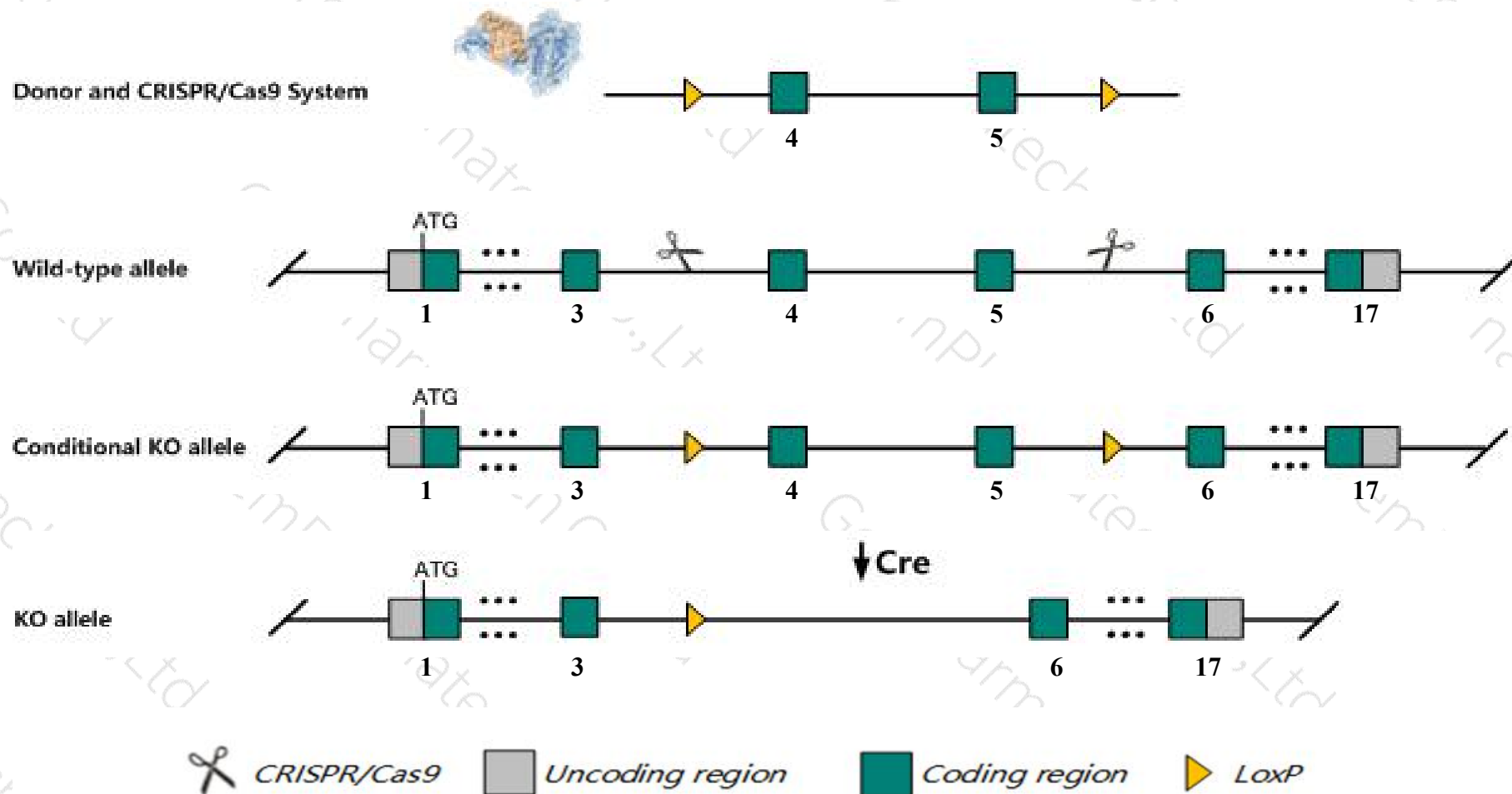
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- The *Prkca* gene has 4 transcripts. According to the structure of *Prkca* gene, exon4-exon5 of *Prkca-201* (ENSMUST00000059595.10) transcript is recommended as the knockout region. The region contains 241bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Prkca* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA 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, Homozygous null mice show no overt macroscopic abnormalities, however examination of one line revealed increased cardiac muscle contractility and protection against heart failure.
- The *Prkca* gene is located on the Chr11. 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)

Prkca protein kinase C, alpha [*Mus musculus* (house mouse)]

Gene ID: 18750, updated on 12-Aug-2019

Summary

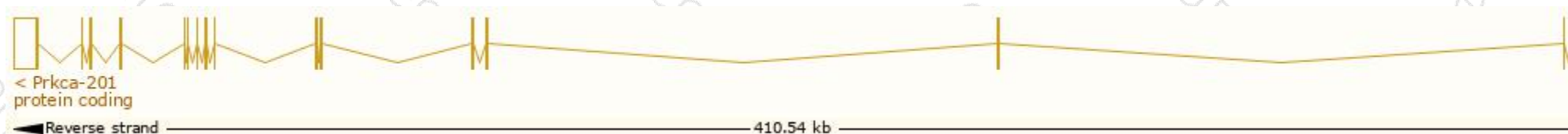
Official Symbol	Prkca provided by MGI
Official Full Name	protein kinase C, alpha provided by MGI
Primary source	MGI:MGI:97595
See related	Ensembl:ENSMUSG00000050965
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Pkca; A1875142
Expression	Broad expression in frontal lobe adult (RPKM 19.9), cortex adult (RPKM 11.1) and 22 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

The gene has 4 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Prkca-201	ENSMUST00000059595.10	8409	672aa	Protein coding	CCDS25573	Q4VA93	TSL:1 GENCODE basic APPRIS P1
Prkca-202	ENSMUST00000100302.3	2265	248aa	Protein coding	-	Q3TQ39	TSL:1 GENCODE basic
Prkca-204	ENSMUST00000134910.1	678	No protein	lncRNA	-	-	TSL:5
Prkca-203	ENSMUST00000134725.1	541	No protein	lncRNA	-	-	TSL:2

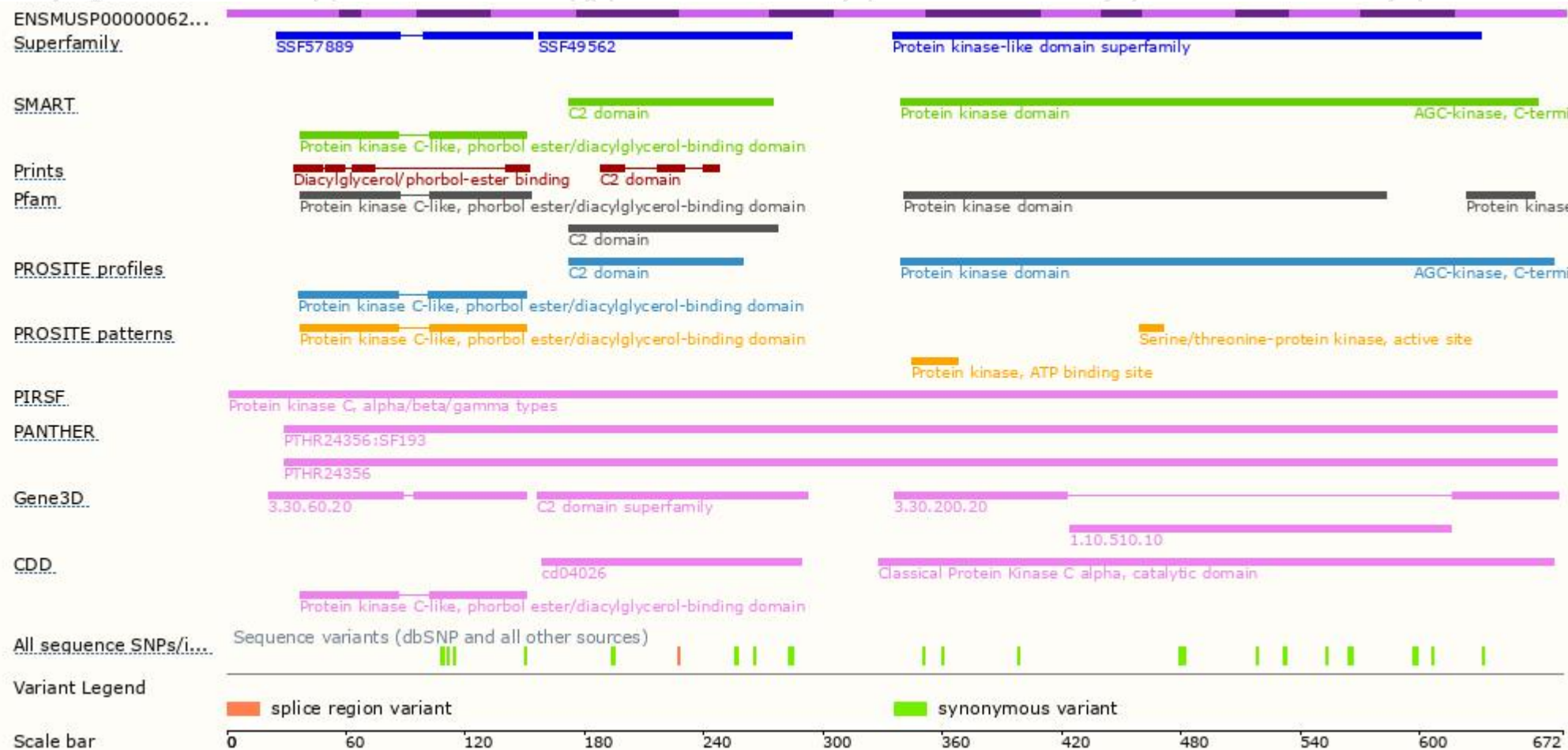
The strategy is based on the design of *Prkca-201* transcript, The transcription is shown below



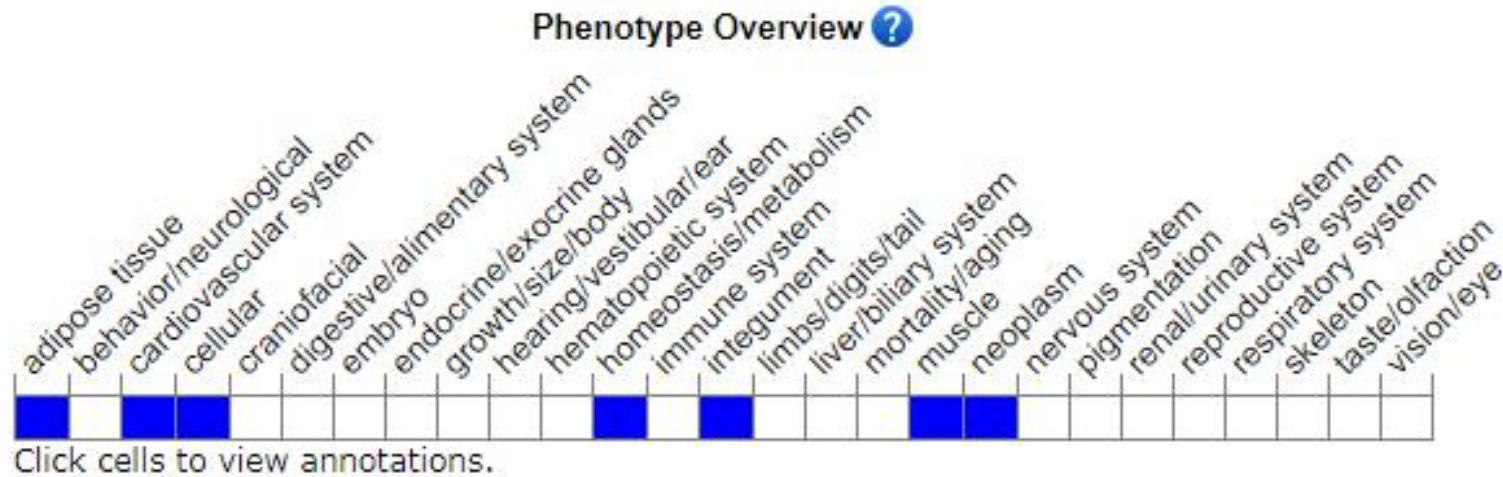
Genomic location distribution



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 null mice show no overt macroscopic abnormalities, however examination of one line revealed increased cardiac muscle contractility and protection against heart failure.

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

Tel: 400-9660890

