

# 70/2/70 Co. 1/2/ Pkn1 Cas9-KO Strategy Rohalmakech College

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# **Project Overview**



**Project Name** 

Pkn1

**Project type** 

Cas9-KO

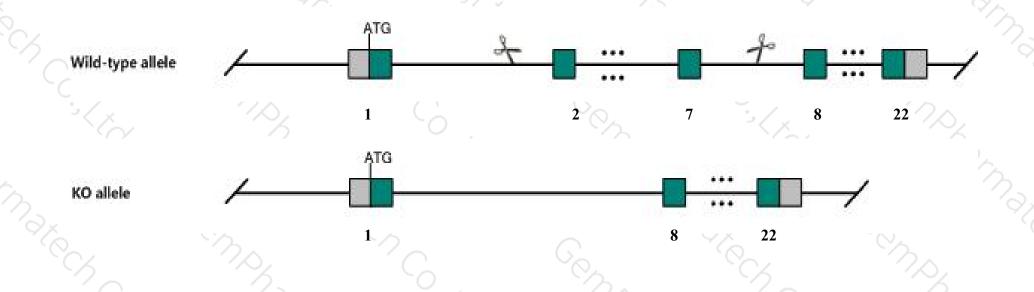
Strain background

C57BL/6JGpt

## **Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the *Pkn1* gene. The schematic diagram is as follows:



CRISPR/Cas9 Uncoding region Coding region

## **Technical routes**



- ➤ The *Pkn1* gene has 9 transcripts. According to the structure of *Pkn1* gene, exon2-exon7 of *Pkn1-208* (
  ENSMUST00000144258.7) transcript is recommended as the knockout region. The region contains 1153bp coding sequence.

  Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Pkn1* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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.

### **Notice**



- > According to the existing MGI data, Homozygotes for a null allele show spontaneous germinal center formation and autoantibody production and develop glomerulonephritis. Homozygotes for a different null allele have mild systolic and diastolic dysfunction, and show increased myocardial infarction size after ischemia-reperfusion injury.
- The *Pkn1* gene is located on the Chr8. 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 gene transcription and translation processes, all risks cannot be predicted under existing information.

## Gene information (NCBI)



#### Pkn1 protein kinase N1 [Mus musculus (house mouse)]

Gene ID: 320795, updated on 2-Mar-2019

#### Summary

↑ ?

Official Symbol Pkn1 provided by MGI

Official Full Name protein kinase N1 provided by MGI

Primary source MGI:MGI:108022

See related Ensembl:ENSMUSG00000057672

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 DBK, F730027O18Rik, PAK1, PRK1, Pkn, Prkcl1, Stk3

Expression Ubiquitous expression in thymus adult (RPKM 55.4), spleen adult (RPKM 49.1) and 28 other tissuesSee more

Orthologs <u>human</u> all

# Transcript information (Ensembl)



The gene has 9 transcript, all transcripts are shown below:

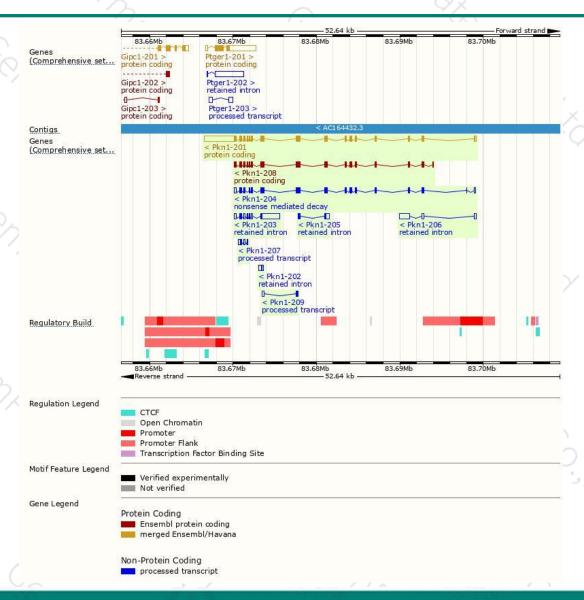
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pkn1-201	ENSMUST00000005616.15	6662	946aa	Protein coding	CCDS22459	P70268	TSL:1 GENCODE basic APPRIS P3
Pkn1-208	ENSMUST00000144258.7	2995	951aa	Protein coding	CCDS57627	P70268	TSL:1 GENCODE basic APPRIS ALT1
Pkn1-204	ENSMUST00000132945.7	3005	796aa	Nonsense mediated decay	825	D6RH37	TSL:1
Pkn1-207	ENSMUST00000138898.1	548	No protein	Processed transcript	5525	20	TSL:2
Pkn1-209	ENSMUST00000146057.1	450	No protein	Processed transcript	127	-	TSL:2
Pkn1-203	ENSMUST00000128523.7	3070	No protein	Retained intron	3.43	l .	TSL:1
Pkn1-206	ENSMUST00000135356.1	1783	No protein	Retained intron	828		TSL:1
Pkn1-205	ENSMUST00000133195.1	694	No protein	Retained intron	5528	95	TSL:3
Pkn1-202	ENSMUST00000124946.1	483	No protein	Retained intron	187	-	TSL:3
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The strategy is based on the design of *Pkn1-208* transcript, The transcription is shown below



## Genomic location distribution





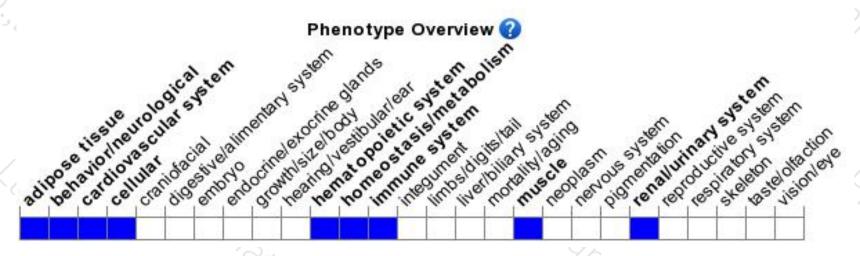
## Protein domain





## 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, Homozygotes for a null allele show spontaneous germinal center formation and autoantibody production and develop glomerulonephritis. Homozygotes for a different null allele have mild systolic and diastolic dysfunction, and show increased myocardial infarction size after ischemia-reperfusion injury.



If you have any questions, you are welcome to inquire. Tel: 400-9660890





