

Mkrn2 Cas9-CKO Strategy

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Project Overview

Project Name

Mkrrn2

Project type

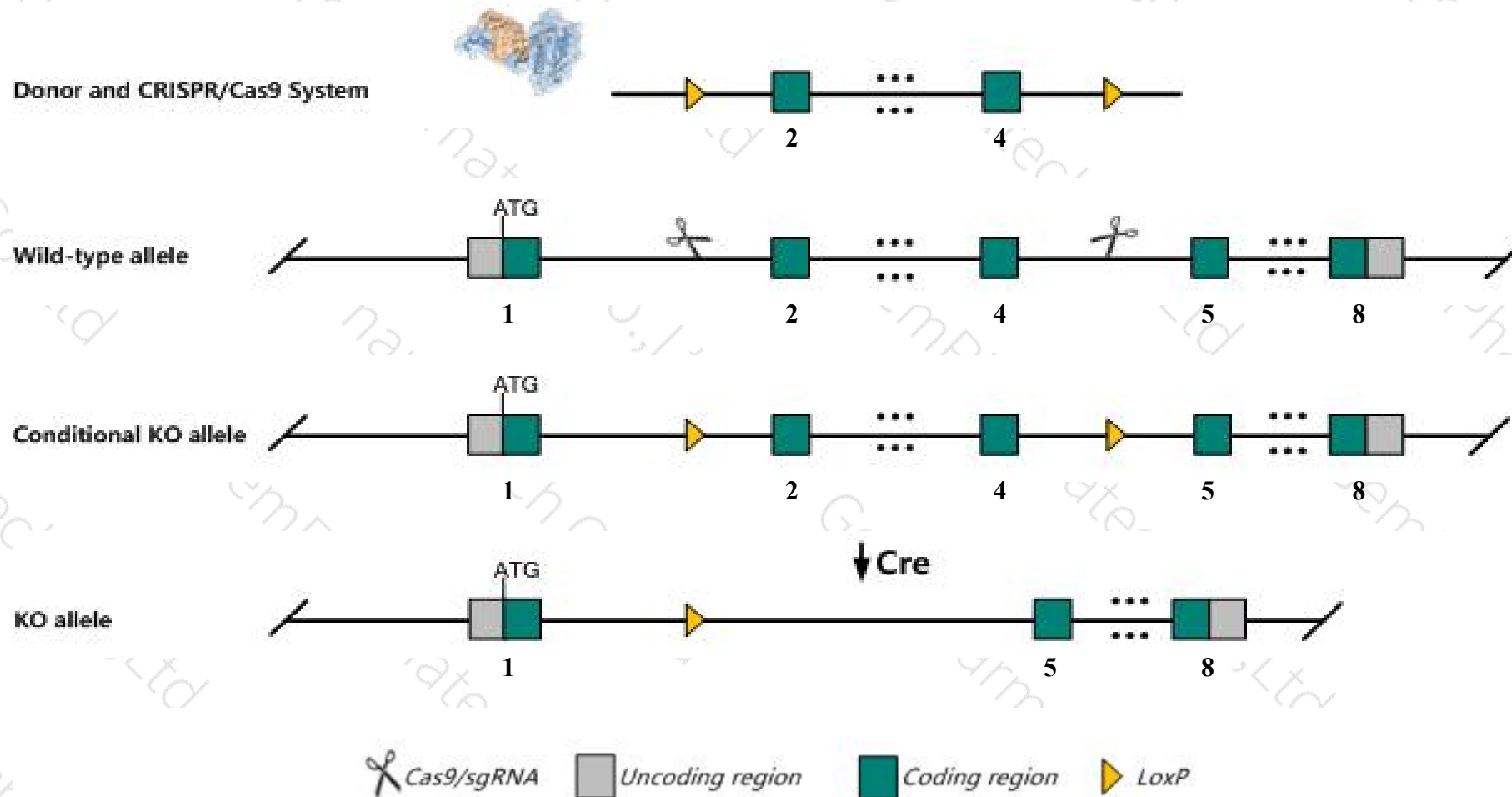
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mktn2* gene. The schematic diagram is as follows:



Technical routes

- The *Mkrn2* gene has 3 transcripts. According to the structure of *Mkrn2* gene, exon2-exon4 of *Mkrn2*-201(ENSMUST00000000449.8) transcript is recommended as the knockout region. The region contains 616bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Mkrn2* 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.

- According to the existing MGI data, homozygous male mice are infertile, with abnormal sperm characterized by low number, poor motility, and aberrant morphology.
- The *Mkrn2* gene is located on the Chr6. 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)

Mkrn2 makorin, ring finger protein, 2 [Mus musculus (house mouse)]

Gene ID: 67027, updated on 13-Mar-2020

Summary



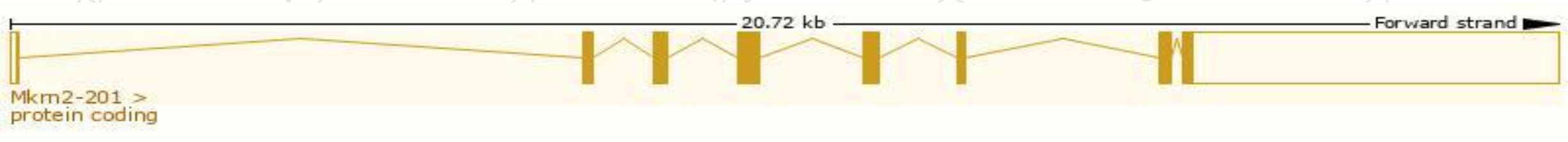
Official Symbol	Mkrn2 provided by MGI
Official Full Name	makorin, ring finger protein, 2 provided by MGI
Primary source	MGI:MGI:1914277
See related	Ensembl:ENSMUSG00000000439
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2610002L04Rik, C81377
Expression	Ubiquitous expression in testis adult (RPKM 33.7), adrenal adult (RPKM 19.9) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

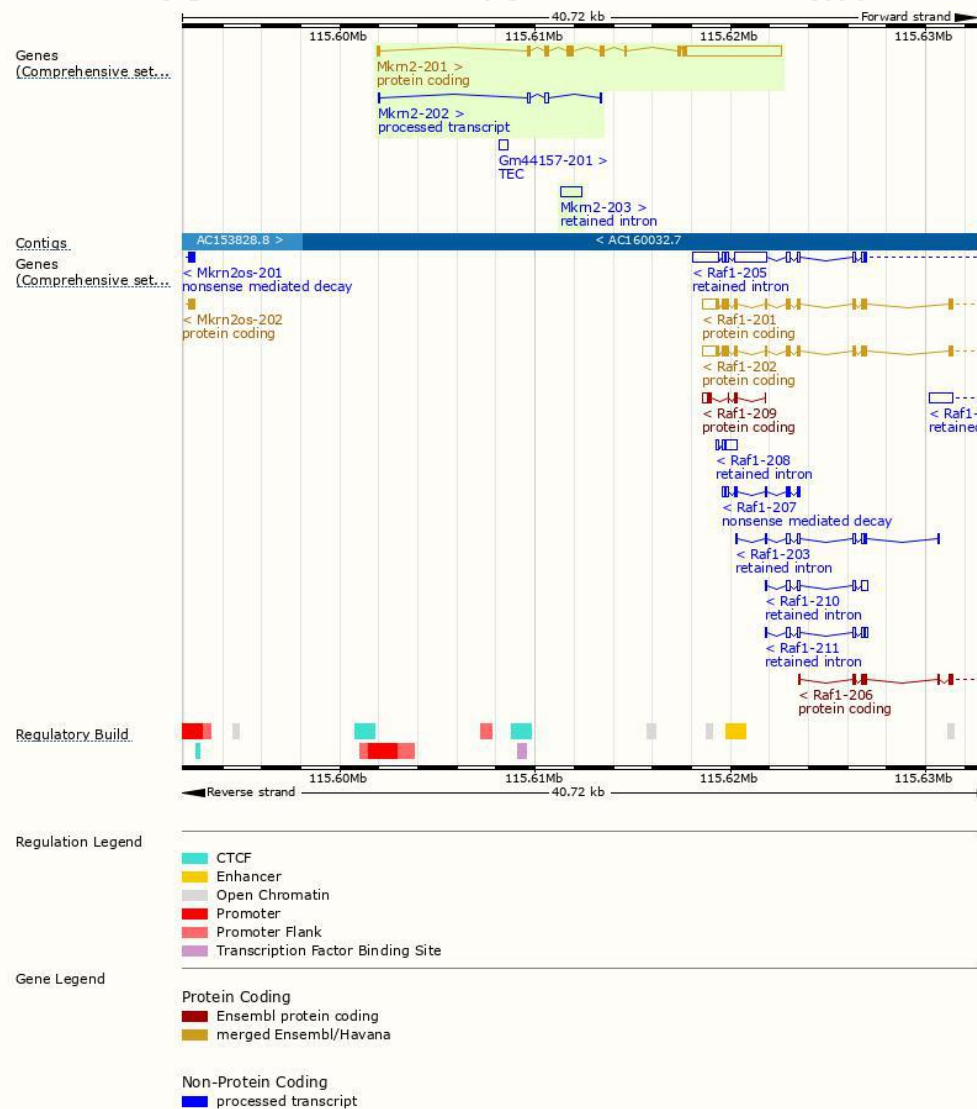
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mkrn2-201	ENSMUST00000000449.8	6230	416aa	Protein coding	CCDS39600	Q9ERV1	TSL:1 GENCODE basic APPRIS P1
Mkrn2-202	ENSMUST00000204409.1	452	No protein	Processed transcript	-	-	TSL:3
Mkrn2-203	ENSMUST00000205248.1	1080	No protein	Retained intron	-	-	TSL:NA

The strategy is based on the design of *Mkrn2-201* transcript,the transcription is shown below:



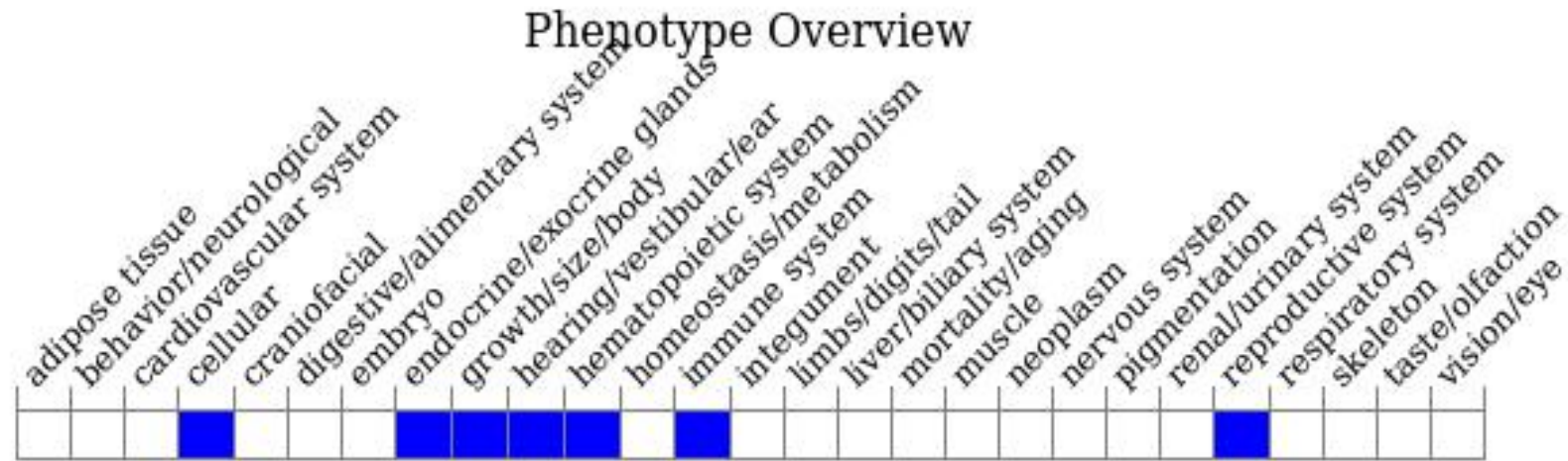
Genomic location distribution



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 male mice are infertile, with abnormal sperm characterized by low number, poor motility, and aberrant morphology.

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

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