

Rock2 Cas9-CKO Strategy

Designer:

Huan Fan

Design Date:

2019-7-25

Project Overview

Project Name

Rock2

Project type

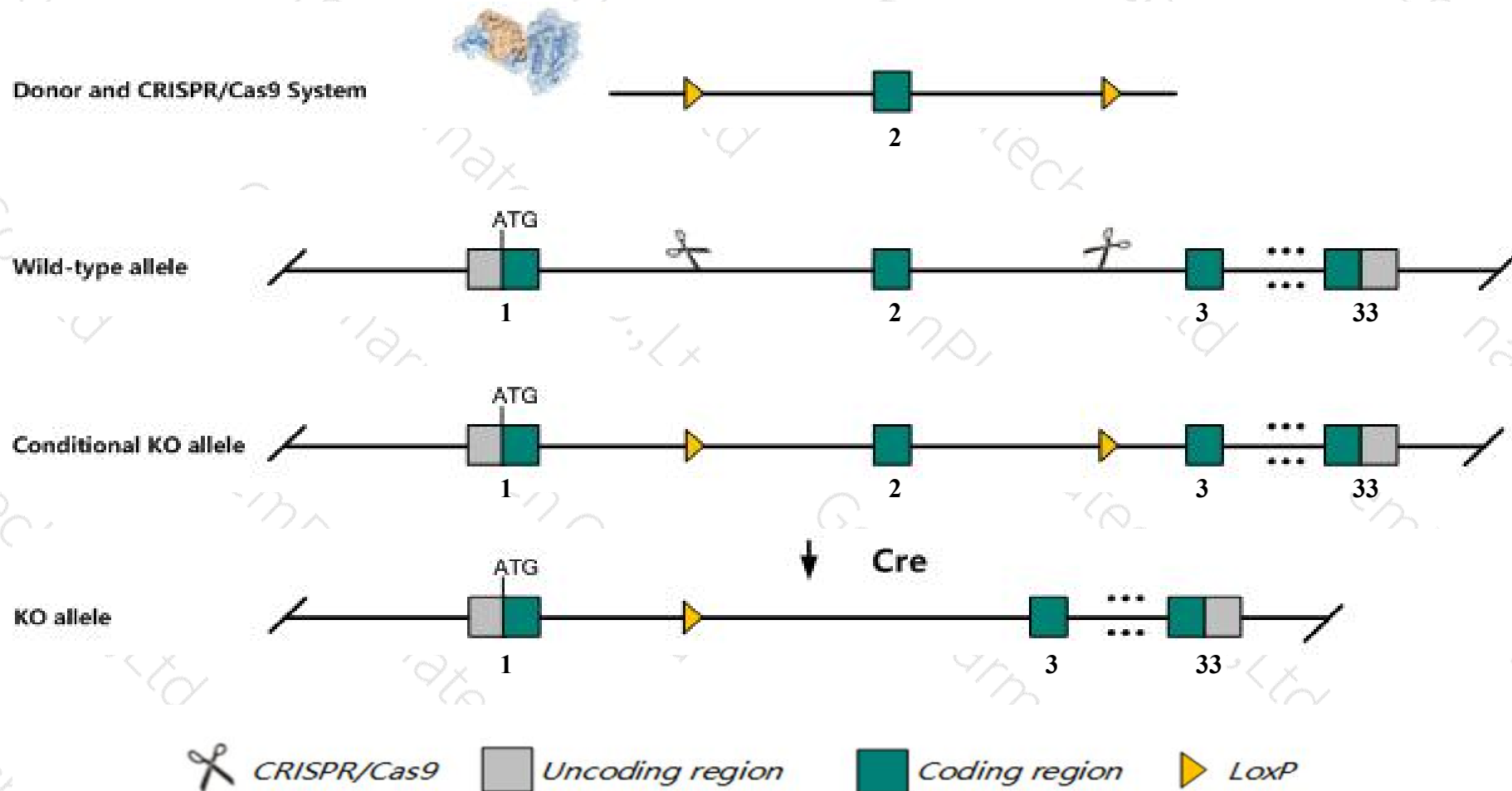
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rock2* gene. The schematic diagram is as follows:



- The *Rock2* gene has 4 transcripts. According to the structure of *Rock2* gene, exon2 of *Rock2-201* (ENSMUST00000020904.7) transcript is recommended as the knockout region. The region contains 82bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rock2* gene. The brief process is as follows: CRISPR/Cas9 system 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, Mice homozygous for disruptions in this genes tend to die before birth; those that survive are small. Hemorrhaging occurs in the placenta, at the tips of hind limb buds and occasionally the tail. Subsequent development is normal and the size deficit is made up. They are fertile as adults.
- Transcript *Rock2-204* may not be affected.
- The *Rock2* gene is located on the Chr12. 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)

Rock2 Rho-associated coiled-coil containing protein kinase 2 [Mus musculus (house mouse)]

Gene ID: 19878, updated on 7-Apr-2019

Summary



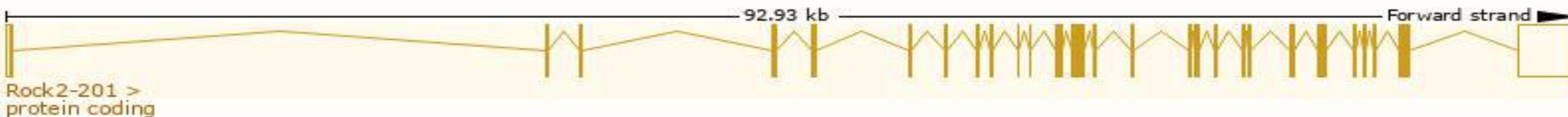
Official Symbol	Rock2 provided by MGI
Official Full Name	Rho-associated coiled-coil containing protein kinase 2 provided by MGI
Primary source	MGI:MGI:107926
See related	Ensembl:ENSMUSG00000020580
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	B230113H15Rik, ROKalpha, Rho-kinase, Rock-II, Rock2m, mKIAA0619
Expression	Ubiquitous expression in bladder adult (RPKM 16.5), cortex adult (RPKM 10.8) and 25 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

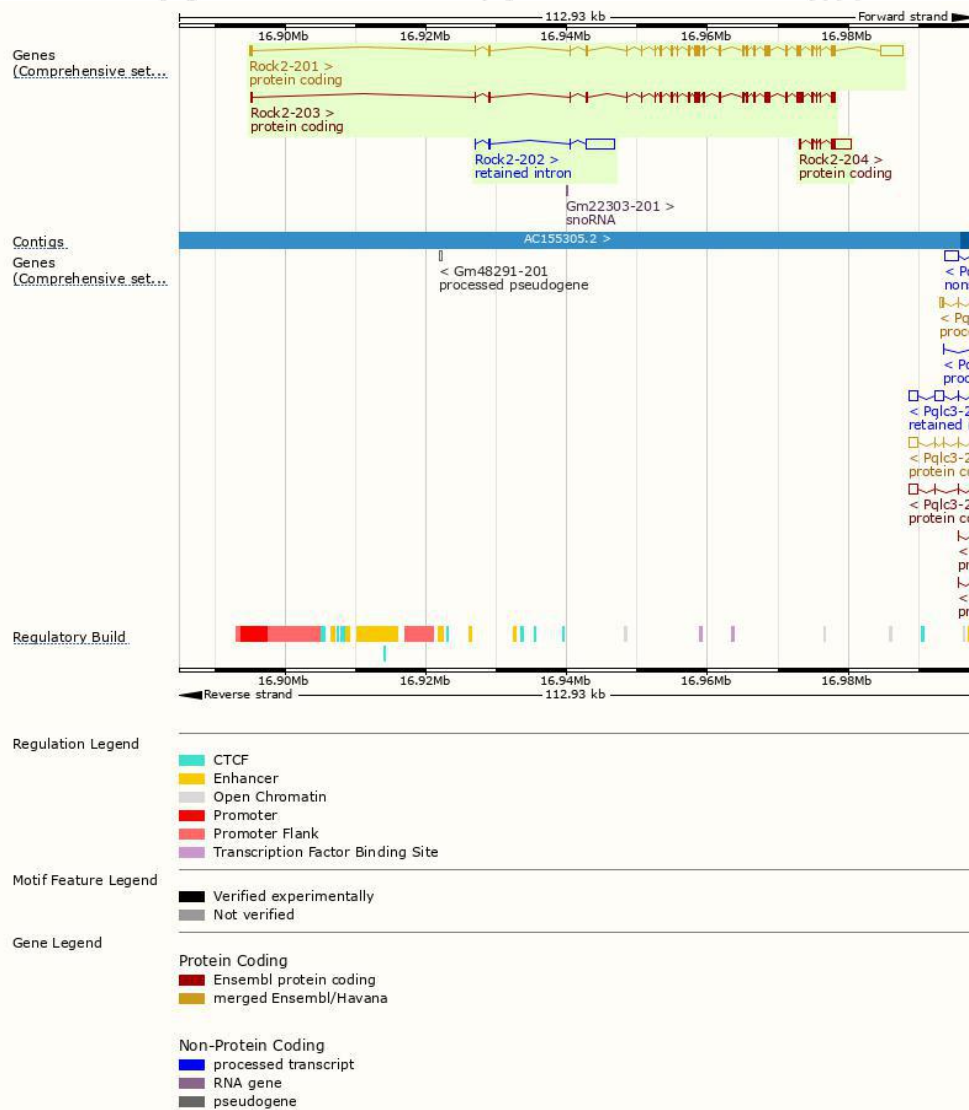
The gene has 4 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rock2-201	ENSMUST00000020904.7	7644	1388aa	Protein coding	CCDS36410	F8VPK5	TSL:5 GENCODE basic APPRIS P1
Rock2-203	ENSMUST00000220688.1	4357	1444aa	Protein coding	-	A0A1Y7VMN0	CDS 3' incomplete TSL:1
Rock2-204	ENSMUST00000221463.1	3002	262aa	Protein coding	-	A0A1Y7VLD1	CDS 5' incomplete TSL:1
Rock2-202	ENSMUST00000220542.1	4380	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Rock2-201* transcript, The transcription is shown below



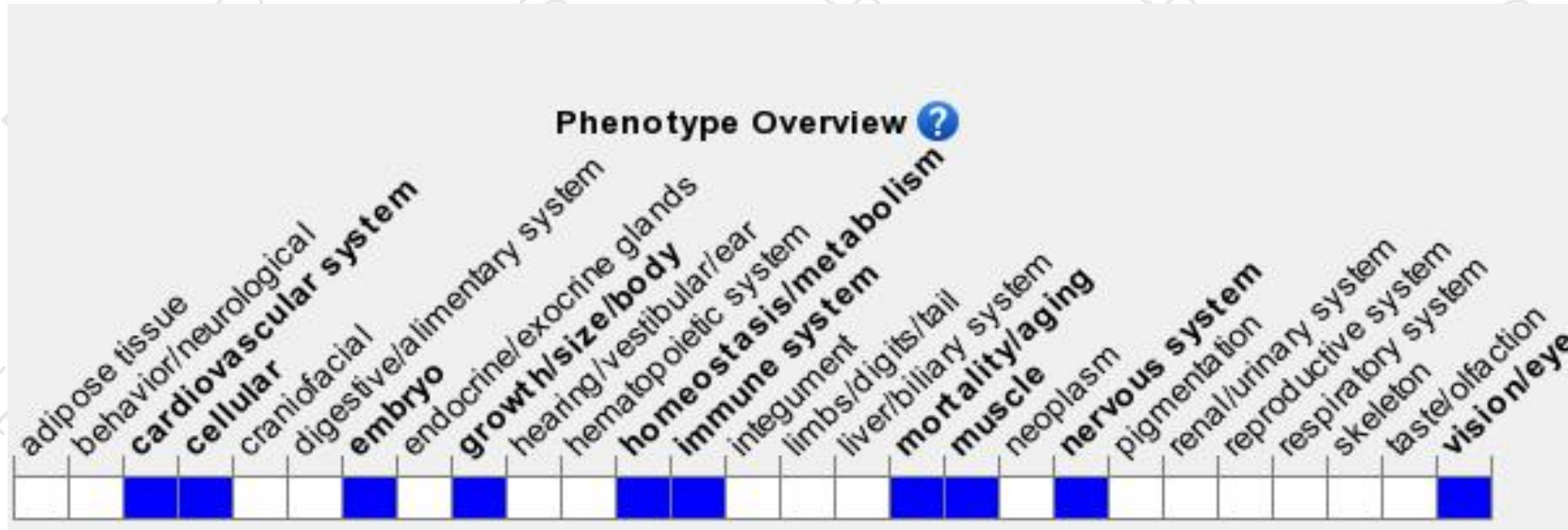
Genomic location distribution



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, Mice homozygous for disruptions in this genes tend to die before birth; those that survive are small. Hemorrhaging occurs in the placenta, at the tips of hind limb buds and occasionally the tail.

Subsequent development is normal and the size deficit is made up. They are fertile as adults.

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

Tel: 400-9660890

