Rhoc Cas9-CKO Strategy

Designer: Jinling Wang

Reviewer: Rui Xiong

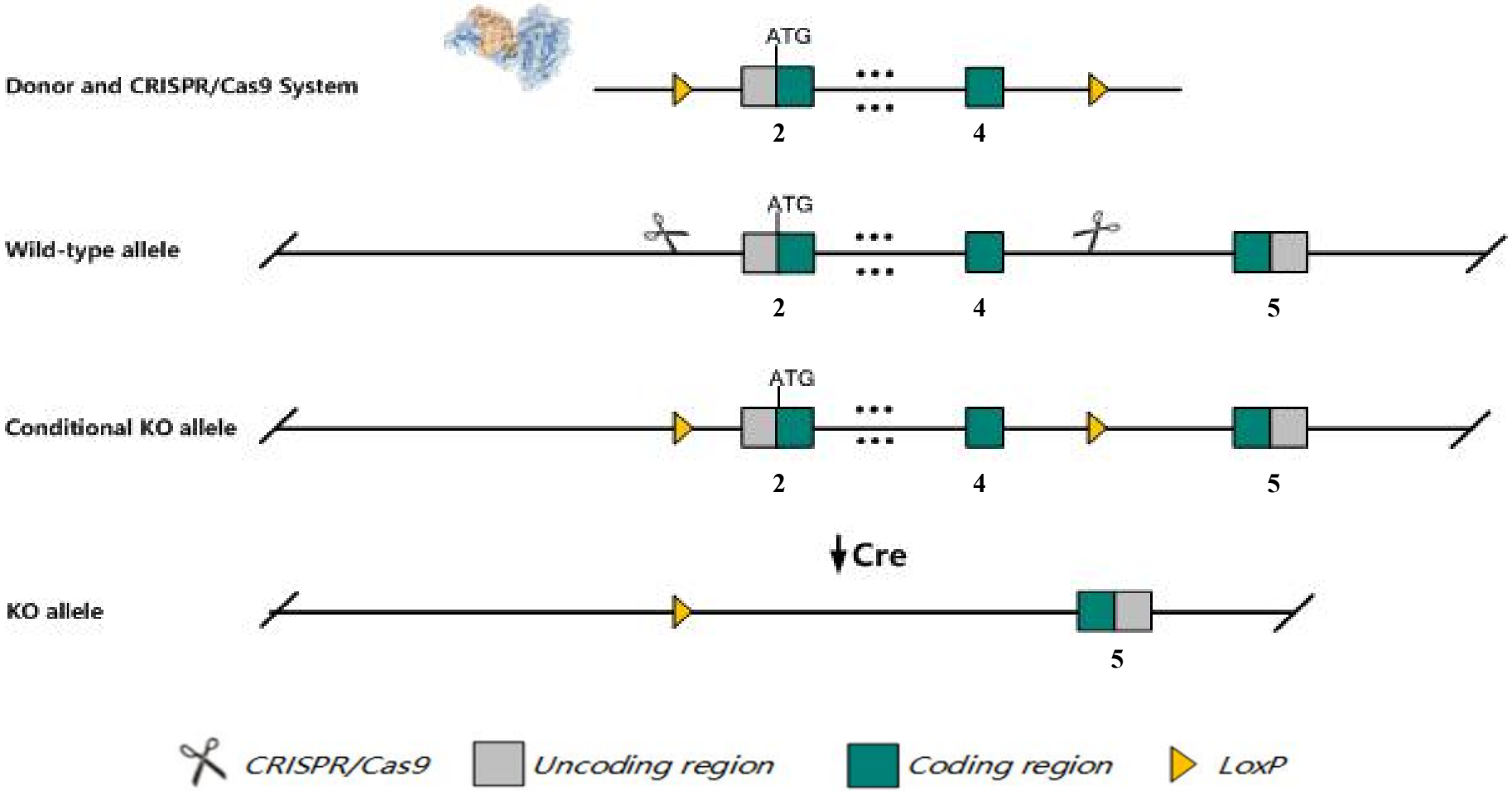
Design Date: 2018-11-9

Project Overview

Project Name	***Rhoc***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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



Technical routes

The *Rhoc* gene has 6 transcripts. According to the structure of *Rhoc* gene, exon2-exon4 of *Rhoc-201*(ENSMUST00000002303.11) transcript is recommended as the knockout region.The region contains start codon ATG.Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Rhoc* 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.

Notice

According to the existing MGI data,homozygous null mutation in this gene results in no abnormal phenotype, except for reduced stress fiber formation in serum starved fibroblasts. However, in combination with Tg(MMTV-PyVT)634Mul mice, metastatic potential of tumors and tumor cell motility are decreased.

The *Rhoc* gene is located on the Chr3. 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.

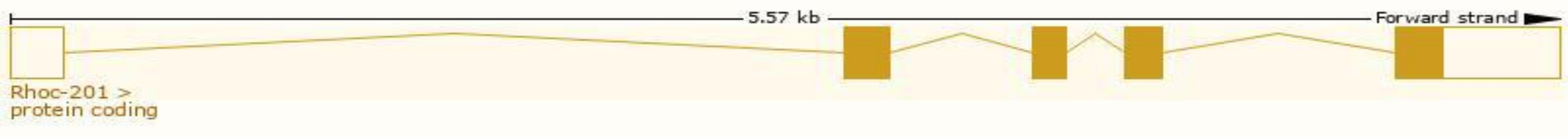
Gene information NCBI

Transcript information Ensembl

The gene has 6 transcripts,all transcripts are shown below:

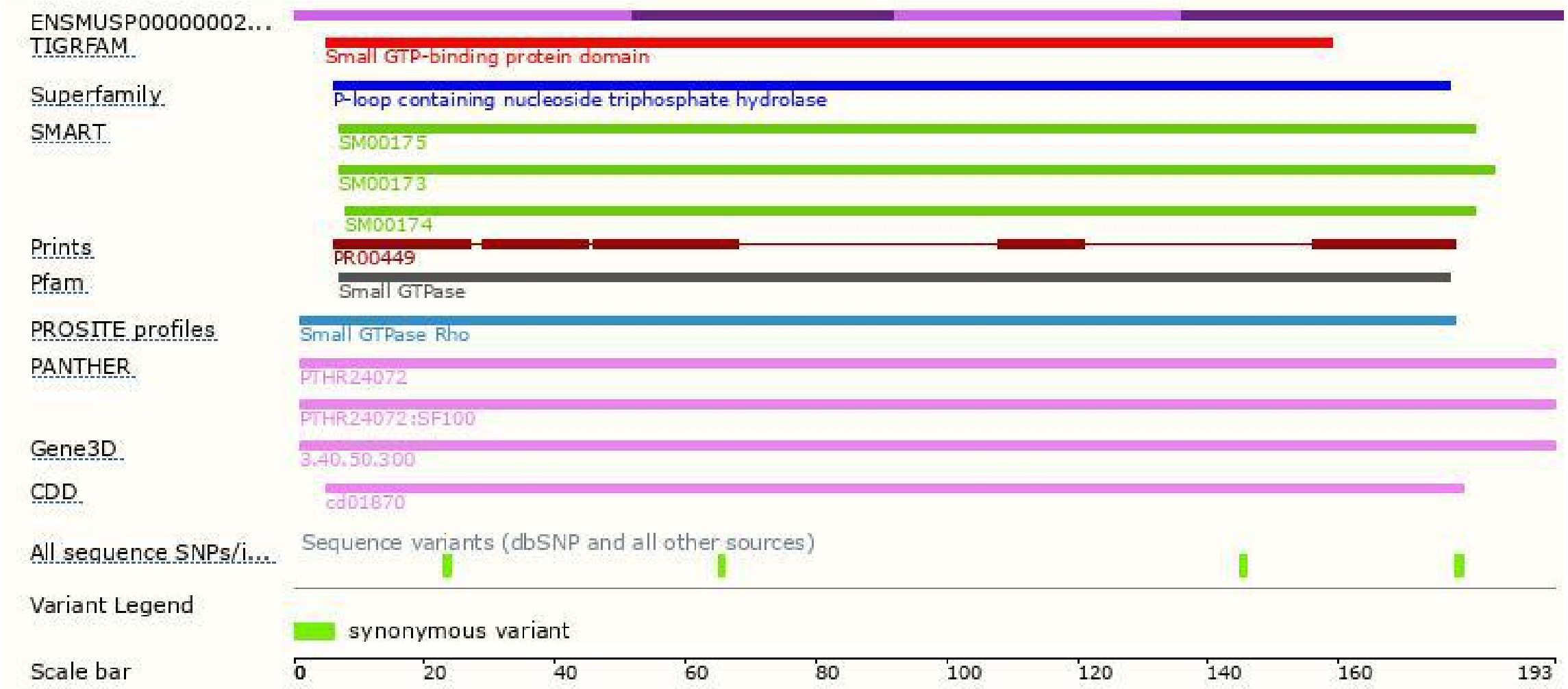
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rhoc-201	ENSMUST00000002303.11	1202	193aa	Protein coding	CCDS17704	Q62159	TSL:1 GENCODE basic APPRIS P1
Rhoc-202	ENSMUST00000106787.7	959	193aa	Protein coding	CCDS17704	Q62159	TSL:2 GENCODE basic APPRIS P1
Rhoc-205	ENSMUST00000196817.4	877	193aa	Protein coding	CCDS17704	Q62159	TSL:3 GENCODE basic APPRIS P1
Rhoc-204	ENSMUST00000176347.5	583	149aa	Protein coding	-	H3BL56	TSL:5 GENCODE basic
Rhoc-206	ENSMUST00000199824.1	518	118aa	Protein coding	-	A0A0G2JEP8	CDS 3' incomplete TSL:5
Rhoc-203	ENSMUST00000132721.1	386	No protein	Retained intron	-	-	TSL:2

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

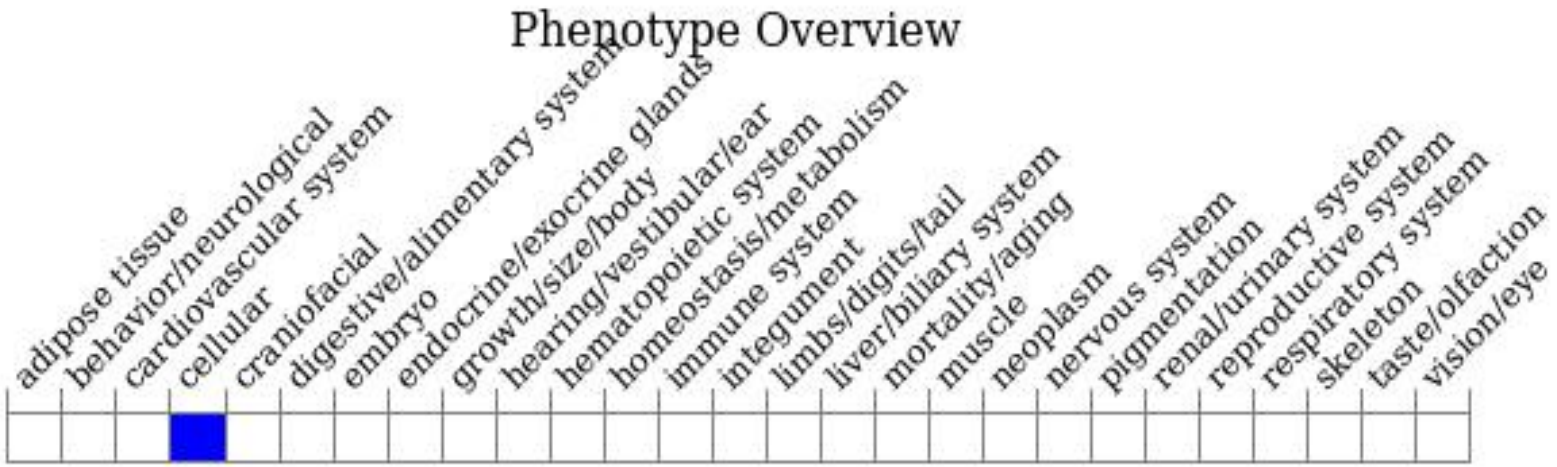


Genomic location distribution

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 mutation in this gene results in no abnormal phenotype, except for reduced stress fiber formation in serum starved fibroblasts. However, in combination with Tg(MMTV-PyVT)634Mul mice, metastatic potential of tumors and tumor cell motility are decreased.

集萃药康
GemPharmatech

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

集萃药康生物科技
GemPharmatech Co.,Ltd