

Arf5 Cas9-CKO Strategy

Designer: Wenjing Li

Reviewer: Jiayuan Yao

Design Date: 2020/11/24

Project Overview

Project Name

Arf5

Project type

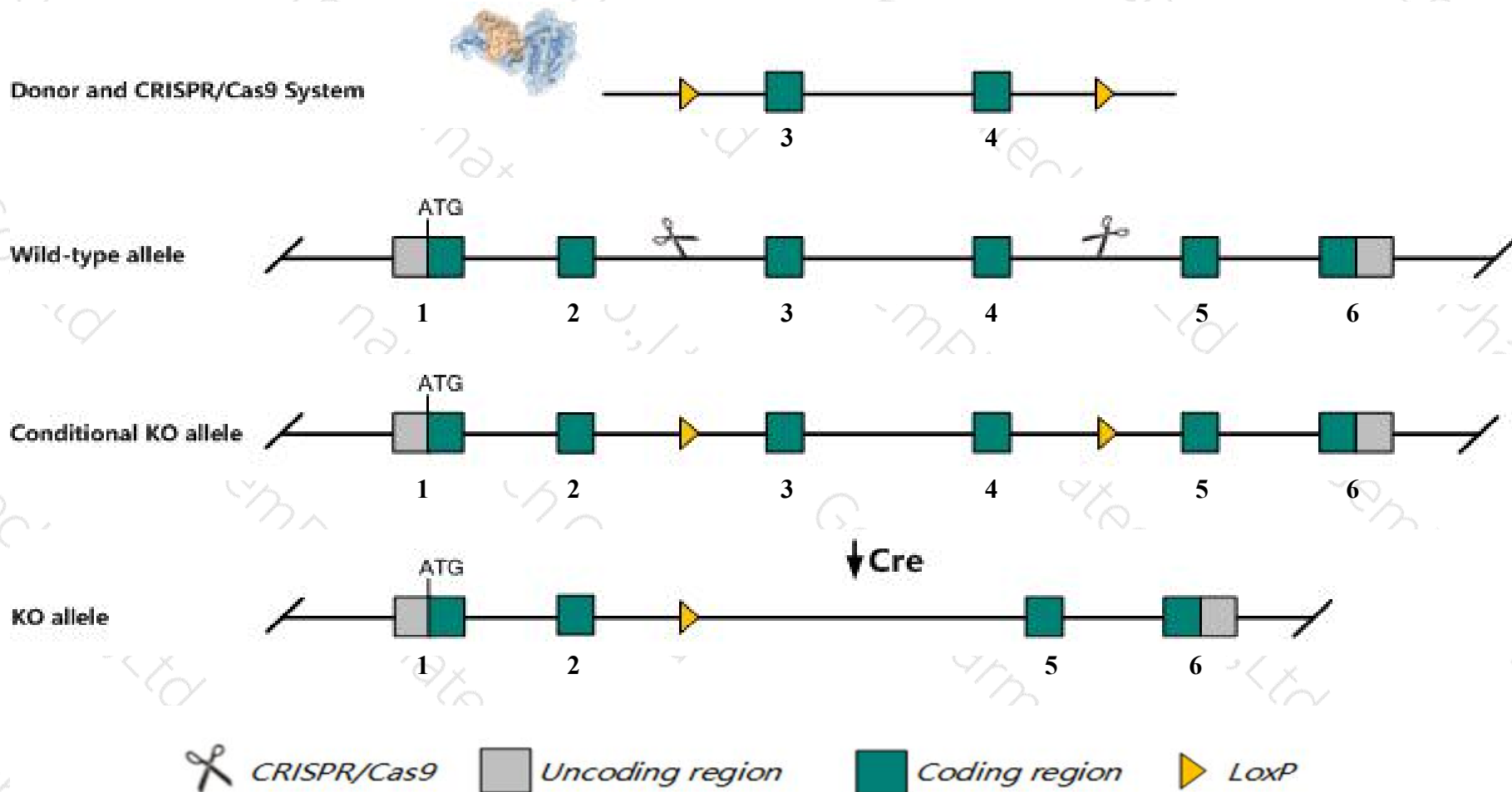
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Arf5* gene. The schematic diagram is as follows:



- The *Arf5* gene has 3 transcripts. According to the structure of *Arf5* gene, exon3-exon4 of *Arf5*-201(ENSMUST00000020717.11) transcript is recommended as the knockout region. The region contains 182bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Arf5* 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 a null allele exhibit no abnormalities. Mice also heterozygous for an *arf4* null allele exhibit impaired coordination, tremors and altered brain wave pattern and Purkinje cell electrophysiology.
- The Intron2 and Intron4 are only 351bp and 494bp, loxp insertion may affect mRNA splicing.
- The KO region contains functional region of the *Gcc1* gene. Knockout of this region may affect the function of *Gcc1* gene. The floxed region is about 2.5kb from the N-terminal of *Fscn3* gene. This strategy may influence the regulatory function of the N-terminal of *Fscn3* gene.
- The *Arf5* 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)

Arf5 ADP-ribosylation factor 5 [Mus musculus (house mouse)]

Gene ID: 11844, updated on 26-Mar-2020

Summary



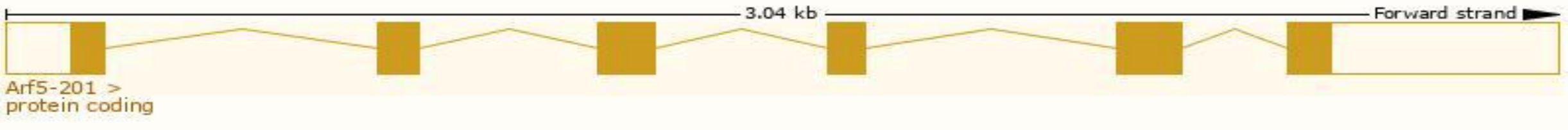
Official Symbol	Arf5 provided by MGI
Official Full Name	ADP-ribosylation factor 5 provided by MGI
Primary source	MGI:MGI:99434
See related	Ensembl:ENSMUSG00000020440
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Ubiquitous expression in duodenum adult (RPKM 584.2), small intestine adult (RPKM 299.8) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

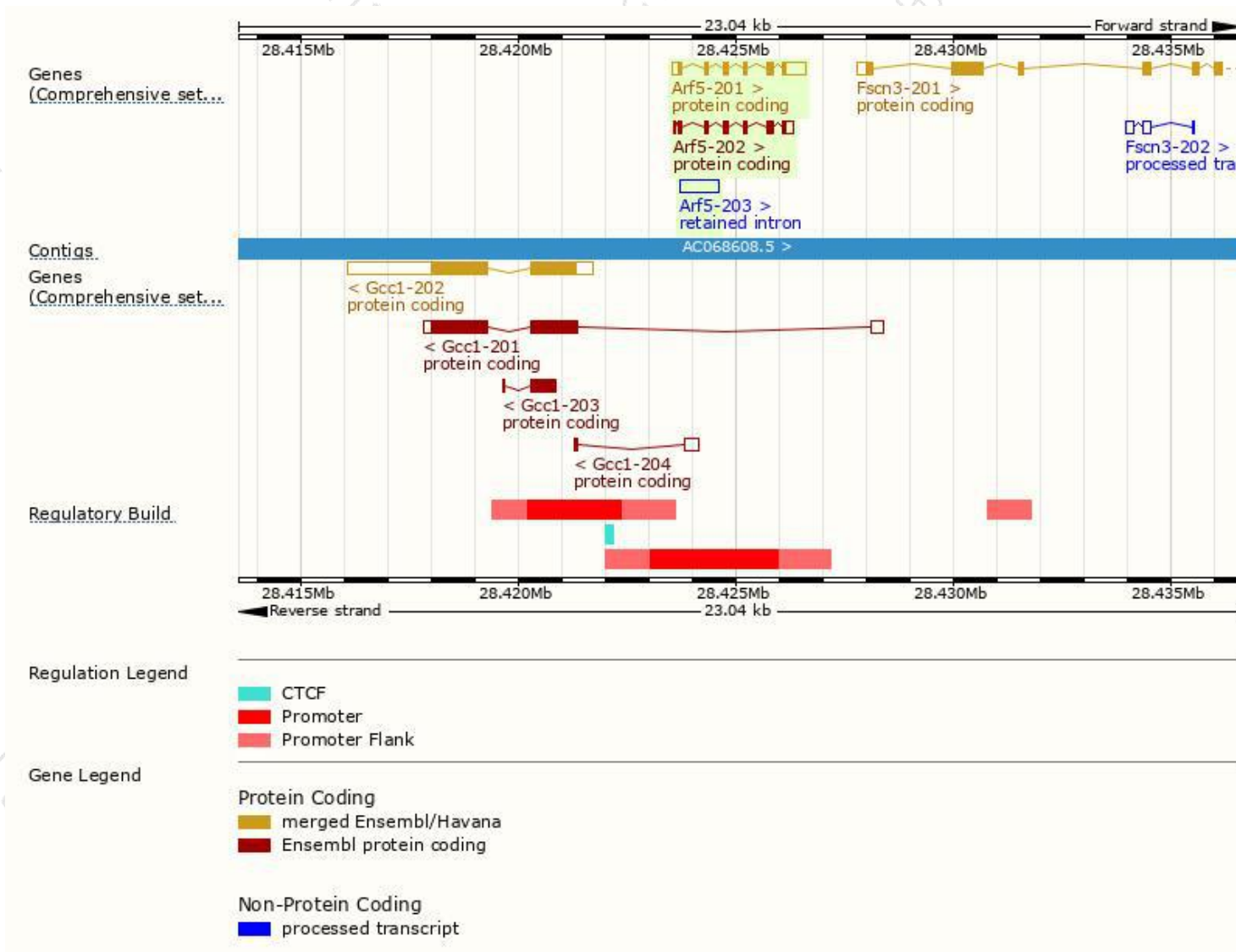
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Arf5-201	ENSMUST00000020717.11	1116	180aa	Protein coding	CCDS19952	P84084	TSL:1 GENCODE basic APPRIS P1
Arf5-202	ENSMUST00000169841.1	768	180aa	Protein coding	CCDS19952	P84084	TSL:5 GENCODE basic APPRIS P1
Arf5-203	ENSMUST00000202028.1	919	No protein	Retained intron	-	-	TSL:NA

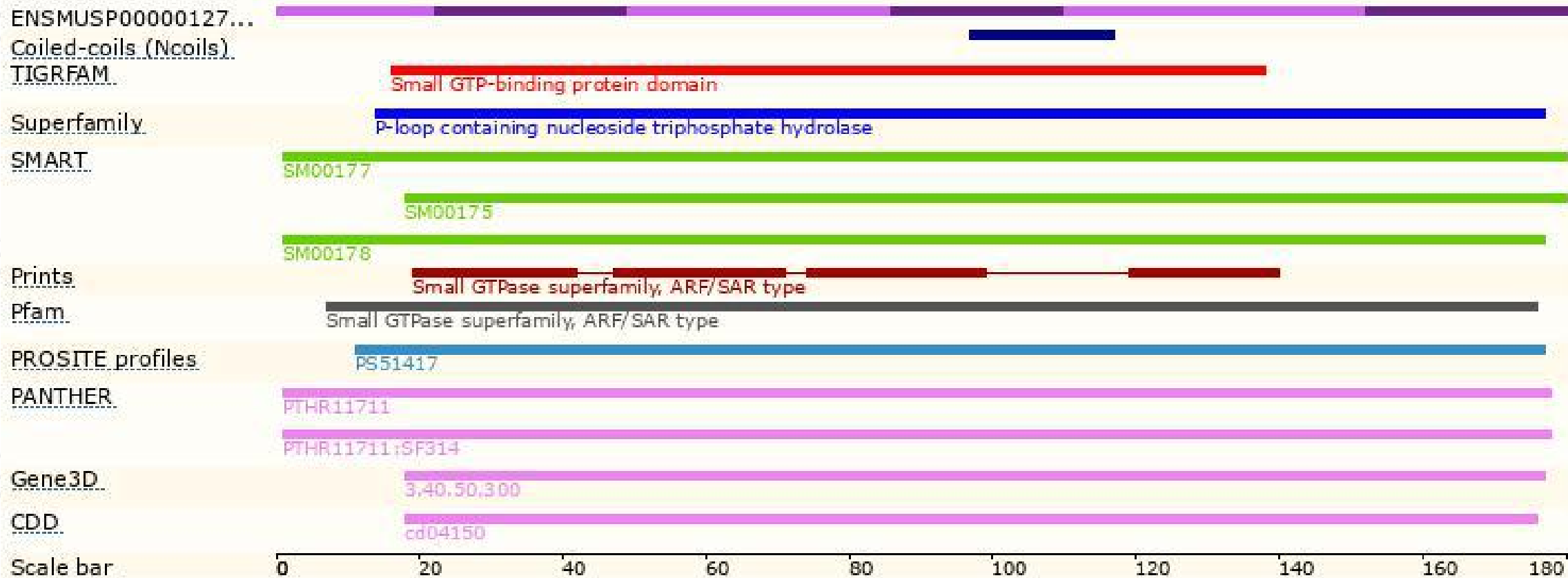
The strategy is based on the design of *Arf5-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

Tel: 400-9660890

