

Agfg2 Cas9-CKO Strategy

Designer: Xiaojing Li

Reviewer: JiaYu

Design Date: 2020-10-14

Project Overview

Project Name

Agfg2

Project type

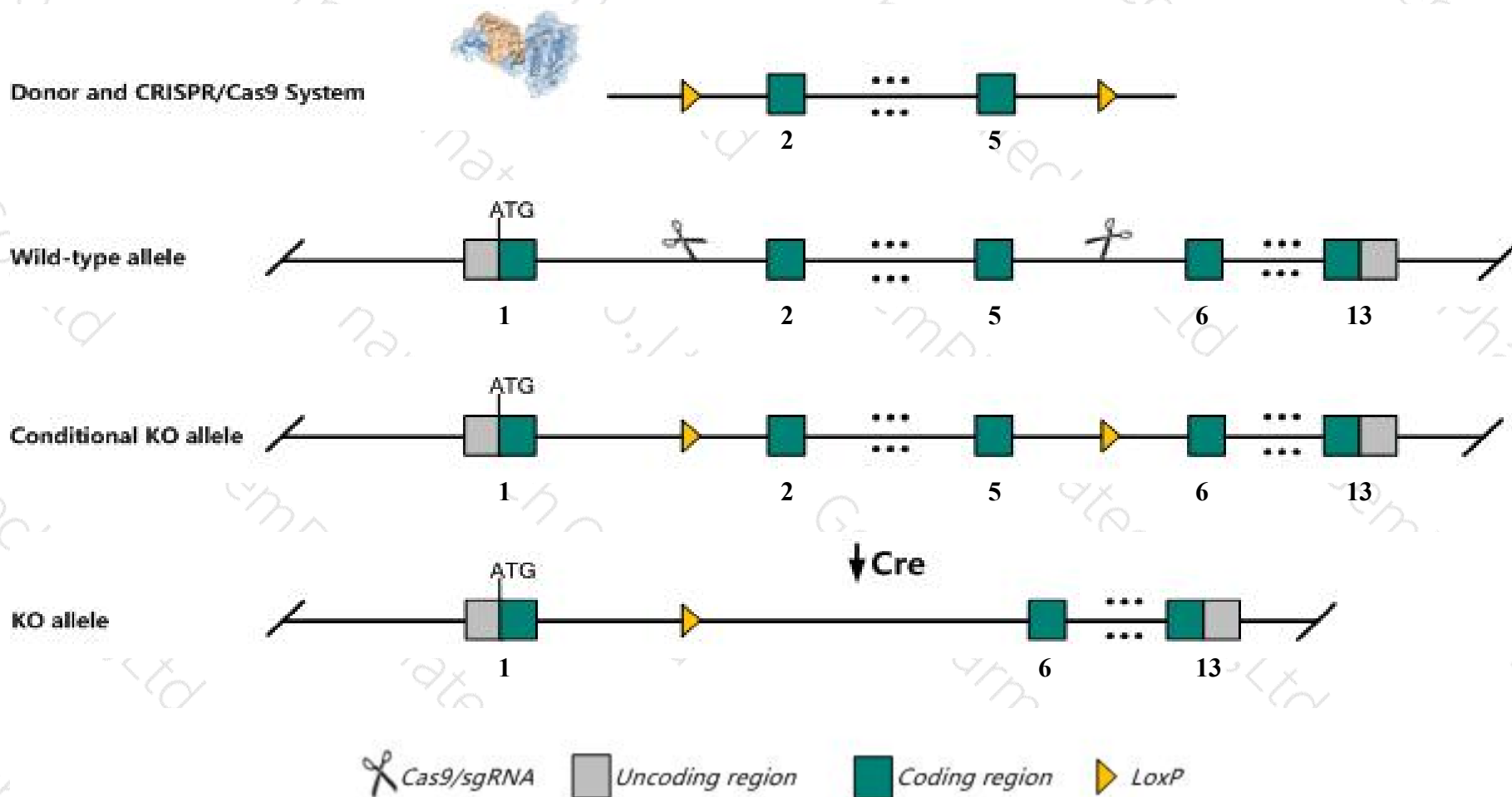
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Agfg2* gene. The schematic diagram is as follows:



Technical routes

- The *Agfg2* gene has 6 transcripts. According to the structure of *Agfg2* gene, exon2-exon5 of *Agfg2*-202(ENSMUST00000100544.10) transcript is recommended as the knockout region. The region contains 536bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Agfg2* 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.

- The *Agfg2* gene is located on the Chr5. 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)

Agfg2 ArfGAP with FG repeats 2 [Mus musculus (house mouse)]

Gene ID: 231801, updated on 13-Mar-2020

Summary



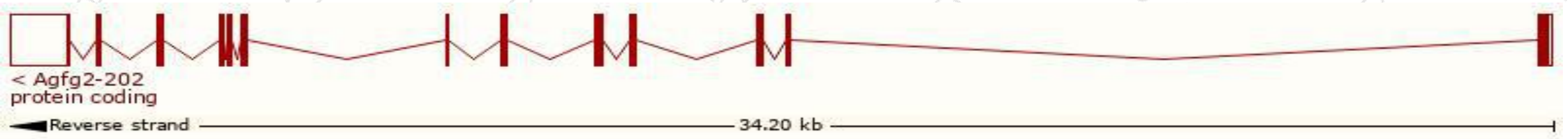
Official Symbol	Agfg2 provided by MGI
Official Full Name	ArfGAP with FG repeats 2 provided by MGI
Primary source	MGI:MGI:2443267
See related	Ensembl:ENSMUSG00000029722
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	A630095P14Rik, Hrbl, RAB-R, RABR
Summary	This gene encodes a paralog of the HIV-1 Rev binding proteins that serve as cellular co-factors for HIV-1 Rev protein in shuttling viral pre-mRNAs from the nucleus to the cytoplasm. The encoded protein contains an ADP-ribosylation factor GTPase activating protein (Arf-GAP) zinc finger domain, several phenylalanine-glycine (FG) motifs and asparagine-proline-phenylalanine (NPF) motifs. Alternate splicing of this gene results in multiple transcript variants. [provided by RefSeq, Dec 2014]
Expression	Ubiquitous expression in thymus adult (RPKM 56.4), adrenal adult (RPKM 28.7) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

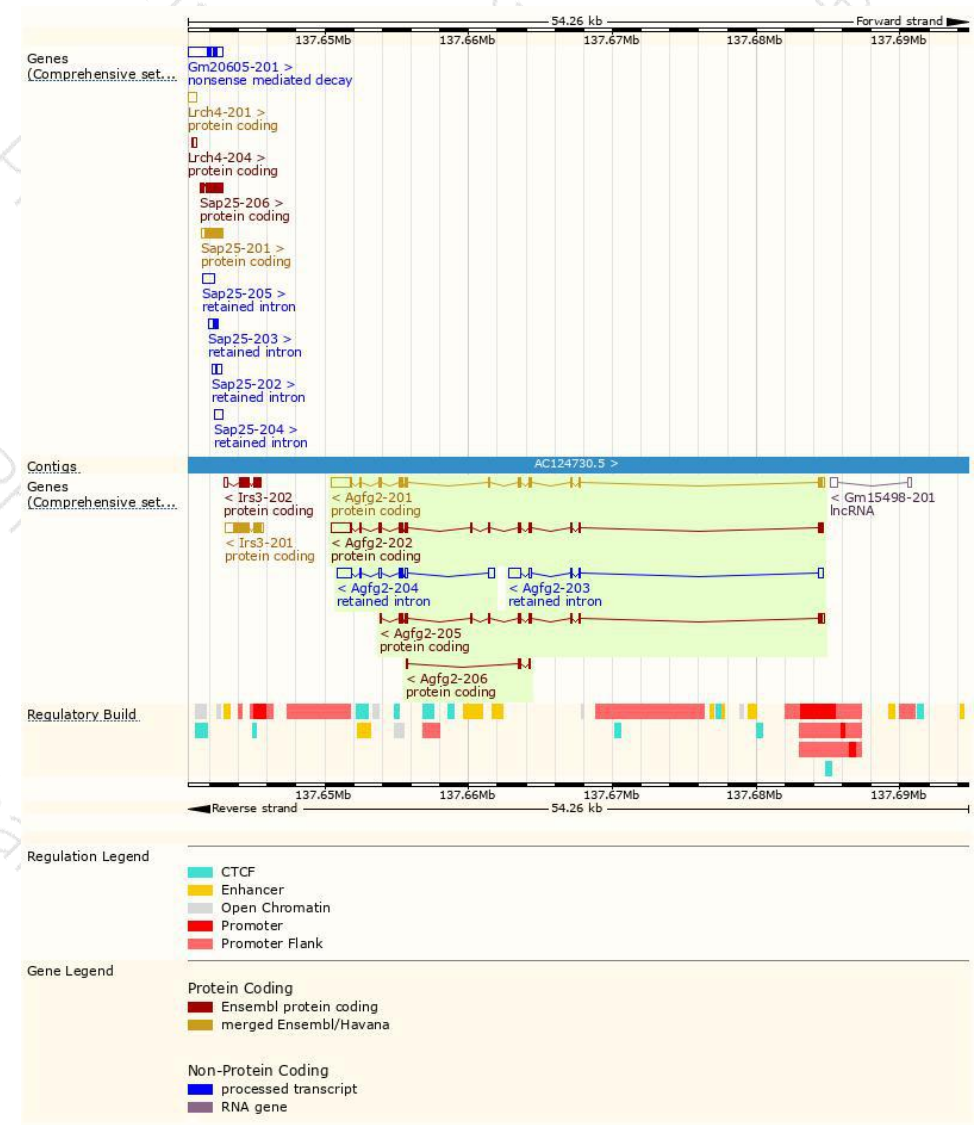
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Agfg2-201	ENSMUST00000031736.15	2893	479aa	Protein coding	CCDS19774	Q80WC7	TSL:1 GENCODE basic APPRIS P3
Agfg2-202	ENSMUST00000100544.10	2858	490aa	Protein coding	CCDS80443	Q3U2K8	TSL:1 GENCODE basic APPRIS ALT2
Agfg2-205	ENSMUST00000151839.1	1365	406aa	Protein coding	-	Q3TBU7	CDS 3' incomplete TSL:1
Agfg2-206	ENSMUST00000198657.1	343	114aa	Protein coding	-	A0A0G2JFR2	CDS 5' and 3' incomplete TSL:3
Agfg2-204	ENSMUST00000147518.1	1863	No protein	Retained intron	-	-	TSL:2
Agfg2-203	ENSMUST00000138604.1	1497	No protein	Retained intron	-	-	TSL:2

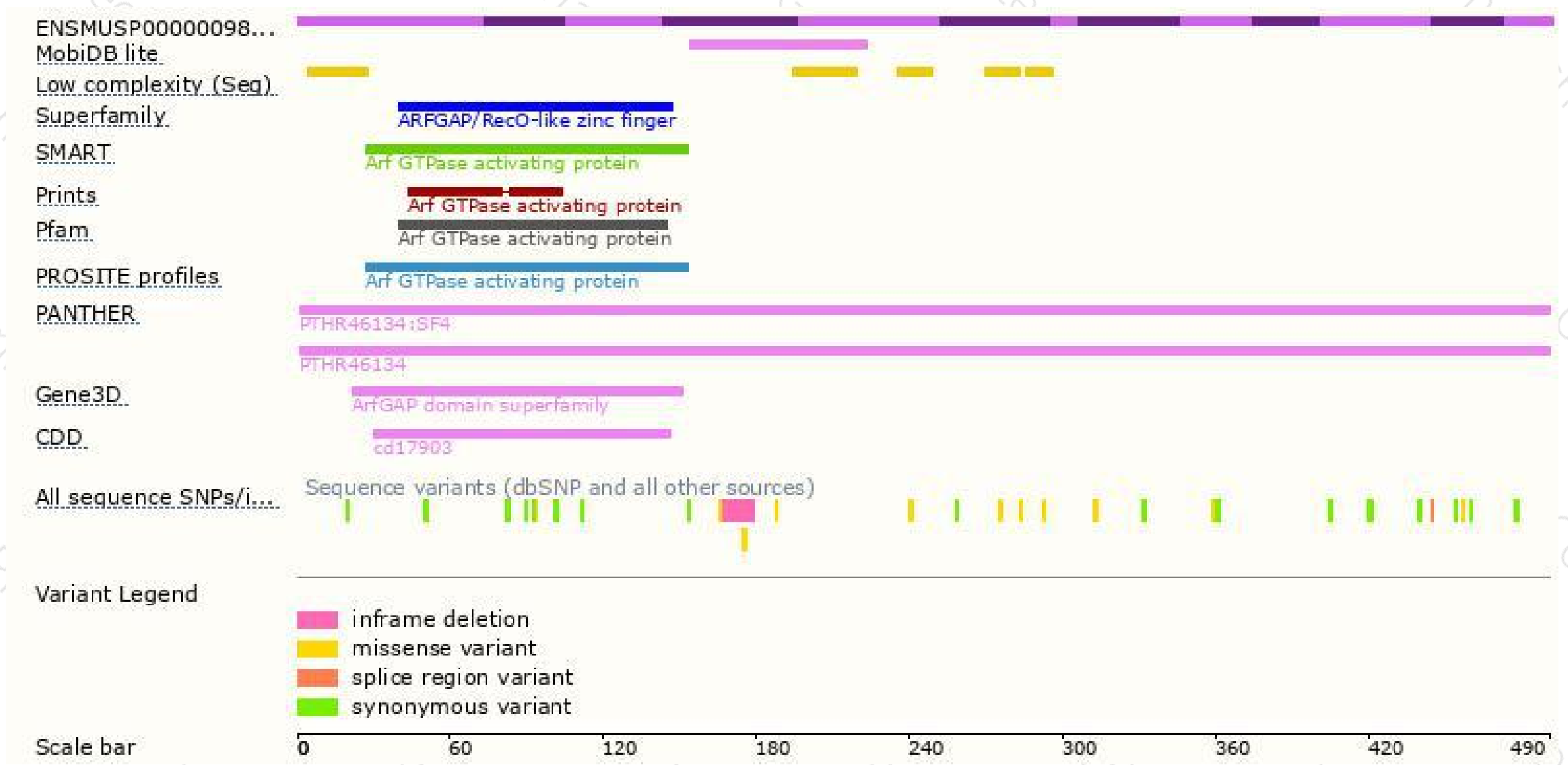
The strategy is based on the design of *Agfg2-202* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

Tel: 025-5864 1534

