

Rffl Cas9-CKO Strategy

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Project Overview

Project Name

Rffl

Project type

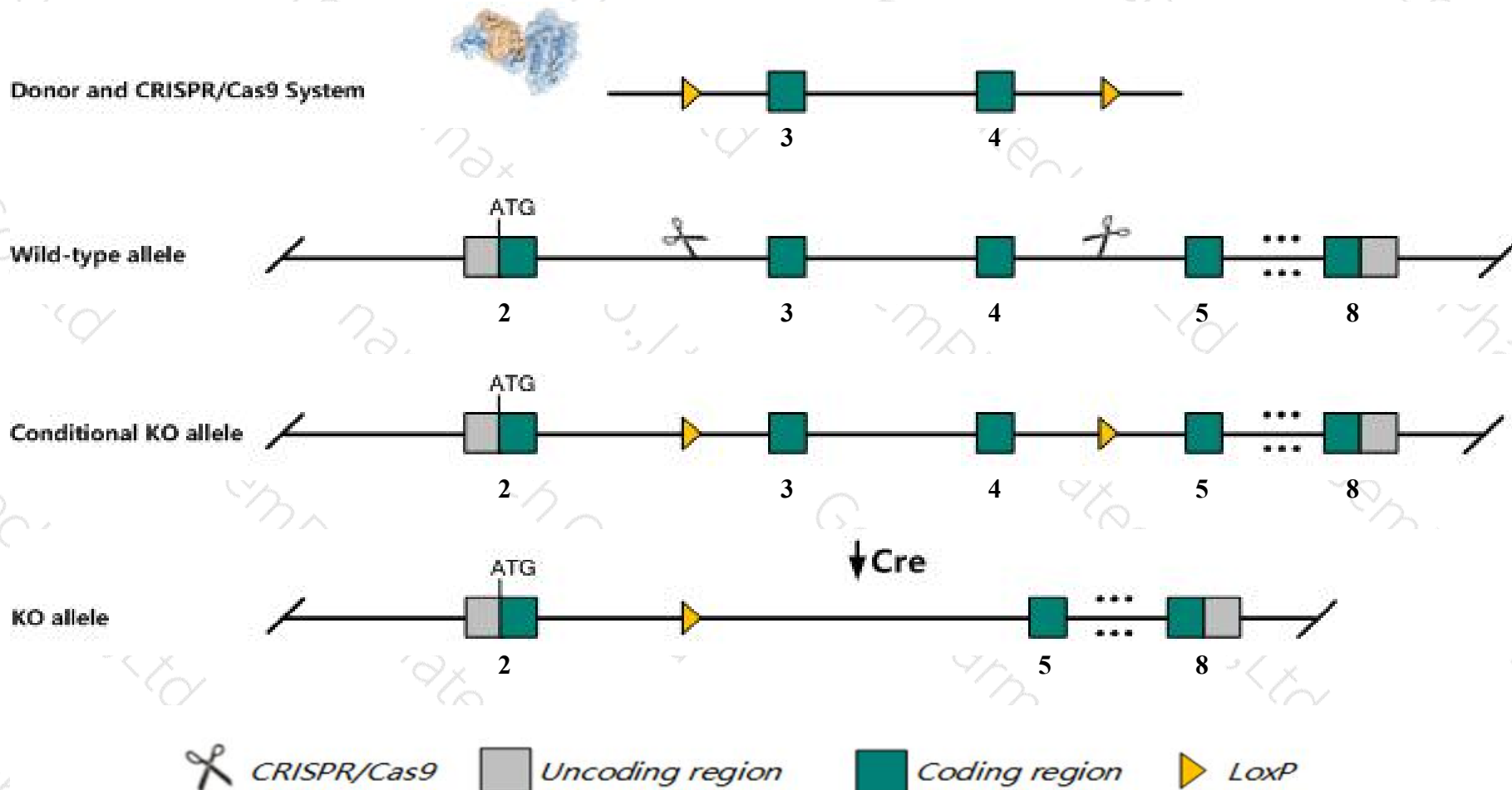
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- The *Rffl* gene has 7 transcripts. According to the structure of *Rffl* gene, exon3-exon4 of *Rffl*-202 (ENSMUST00000071152.13) transcript is recommended as the knockout region. The region contains 599bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rffl* 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 an inactivating targeted mutation of this gene are born at the expected Mendelian frequency; they are viable and fertile and exhibit no apparent abnormal phenotype.
- The *Rffl* gene is located on the Chr11. 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)

Rffl ring finger and FYVE like domain containing protein [Mus musculus (house mouse)]

Gene ID: 67338, updated on 13-Mar-2020

Summary



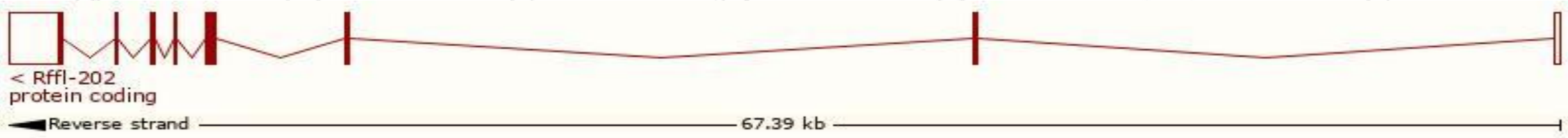
Official Symbol	Rffl provided by MGI
Official Full Name	ring finger and FYVE like domain containing protein provided by MGI
Primary source	MGI:MGI:1914588
See related	Ensembl:ENSMUSG00000020696
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1700051E09Rik, 4930516L10Rik, BG080975, Carp2
Expression	Ubiquitous expression in testis adult (RPKM 17.6), placenta adult (RPKM 7.7) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

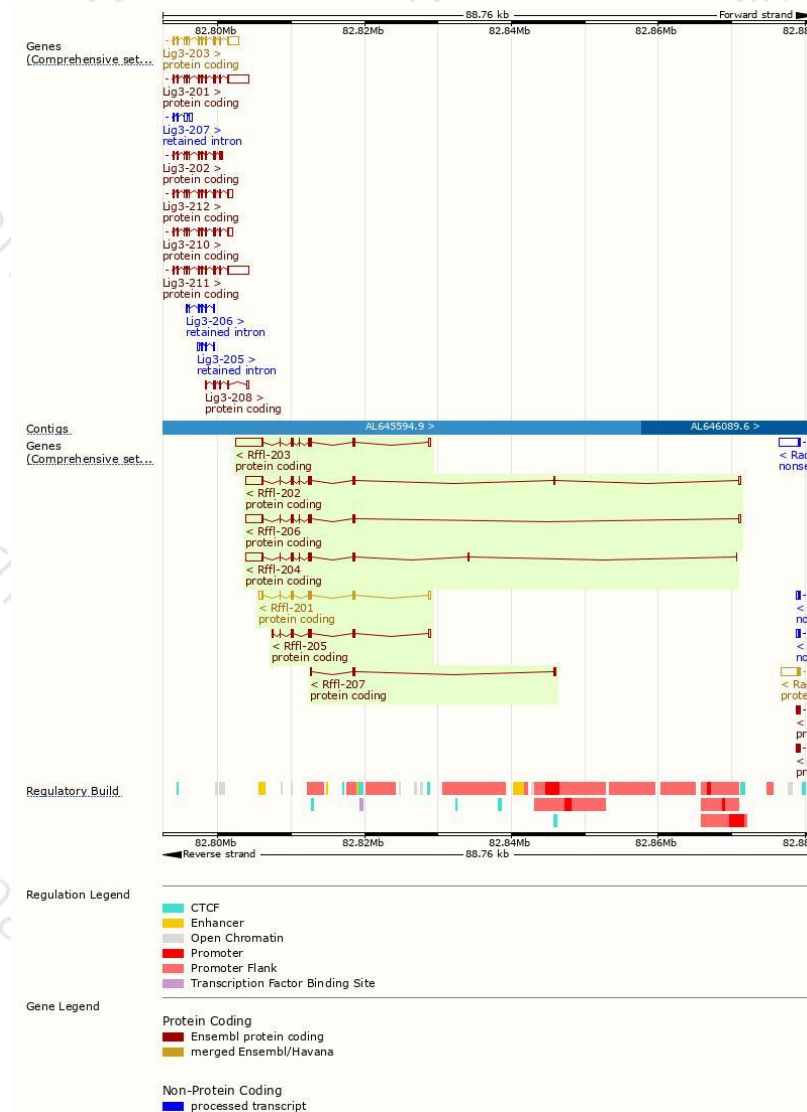
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rffl-203	ENSMUST00000074515.10	4973	363aa	Protein coding	CCDS25148	Q3UD78 Q6ZQM0	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Rffl-202	ENSMUST00000071152.13	3633	398aa	Protein coding	CCDS48868	Q3UCG9	TSL:1 GENCODE basic
Rffl-204	ENSMUST00000093975.11	3575	377aa	Protein coding	CCDS48867	Q6ZQM0	TSL:1 GENCODE basic
Rffl-206	ENSMUST00000108173.9	3489	363aa	Protein coding	CCDS25148	Q3UD78 Q6ZQM0	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Rffl-201	ENSMUST00000021036.12	1719	335aa	Protein coding	CCDS25149	Q148A8 Q6ZQM0	TSL:1 GENCODE basic
Rffl-205	ENSMUST00000103218.2	1264	293aa	Protein coding	-	Q6ZQM0	TSL:1 GENCODE basic
Rffl-207	ENSMUST00000126660.1	671	142aa	Protein coding	-	B1AT06	CDS 3' incomplete TSL:2

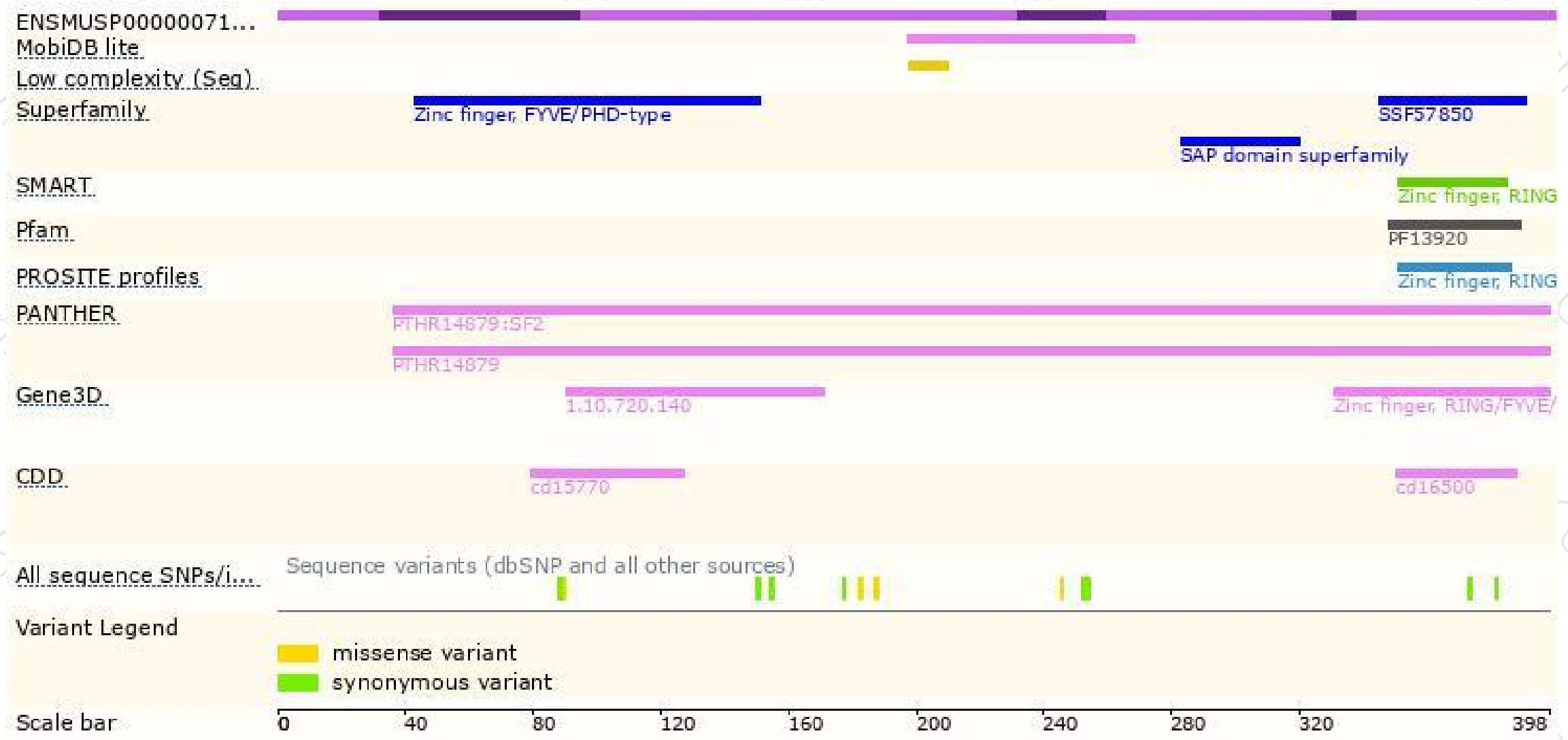
The strategy is based on the design of *Rffl-202* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

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