Rfc3 Cas9-CKO Strategy

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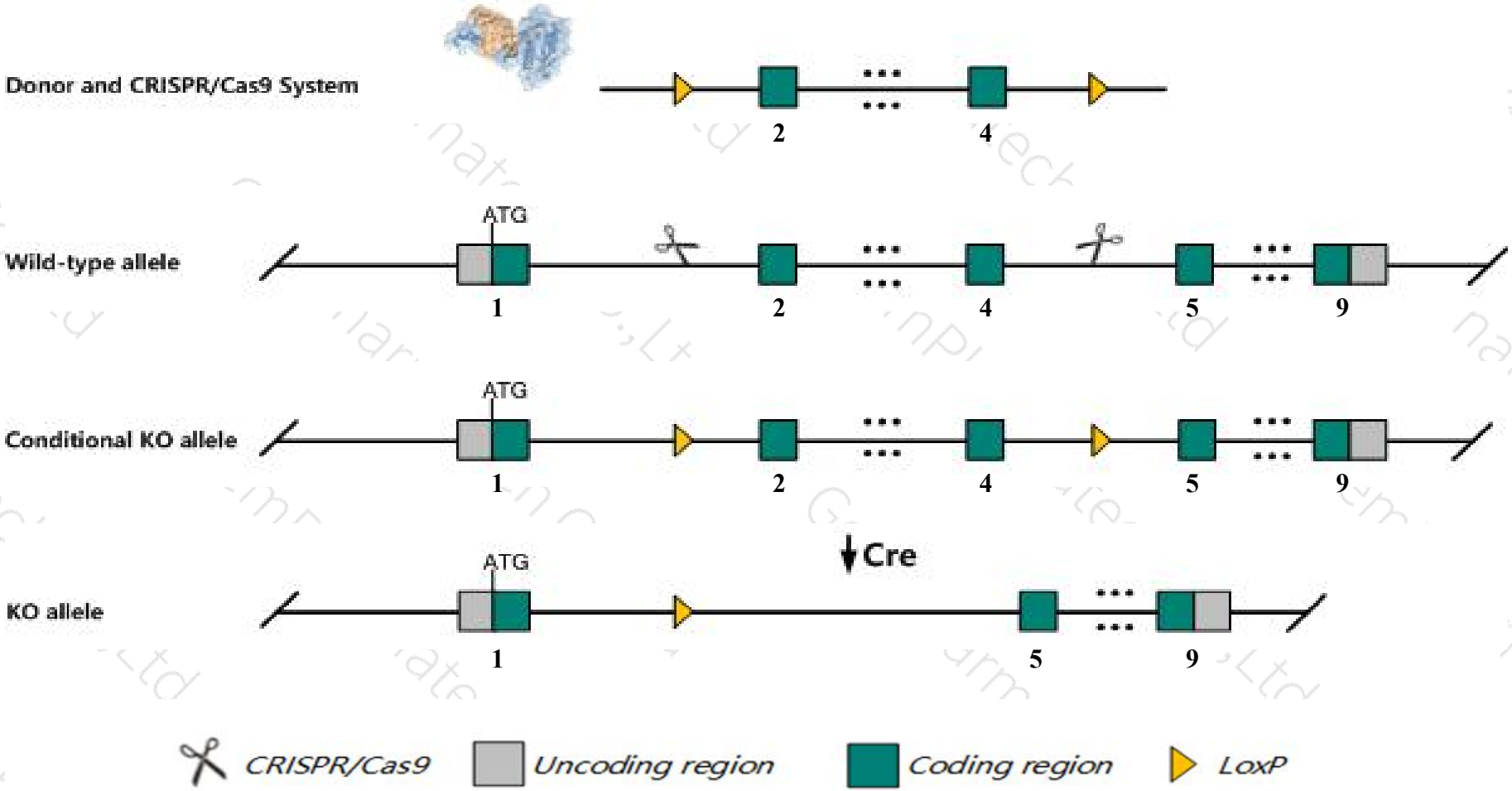
Project Overview

Project Name	***Rfc3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rfc3* gene. The schematic diagram is as follows:



Technical routes

- The *Rfc3* gene has 7 transcripts. According to the structure of *Rfc3* gene, exon2-exon4 of *Rfc3-201* (ENSMUST00000038131.9) transcript is recommended as the knockout region.The region contains 304bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Rfc3* 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

➢ Transcript *Rfc3*-202&207 may not be affected.

➢ The *Rfc3* 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）

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Rfc3 replication factor C (activator 1) 3 [*Mus musculus* (house mouse)]

Gene ID: 69263, updated on 13-Mar-2020

Summary

Official Symbol	Rfc3 provided by MGI
Official Full Name	replication factor C (activator 1) 3 provided by MGI
Primary source	MGI:MGI:1916513
See related	Ensembl:ENSMUSG00000033970
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	38kDa; Recc3; AU022547; 2810416I22Rik
Expression	Broad expression in CNS E11.5 (RPKM 28.7), liver E14 (RPKM 20.7) and 26 other tissues See more
Orthologs	human all

Genomic context

Location: 5; 5 G3

See Rfc3 in Genome Data Viewer

Exon count: 10

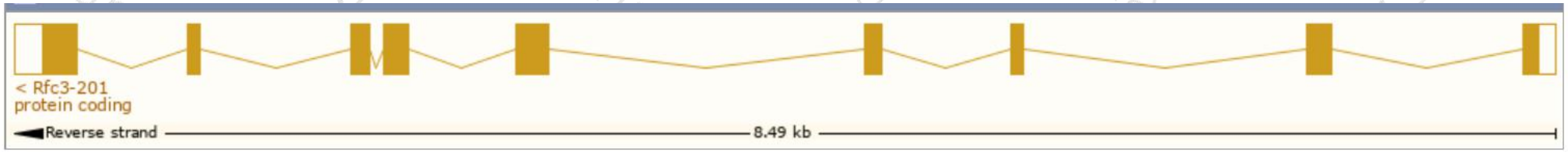
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	5	NC_000071.6 (151642817..151651208, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	5	NC_000071.5 (152445399..152453783, complement)

Transcript information（Ensembl）

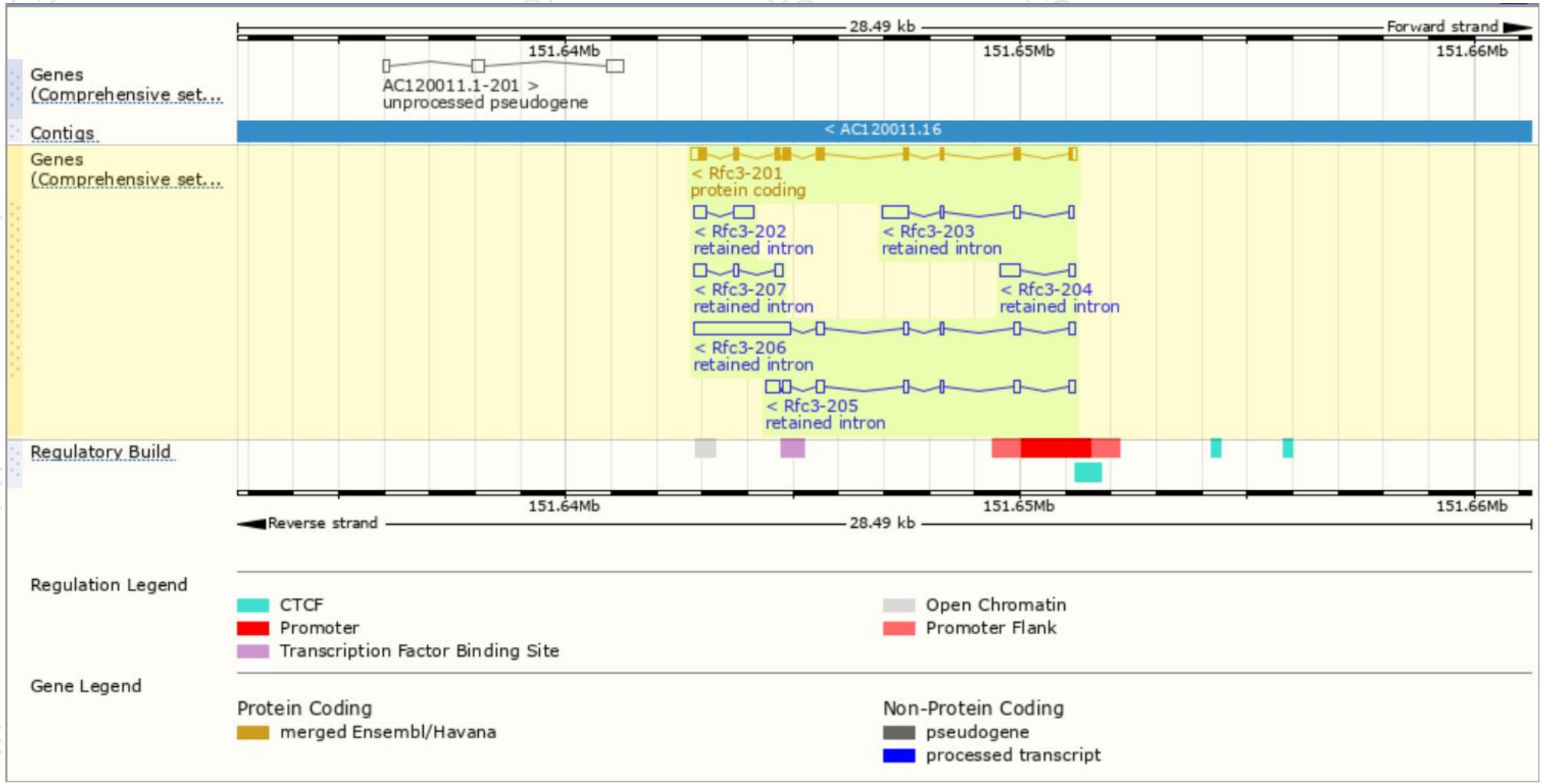
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rfc3-201	ENSMUST00000038131.9	1317	356aa	Protein coding	CCDS39413	Q3TKD1 Q8R323	TSL:1 GENCODE basic APPRIS P1
Rfc3-206	ENSMUST00000145106.7	2726	No protein	Retained intron	-	-	TSL:1
Rfc3-205	ENSMUST00000140067.7	1062	No protein	Retained intron	-	-	TSL:1
Rfc3-203	ENSMUST00000132709.1	906	No protein	Retained intron	-	-	TSL:1
Rfc3-202	ENSMUST00000127366.1	706	No protein	Retained intron	-	-	TSL:2
Rfc3-204	ENSMUST00000136752.1	605	No protein	Retained intron	-	-	TSL:2
Rfc3-207	ENSMUST00000156667.1	503	No protein	Retained intron	-	-	TSL:2

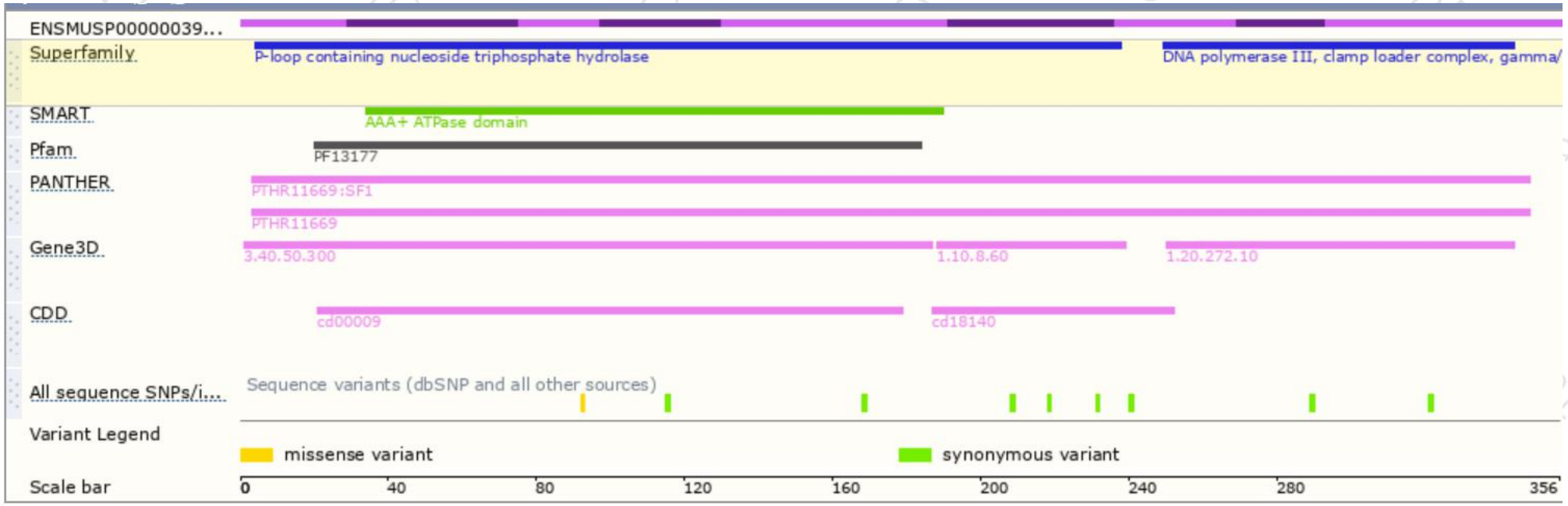
The strategy is based on the design of *Rfc3-201* transcript,The transcription is shown below



Genomic location distribution



Protein domain



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

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