Prpf4 Cas9-CKO Strategy

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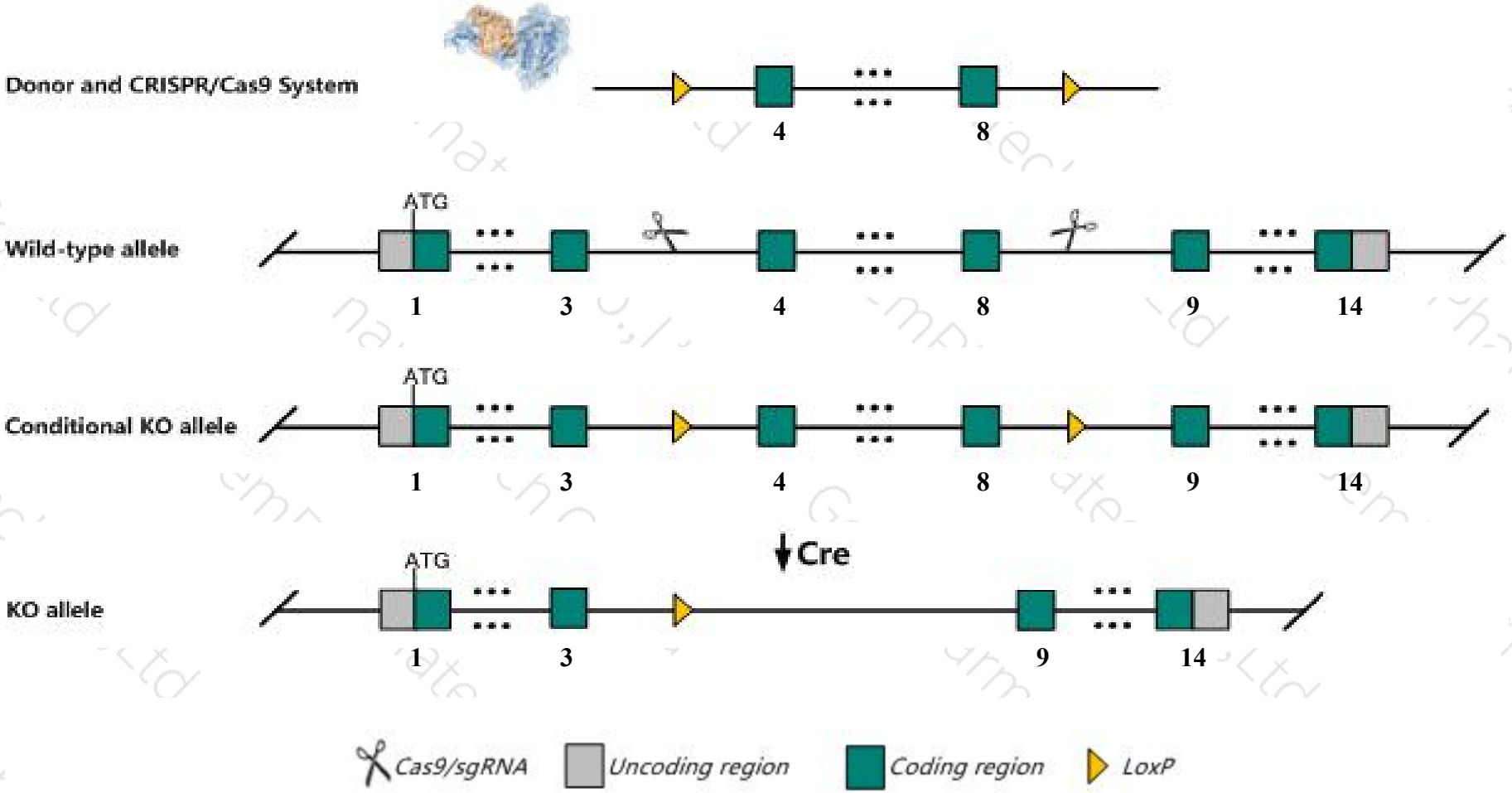
Project Overview

Project Name	***Prpf4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Prpf4* gene. The schematic diagram is as follows:



Technical routes

➢ The *Prpf4* gene has 2 transcripts. According to the structure of *Prpf4* gene, exon4-exon8 of *Prpf4-201*(ENSMUST00000084524.3) transcript is recommended as the knockout region.The region contains 416bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Prpf4* 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.

Notice

➢ The N-terminal of *Prpf4* gene will remain several amino acids, it may remain the partial function of *Prpf4* gene.

➢ The *Prpf4* gene is located on the Chr4. 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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Prpf4 pre-mRNA processing factor 4 [Mus musculus (house mouse)]

Gene ID: 70052, updated on 13-Mar-2020

Summary

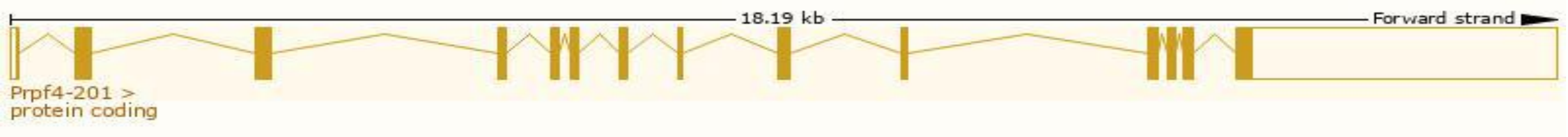
Official Symbol	Prpf4 provided by MGI
Official Full Name	pre-mRNA processing factor 4 provided by MGI
Primary source	MGI:MGI:1917302
See related	Ensembl:ENSMUSG00000066148
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1600015H11Rik, AI874830, AW047464, bN189G18.1
Expression	Ubiquitous expression in CNS E11.5 (RPKM 7.2), limb E14.5 (RPKM 6.9) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

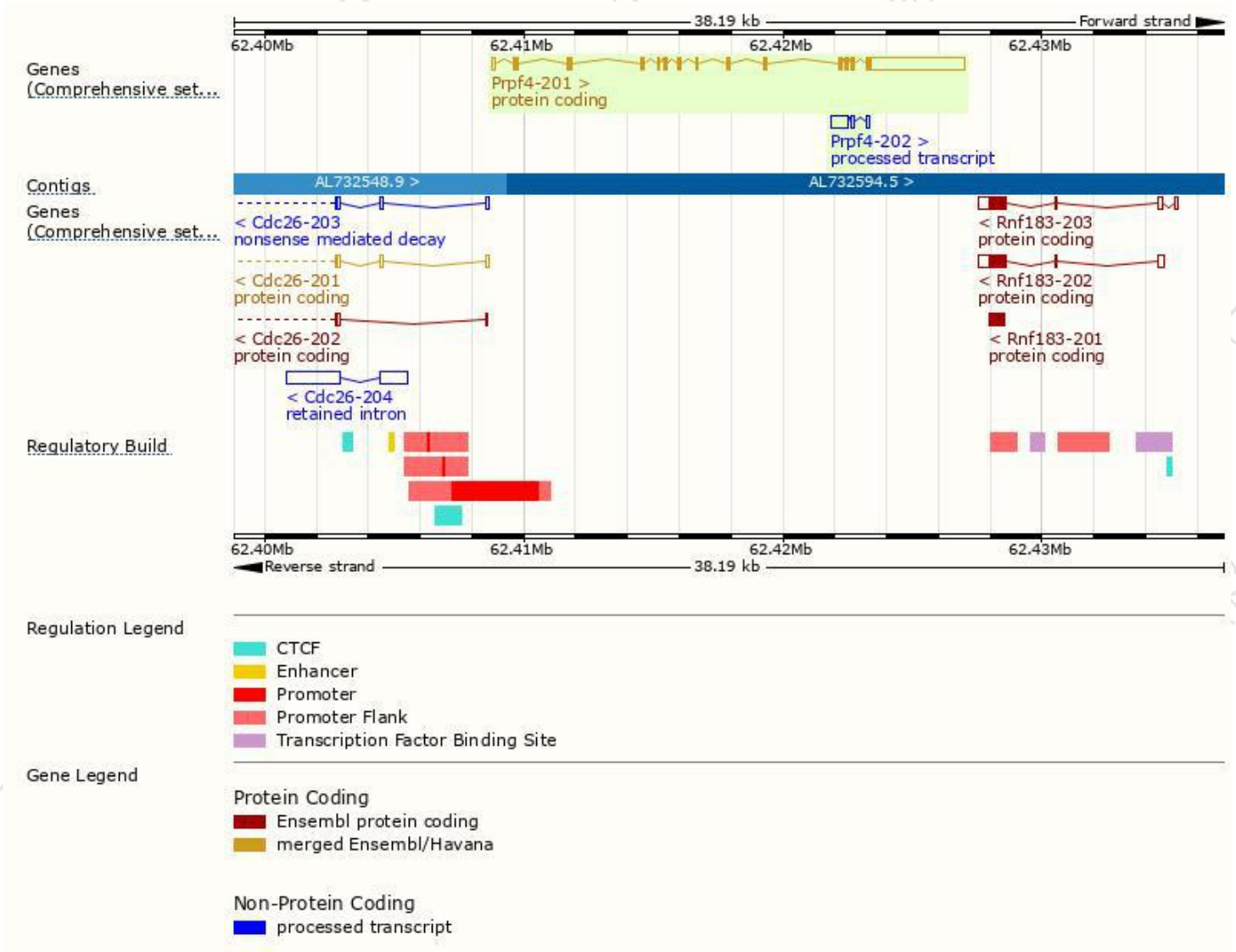
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Prpf4-201	ENSMUST00000084524.3	5223	521aa	Protein coding	CCDS18239	Q059T9 Q9DAW6	TSL:1 GENCODE basic APPRIS P1
Prpf4-202	ENSMUST00000148774.1	912	No protein	Processed transcript	-	-	TSL:2

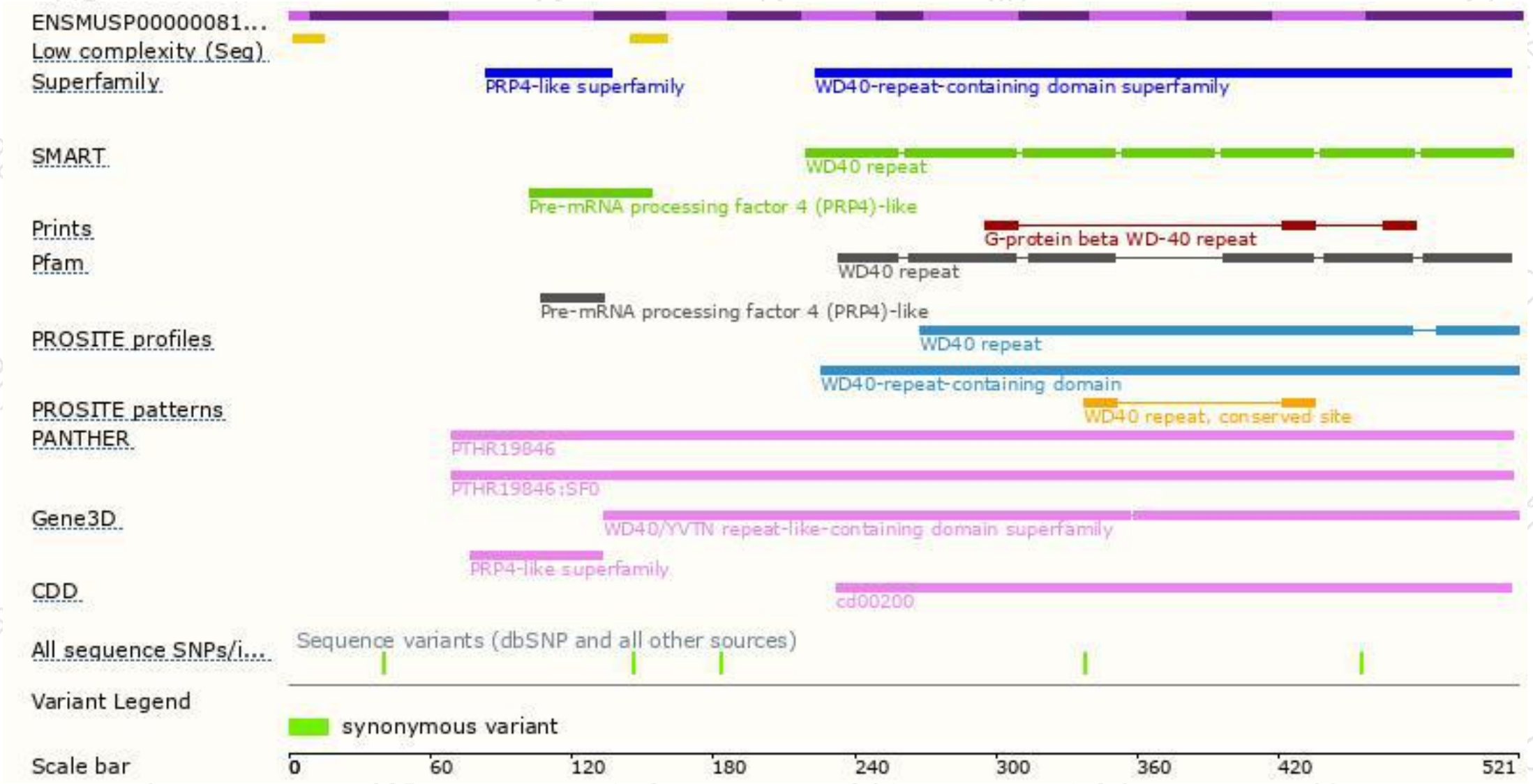
The strategy is based on the design of *Prpf4-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

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