Prf1 Cas9-KO Strategy

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Reviewer: Yumeng Wang

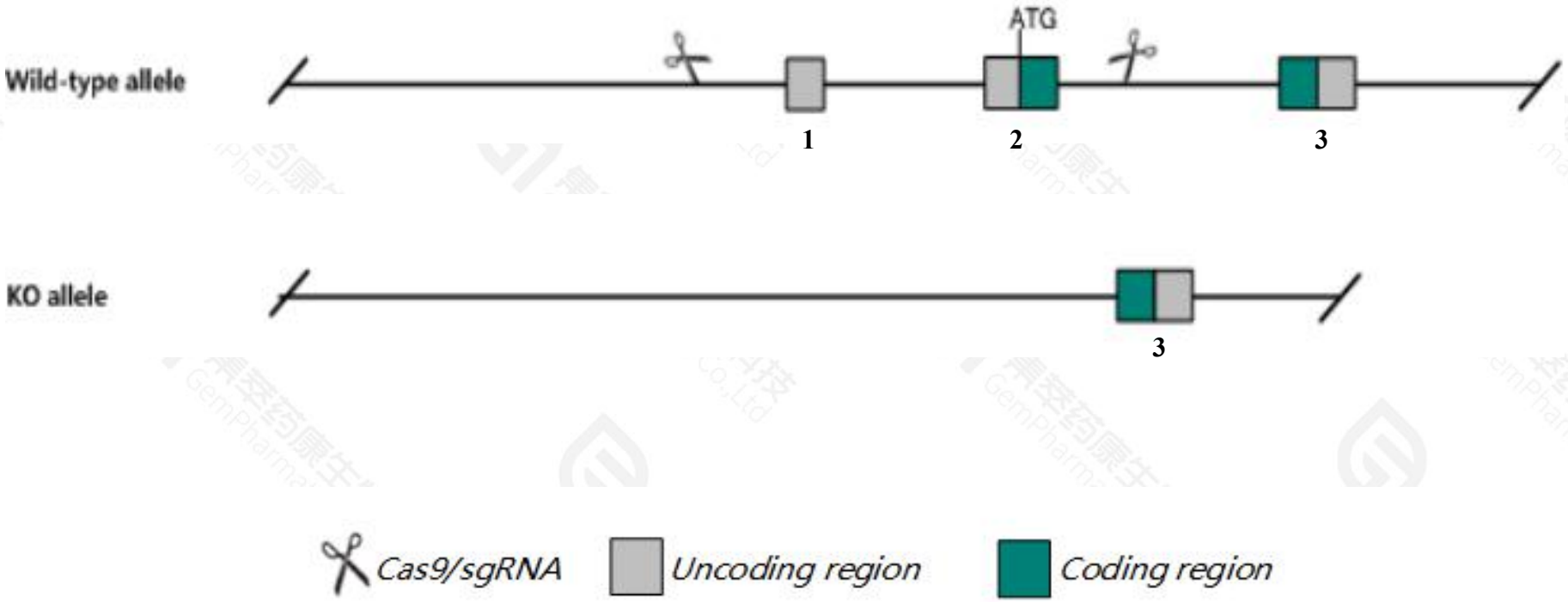
Design Date: 2021-7-15

Project Overview

Project Name	***Prf1***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Prf1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Prf1* gene has 2 transcripts. According to the structure of *Prf1* gene, exon1-exon2 of *Prf1-202*(ENSMUST00000219375.2) transcript is recommended as the knockout region.The region contains start coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Prf1* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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.

Notice

➢ According to the existing MGI data,homozygous null mice exhibit increased susceptibility to viral infection and defective cytotoxic T cell cytolysis and NK cell cytolysis.

➢ The *Prf1* gene is located on the Chr10. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

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Prf1 perforin 1 (pore forming protein) [Mus musculus (house mouse)]

Gene ID: 18646, updated on 13-Mar-2020

Summary

Official Symbol	Prf1 provided by MGI
Official Full Name	perforin 1 (pore forming protein) provided by MGI
Primary source	MGI:MGI:97551
See related	Ensembl:ENSMUSG00000037202
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	Pfn, Pfp, Prf-1
Expression	Biased expression in spleen adult (RPKM 7.1), mammary gland adult (RPKM 3.1) and 7 other tissues See more
Orthologs	human all

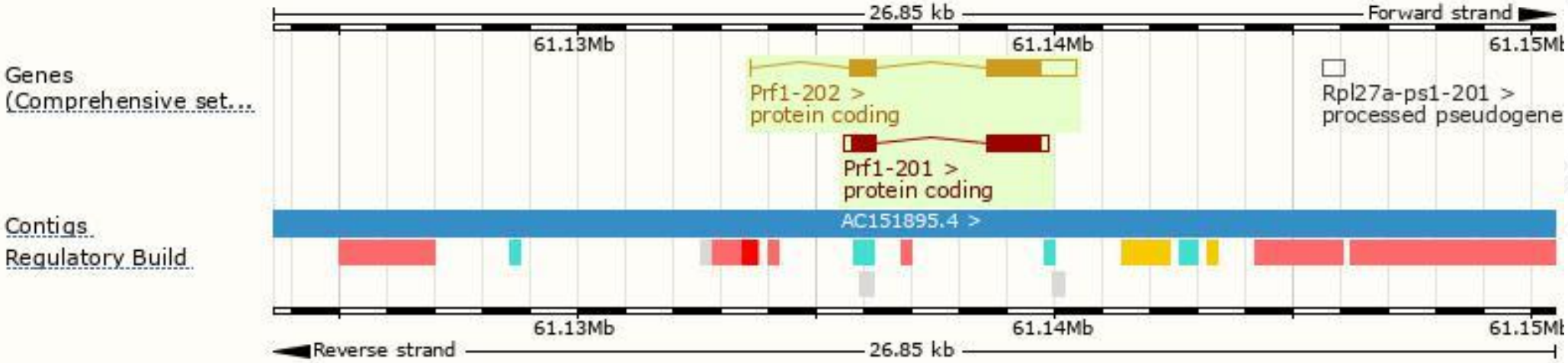
Transcript information（Ensembl）

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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Prf1-202	ENSMUST00000219375.1	2474	554aa	Protein coding	CCDS23875	A2RSY7 P10820	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
Prf1-201	ENSMUST00000035419.6	1968	554aa	Protein coding	CCDS23875	A2RSY7 P10820	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

The strategy is based on the design of *Prf1-202* transcript,the transcription is shown below:

Genomic location distribution



Regulation Legend

- CTCF
- Enhancer
- Open Chromatin
- Promoter
- Promoter Flank

Gene Legend

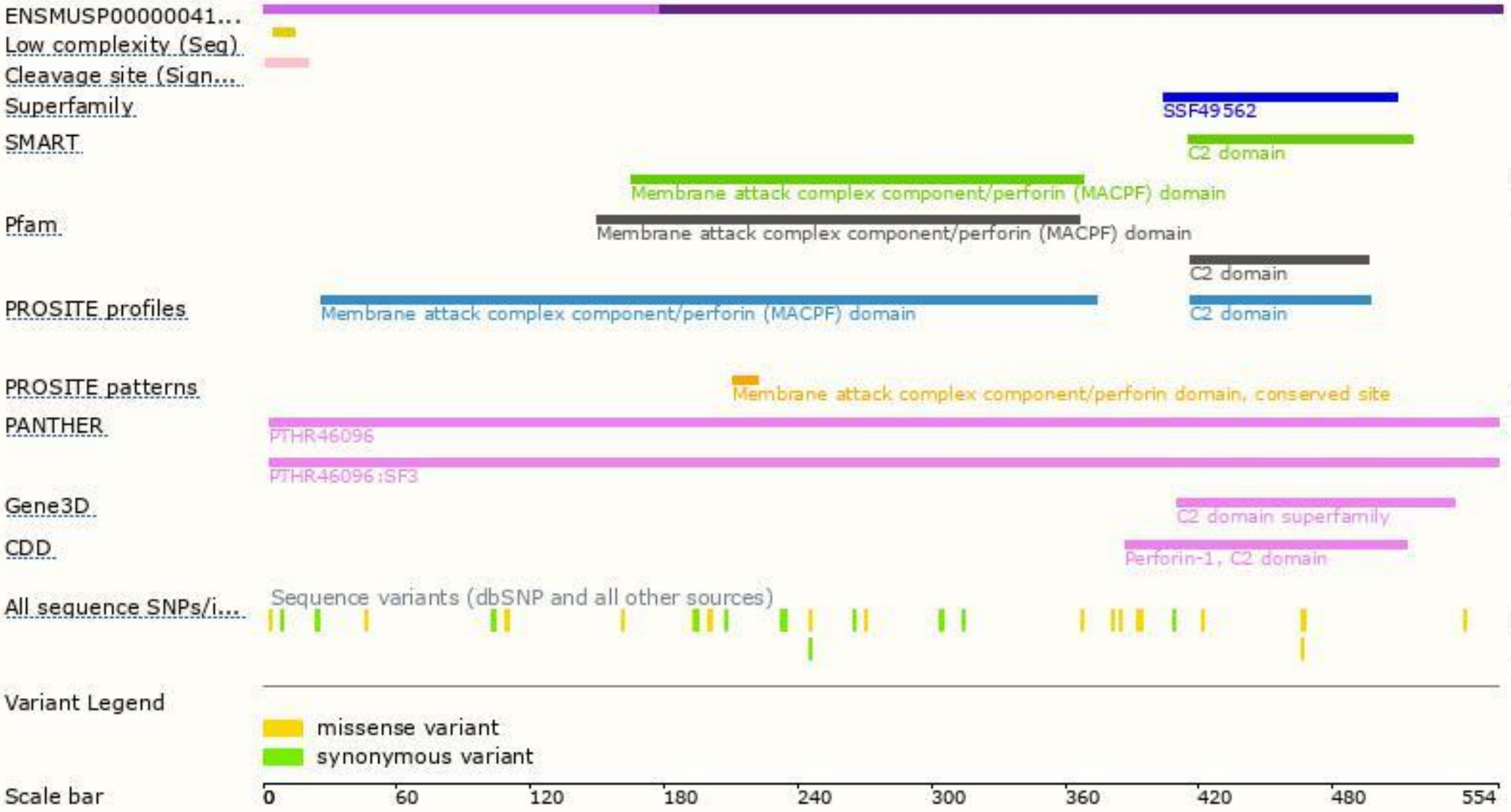
Protein Coding

- merged Ensembl/Havana
- Ensembl protein coding

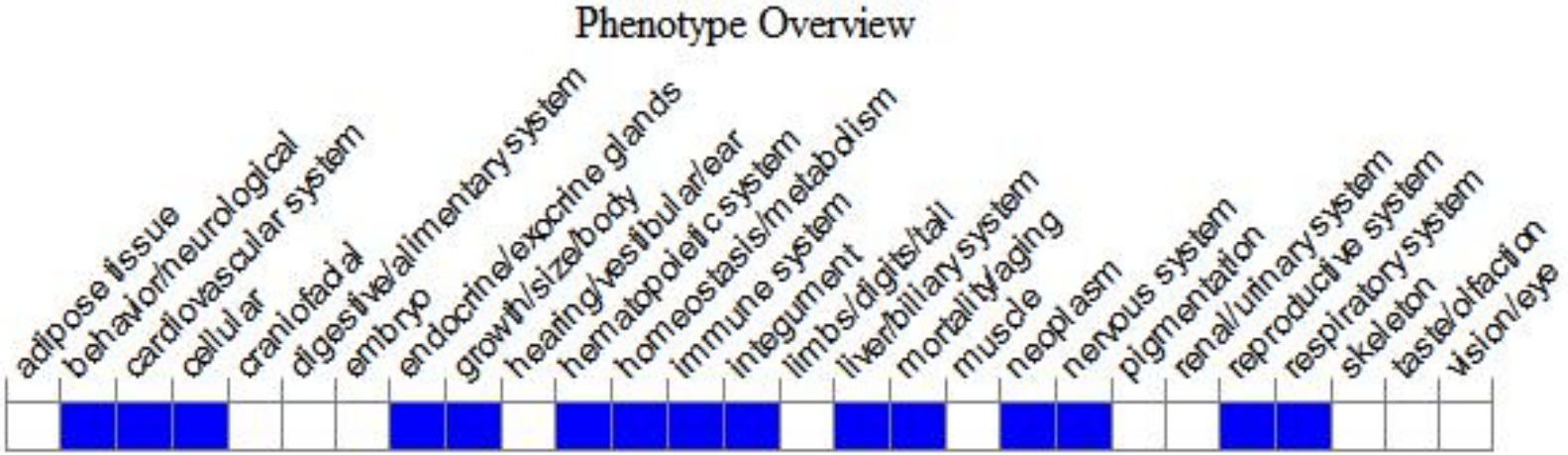
Non-Protein Coding

- pseudogene

Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

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

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