

Prfl Cas9-CKO Strategy

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Project Overview

Project Name

Prf1

Project type

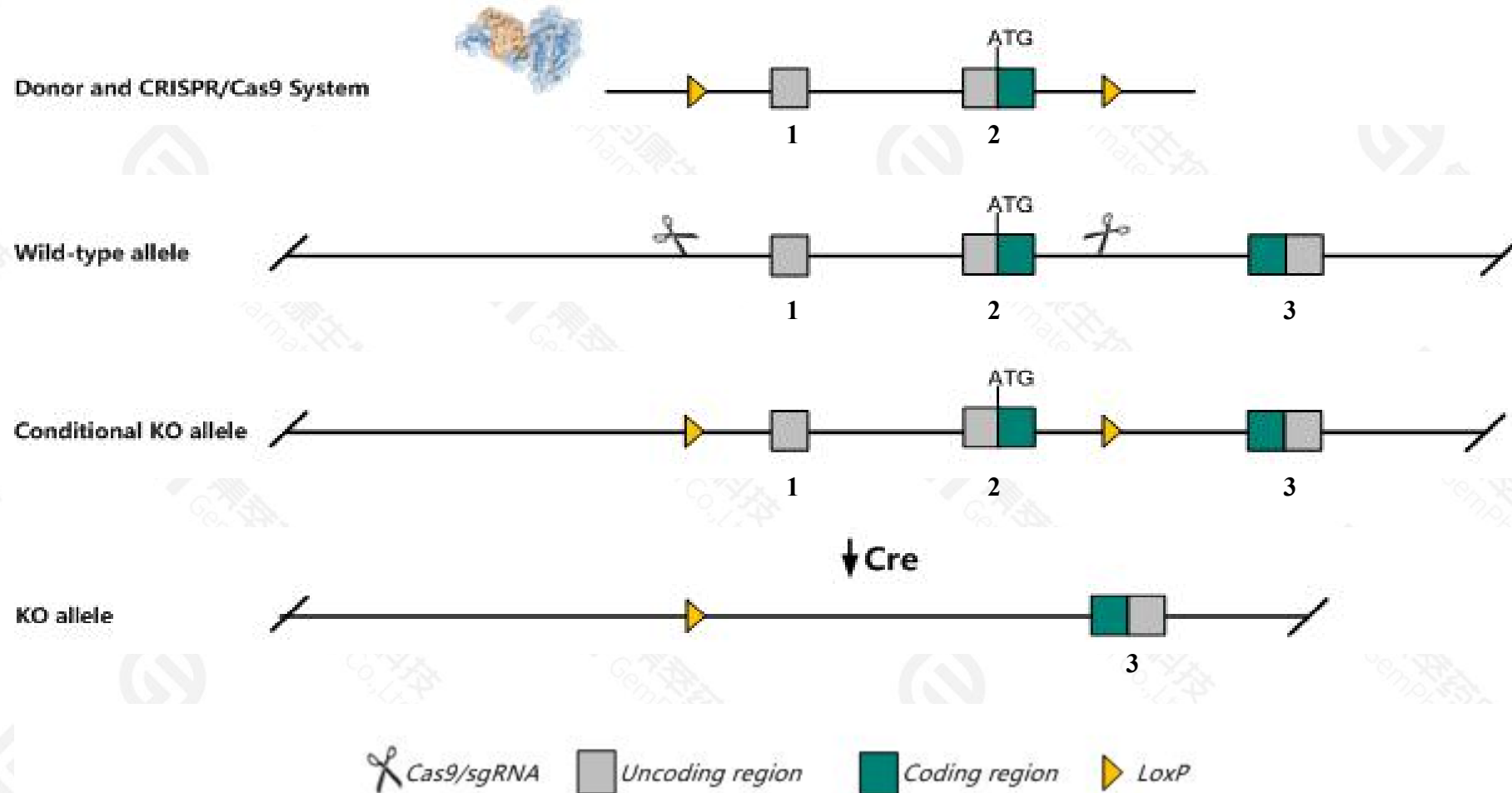
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- The *Prfl* gene has 2 transcripts. According to the structure of *Prfl* gene, exon1-exon2 of *Prfl*-202(ENSMUST00000219375.2) transcript is recommended as the knockout region. The region contains ATG start codon. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Prfl* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor 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.

- 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 *Prfl* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)

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](#)

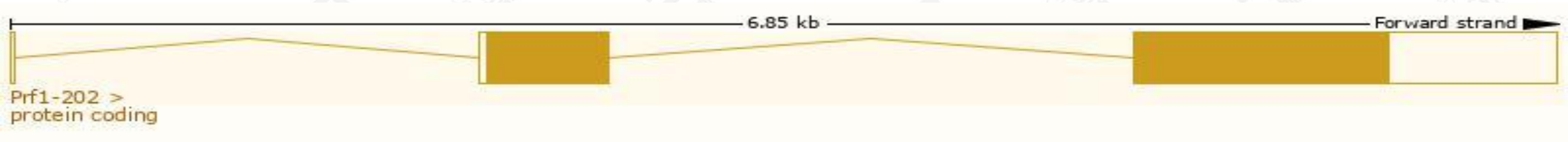
Orthologs [human](#) [all](#)

Transcript information (Ensembl)

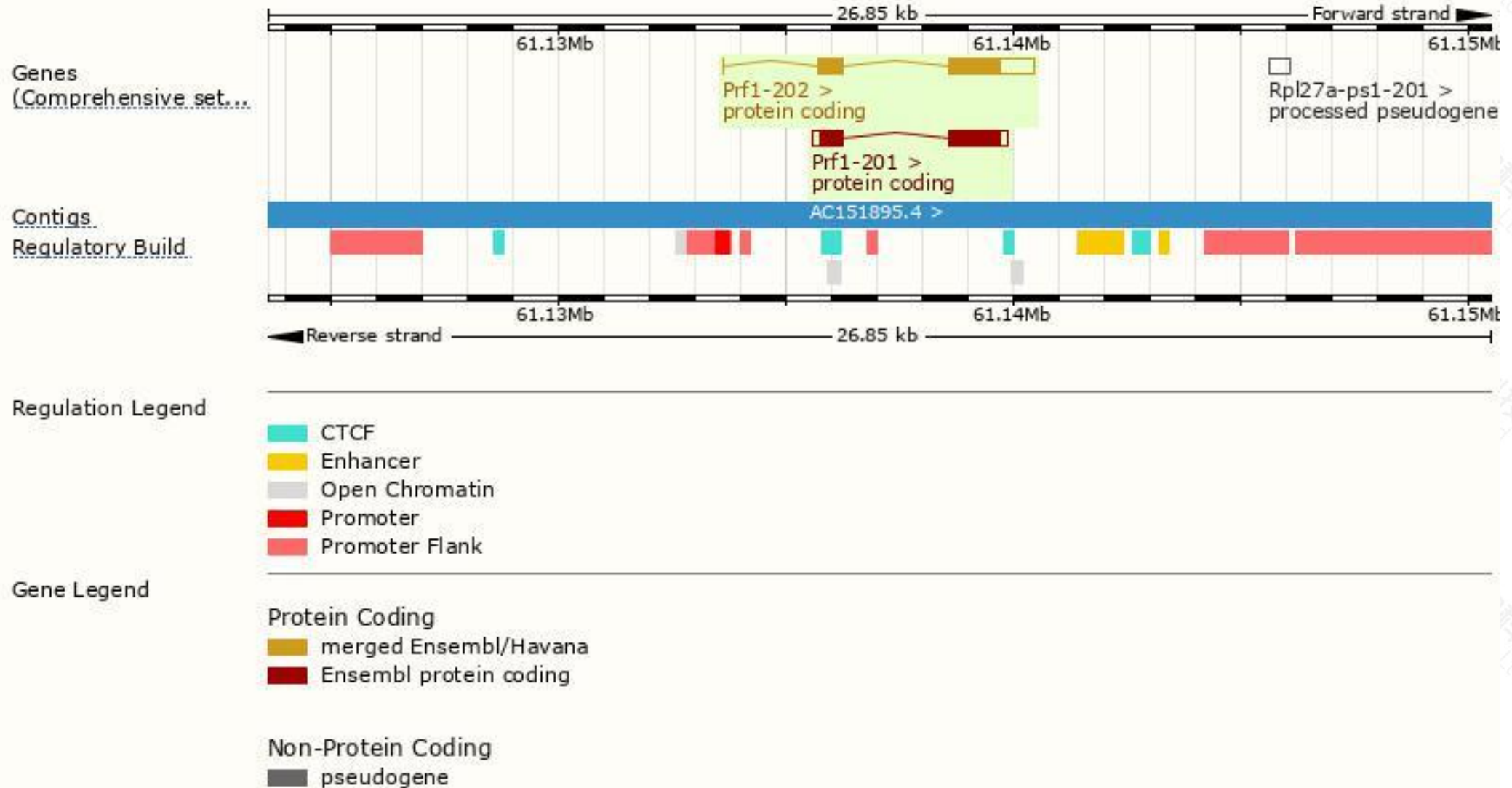
The gene has 2 transcripts,all transcripts are shown below:

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:

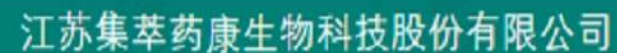


Genomic location distribution

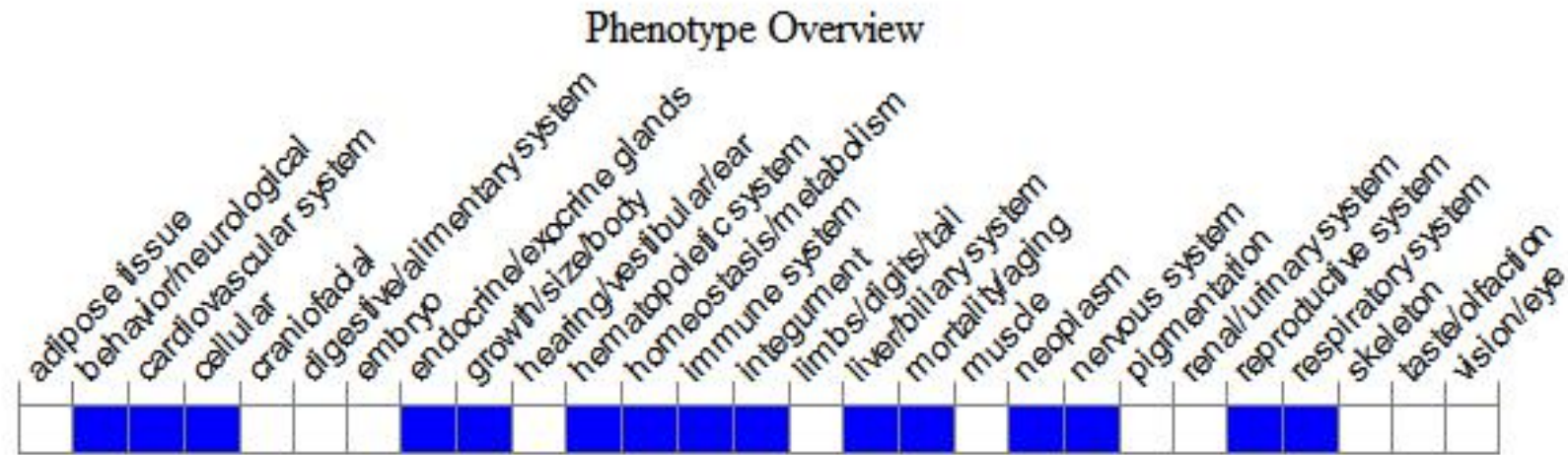




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Mouse phenotype description(MGI)



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

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

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

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