

Pfkfb1 Cas9-CKO Strategy

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Project Overview

Project Name

Pfkfb1

Project type

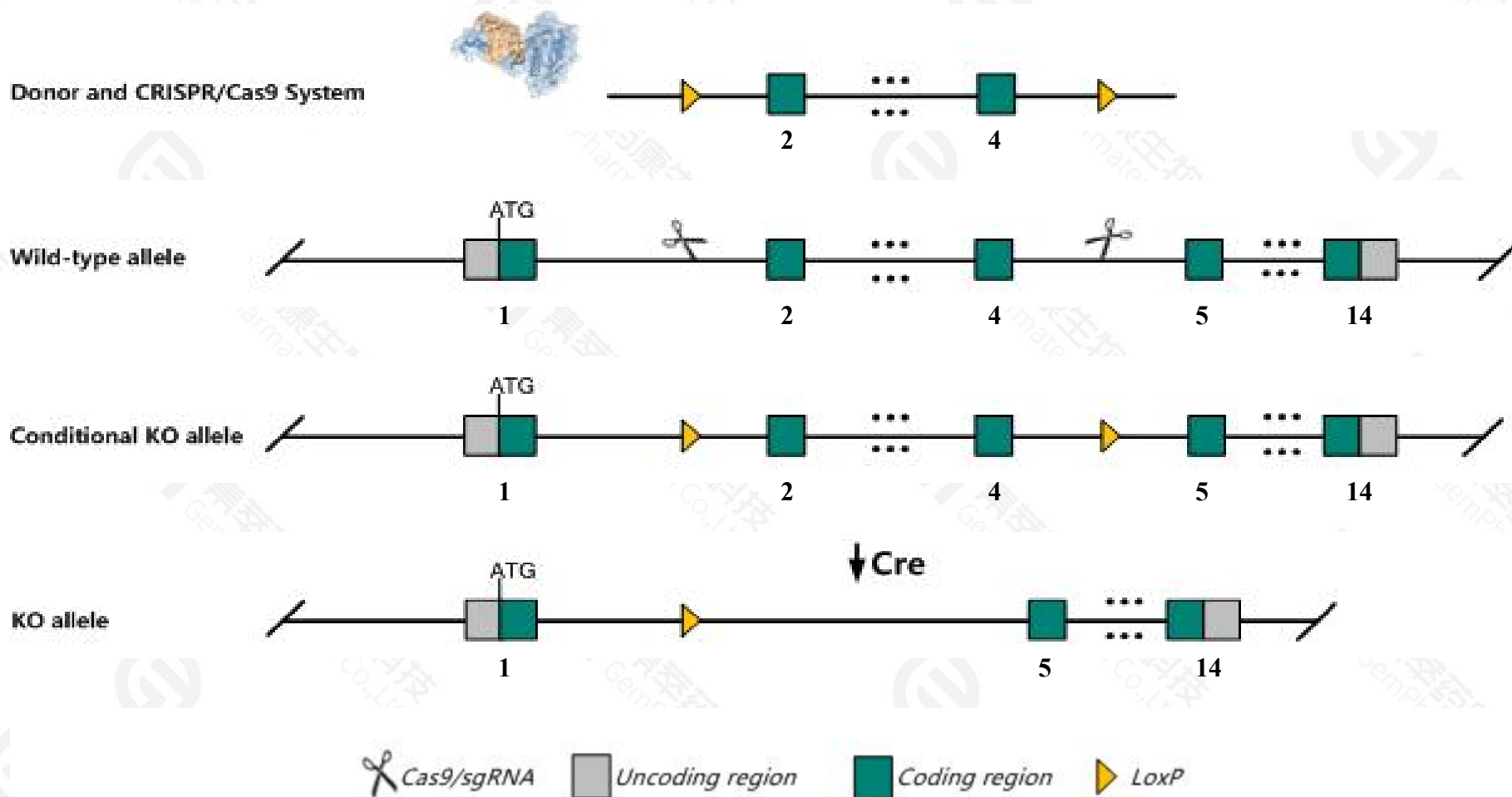
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pfkfb1* gene. The schematic diagram is as follows:



Technical routes

- The *Pfkfb1* gene has 7 transcripts. According to the structure of *Pfkfb1* gene, exon2-exon4 of *Pfkfb1-201*(ENSMUST00000080884.11) transcript is recommended as the knockout region. The region contains 287bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Pfkfb1* 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.

- The *Pfkfb1* gene is located on the ChrX. 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)

Pfkfb1 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1 [Mus musculus (house mouse)]

Gene ID: 18639, updated on 17-Feb-2021

Summary



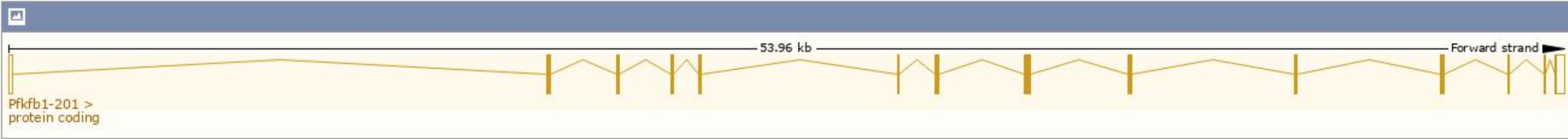
Official Symbol	Pfkfb1 provided by MGI
Official Full Name	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1 provided by MGI
Primary source	MGI:MGI:107816
See related	Ensembl:ENSMUSG00000025271
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	PFK/FBPase 1
Expression	Biased expression in liver E18 (RPKM 56.0), mammary gland adult (RPKM 15.0) and 5 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

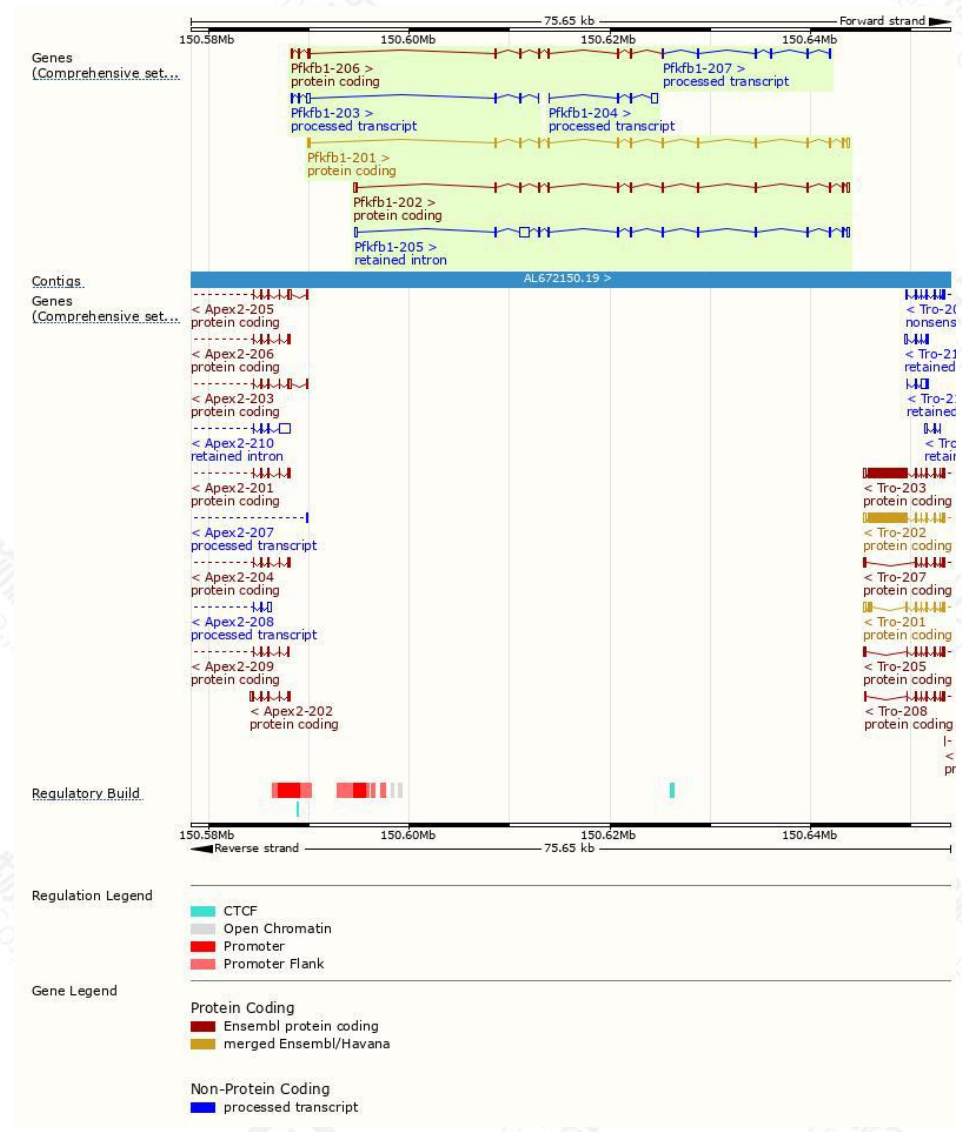
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pfkfb1-201	ENSMUST00000080884.11	1739	448aa	Protein coding	CCDS30464		TSL:1 , GENCODE basic , APPRIS P2 ,
Pfkfb1-202	ENSMUST00000112713.3	1872	471aa	Protein coding	-		TSL:5 , GENCODE basic , APPRIS ALT1 ,
Pfkfb1-206	ENSMUST00000153221.8	914	198aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Pfkfb1-204	ENSMUST00000142848.2	757	No protein	Processed transcript	-		TSL:5 ,
Pfkfb1-203	ENSMUST00000124381.8	715	No protein	Processed transcript	-		TSL:5 ,
Pfkfb1-207	ENSMUST00000154528.2	589	No protein	Processed transcript	-		TSL:5 ,
Pfkfb1-205	ENSMUST00000150783.8	2681	No protein	Retained intron	-		TSL:5 ,

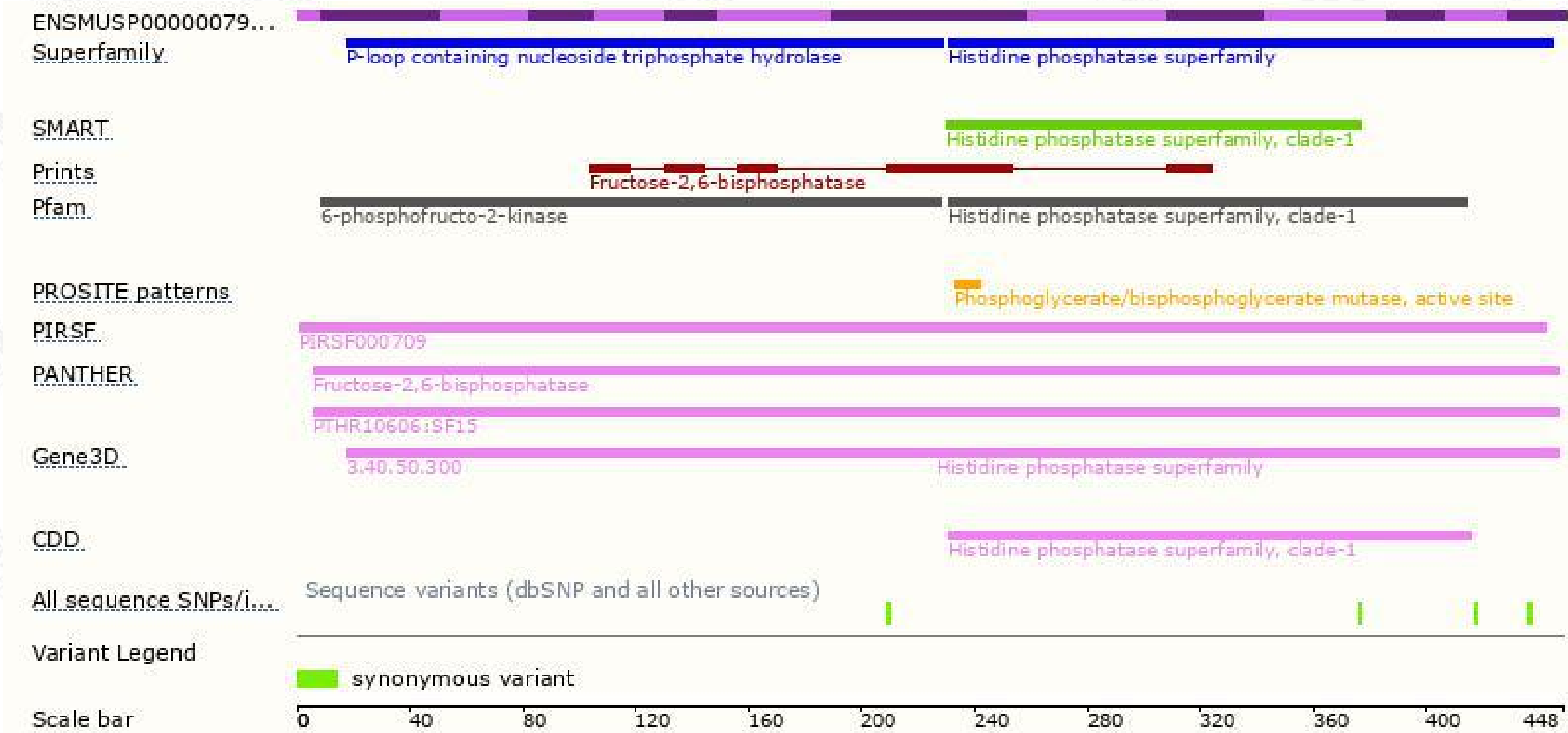
The strategy is based on the design of *Pfkfb1-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

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