

***Pfkfb4* Cas9-KO Strategy**

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Project Overview

Project Name

Pfkfb4

Project type

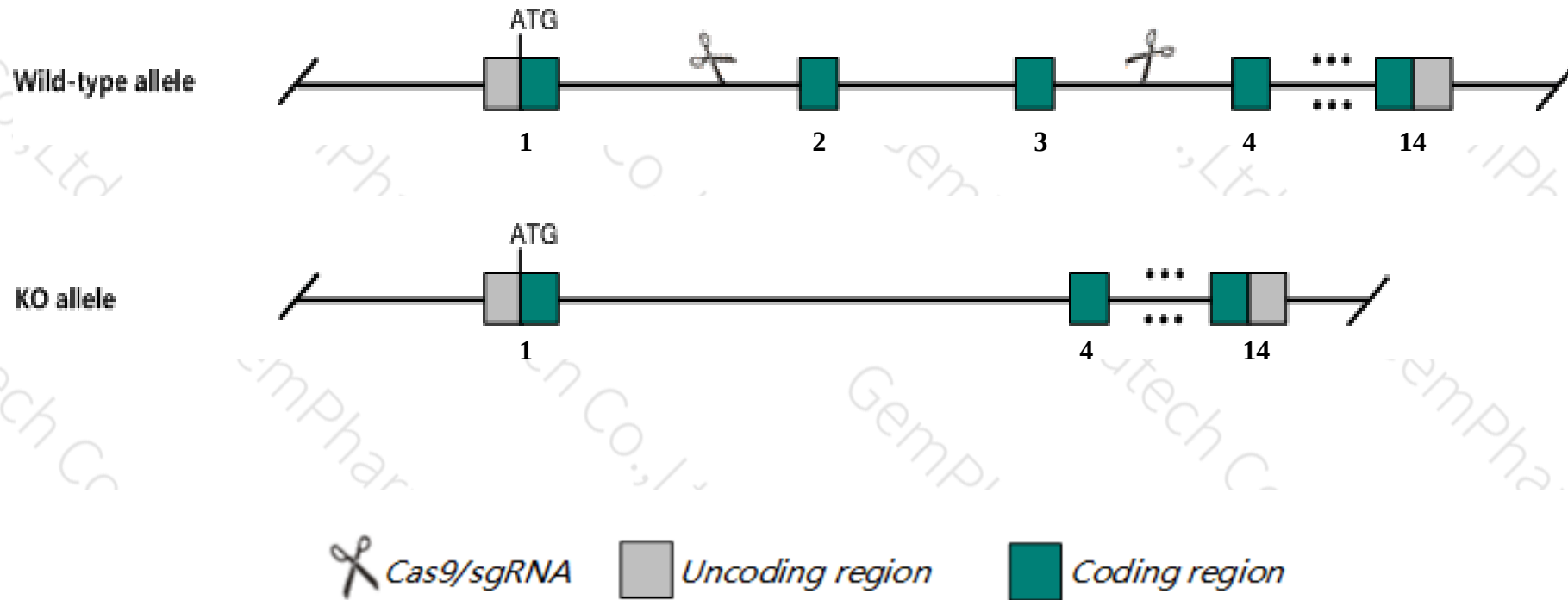
Cas9-KO

Strain background

C57BL/6JGpt

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pfkfb4* gene. The schematic diagram is as follows:



Technical routes

- The *Pfkfb4* gene has 10 transcripts. According to the structure of *Pfkfb4* gene, exon2-exon3 of *Pfkfb4*-203(ENSMUST00000198140.4) transcript is recommended as the knockout region. The region contains 214bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Pfkfb4* 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.

- Transcript 209 may not be affected.
- The *Pfkfb4* gene is located on the Chr9. 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)

Pfkfb4 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4 [Mus musculus (house mouse)]

Gene ID: 270198, updated on 13-Mar-2020

Summary



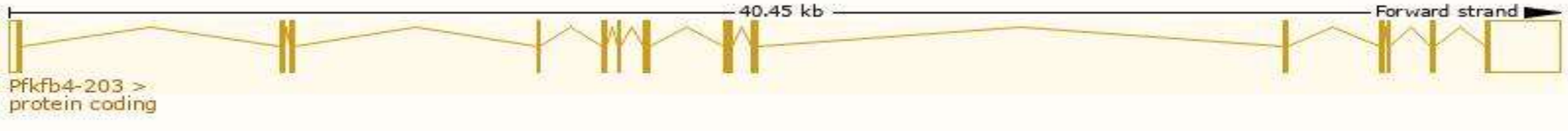
Official Symbol	Pfkfb4 provided by MGI
Official Full Name	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4 provided by MGI
Primary source	MGI:MGI:2687284
See related	Ensembl:ENSMUSG00000025648
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	C230090D14
Expression	Broad expression in large intestine adult (RPKM 18.1), small intestine adult (RPKM 17.4) and 25 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

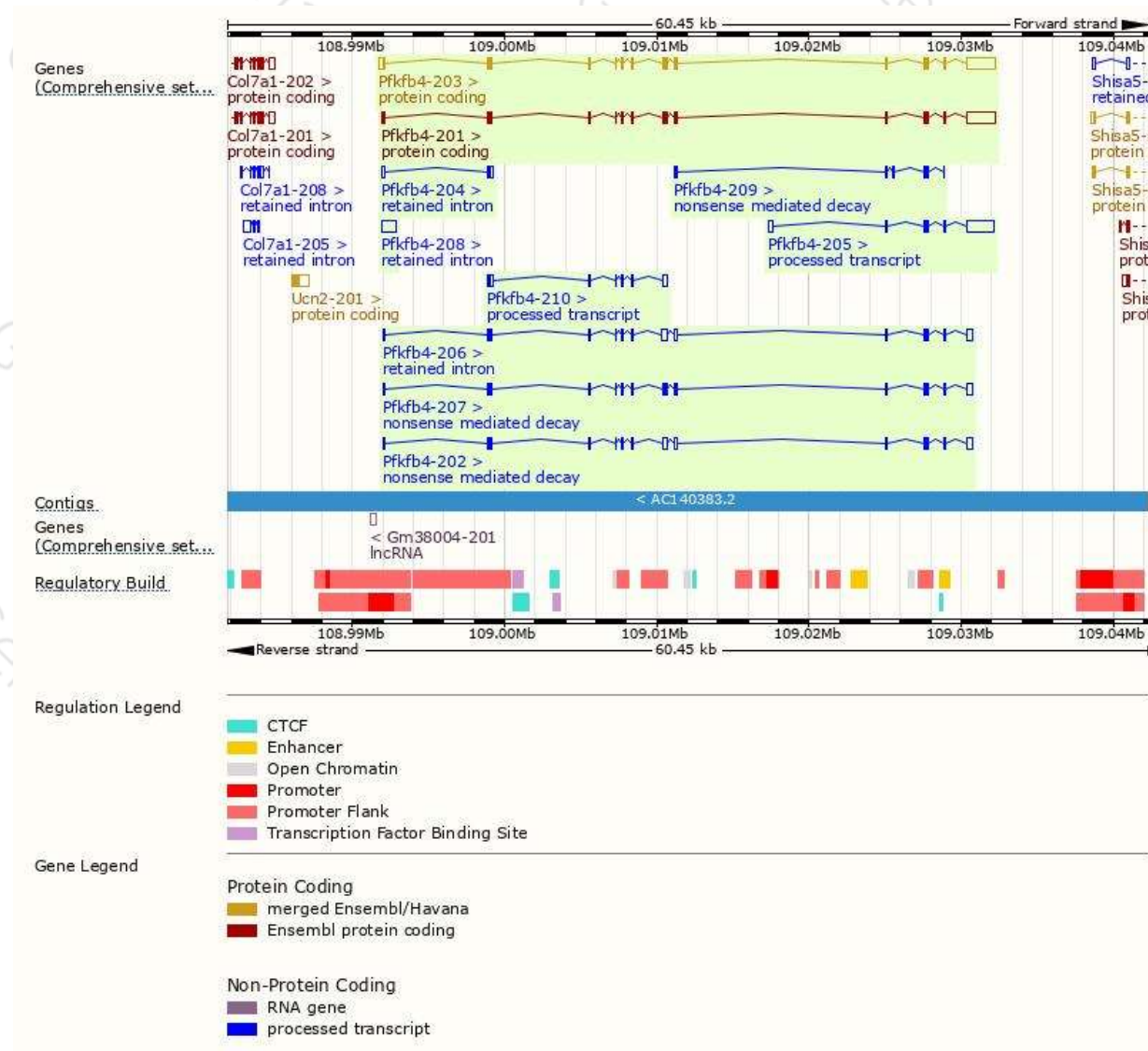
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pfkfb4-203	ENSMUST00000198140.4	3488	469aa	Protein coding	CCDS23541	Q6DTY7	TSL:1 GENCODE basic APPRIS P1
Pfkfb4-201	ENSMUST00000051873.14	3316	453aa	Protein coding	-	Q6DTY7	TSL:1 GENCODE basic
Pfkfb4-202	ENSMUST00000196249.4	1751	40aa	Nonsense mediated decay	-	A0A0G2JFP0	TSL:1
Pfkfb4-207	ENSMUST00000199591.4	1712	413aa	Nonsense mediated decay	-	Q6DTY7	TSL:1
Pfkfb4-209	ENSMUST00000200015.5	591	95aa	Nonsense mediated decay	-	A0A0G2JDE6	CDS 5' incomplete TSL:5
Pfkfb4-205	ENSMUST00000198763.1	2446	No protein	Processed transcript	-	-	TSL:1
Pfkfb4-210	ENSMUST00000200229.1	811	No protein	Processed transcript	-	-	TSL:5
Pfkfb4-206	ENSMUST00000199184.4	1841	No protein	Retained intron	-	-	TSL:1
Pfkfb4-208	ENSMUST00000199620.1	910	No protein	Retained intron	-	-	TSL:NA
Pfkfb4-204	ENSMUST00000198277.4	439	No protein	Retained intron	-	-	TSL:2

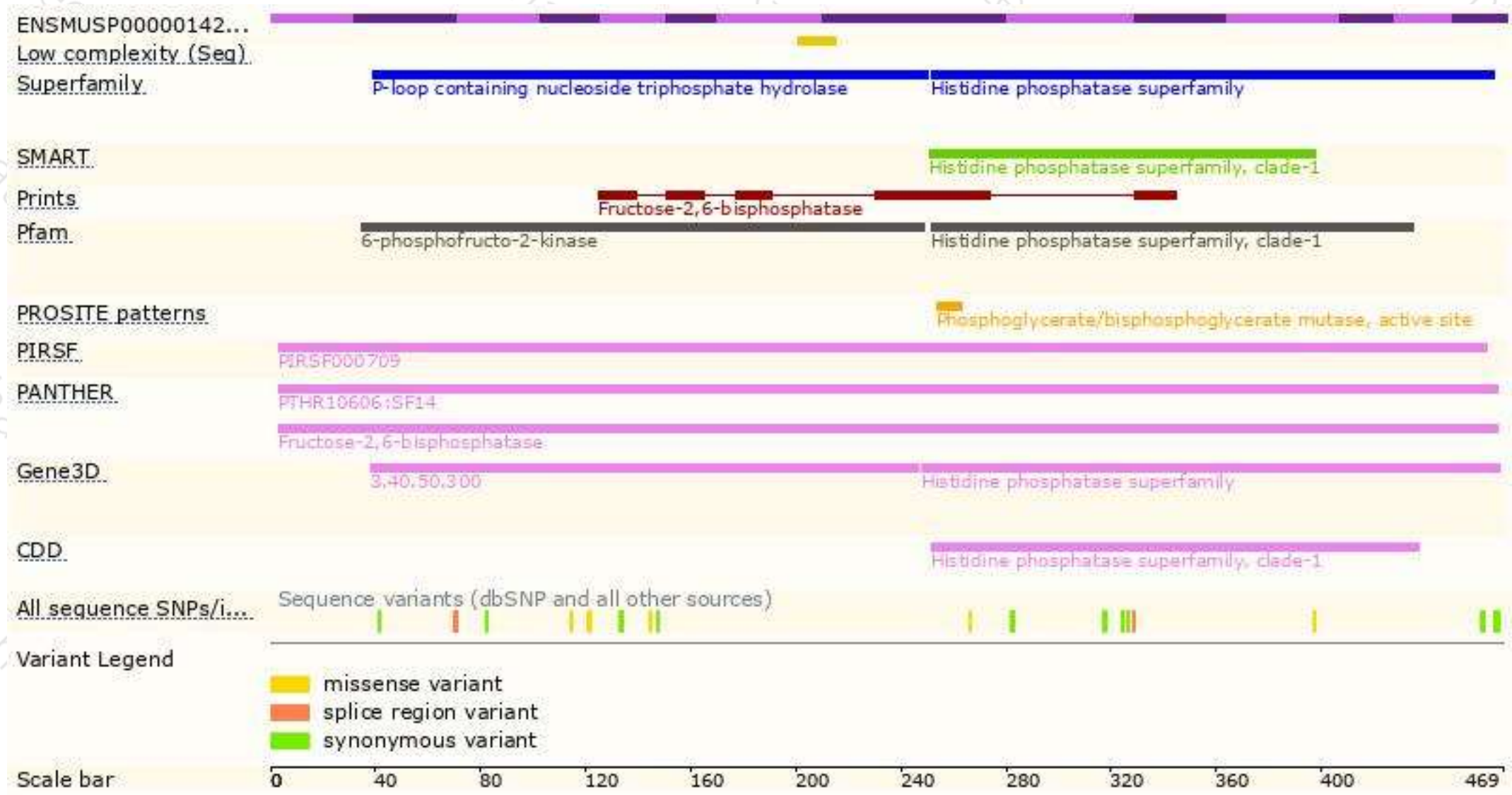
The strategy is based on the design of *Pfkfb4-203* transcript,the transcription is shown below:



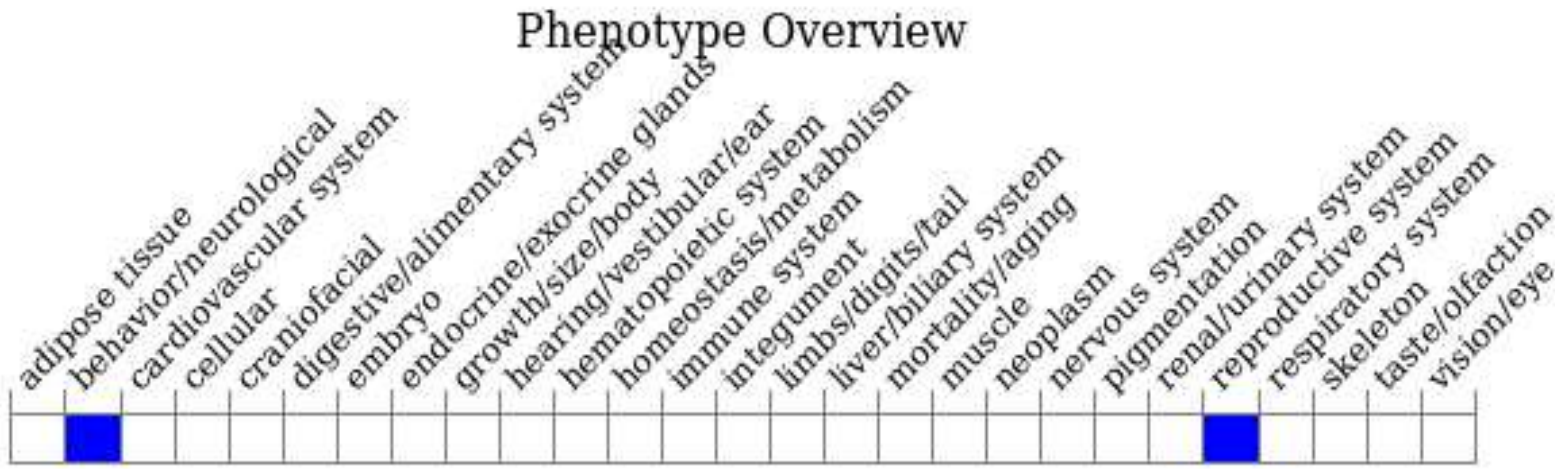
Genomic location distribution



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/>).

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

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