

# *Pfkm* Cas9-KO Strategy

**Designer: Longyun Hu**

**Reviewer: Rui Xiong**

**Design Date: 2021-3-22**

# Project Overview

---

**Project Name**

*Pfkm*

---

**Project type**

**Cas9-KO**

---

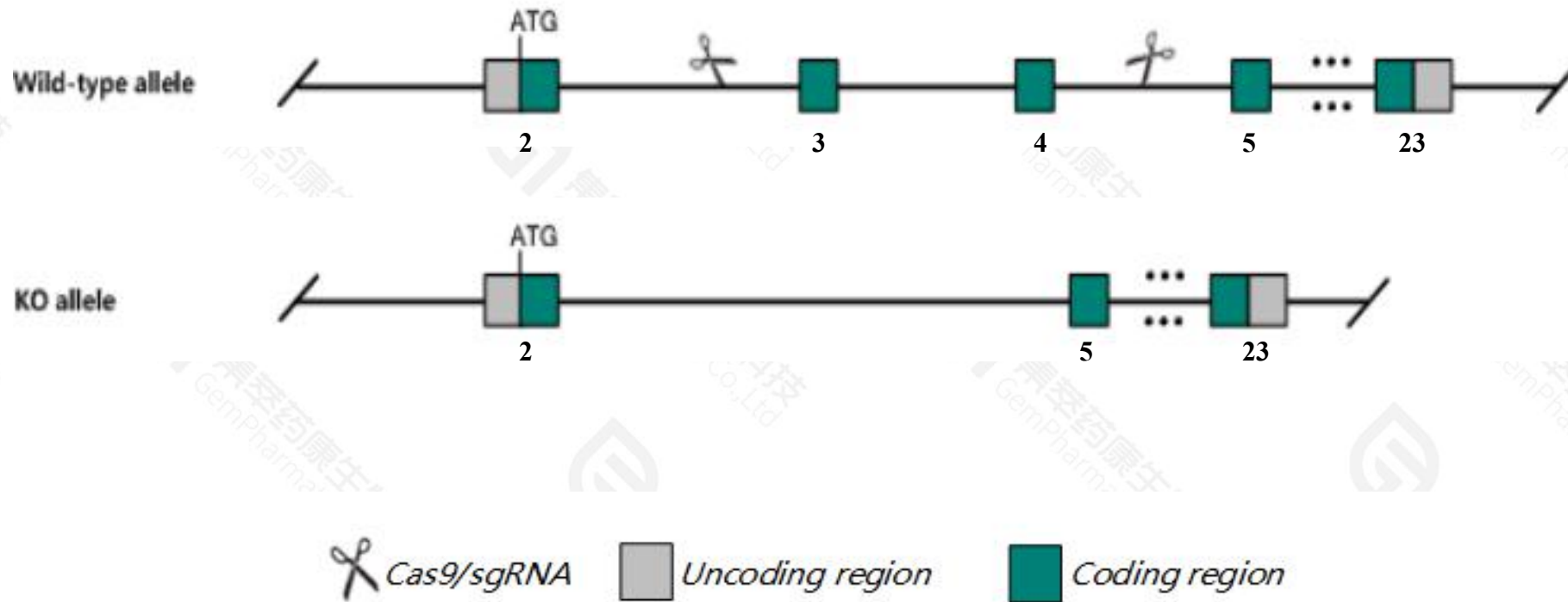
**Strain background**

**C57BL/6JGpt**

---

# Knockout strategy

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



- The *Pfkm* gene has 5 transcripts. According to the structure of *Pfkm* gene, exon3-exon4 of *Pfkm*-201(ENSMUST00000051226.8) transcript is recommended as the knockout region. The region contains 152bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Pfkm* 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.



- According to the existing MGI data, mice homozygous for a gene trapped allele exhibit abnormal glucose homeostasis. Mice homozygous for a knock-out allele exhibit premature death, exercise intolerance, abnormal glucose homeostasis, cardiomegaly, splenomegaly, and abnormal muscle morphology and physiology.
- The *Pfkm* gene is located on the Chr15. 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.

## Pfkm phosphofructokinase, muscle [Mus musculus (house mouse)]

Gene ID: 18642, updated on 10-Jan-2021

### Summary



**Official Symbol** Pfkm provided by [MGI](#)

**Official Full Name** phosphofructokinase, muscle provided by [MGI](#)

**Primary source** [MGI:MGI:97548](#)

**See related** [Ensembl:ENSMUSG00000033065](#)

**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** AI131669, ATP-PFK, PFK-A, PFK-M, Pfk, Pfk-, Pfk-4, Pfk4, PfkA, Pfkx

**Expression** Broad expression in heart adult (RPKM 178.4), cerebellum adult (RPKM 100.9) and 18 other tissues [See more](#)

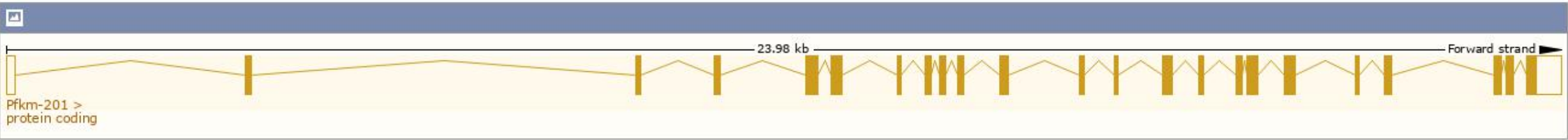
**Orthologs** [human](#) [all](#)

# Transcript information (Ensembl)

The gene has 5 transcripts,all transcripts are shown below:

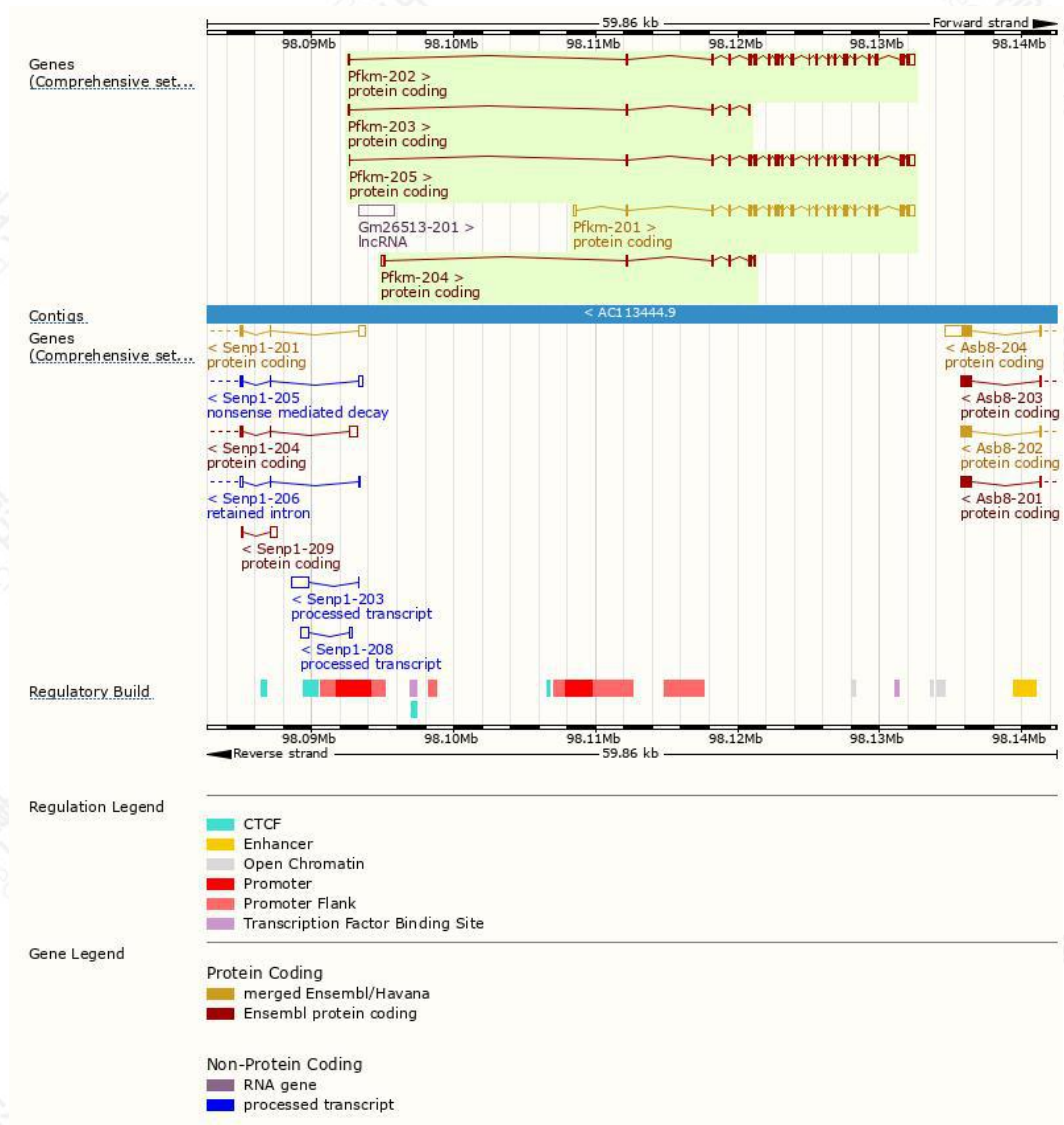
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pfkm-201	<a href="#">ENSMUST00000051226.8</a>	2879	<a href="#">780aa</a>	Protein coding	<a href="#">CCDS27786</a>		TSL:1 , GENCODE basic , APPRIS P1 ,
Pfkm-202	<a href="#">ENSMUST00000163507.8</a>	2816	<a href="#">780aa</a>	Protein coding	<a href="#">CCDS27786</a>		TSL:5 , GENCODE basic , APPRIS P1 ,
Pfkm-205	<a href="#">ENSMUST00000230445.2</a>	2798	<a href="#">780aa</a>	Protein coding	<a href="#">CCDS27786</a>		GENCODE basic , APPRIS P1 ,
Pfkm-204	<a href="#">ENSMUST00000229344.2</a>	785	<a href="#">197aa</a>	Protein coding	-		CDS 3' incomplete ,
Pfkm-203	<a href="#">ENSMUST00000229280.2</a>	438	<a href="#">117aa</a>	Protein coding	-		CDS 3' incomplete ,

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



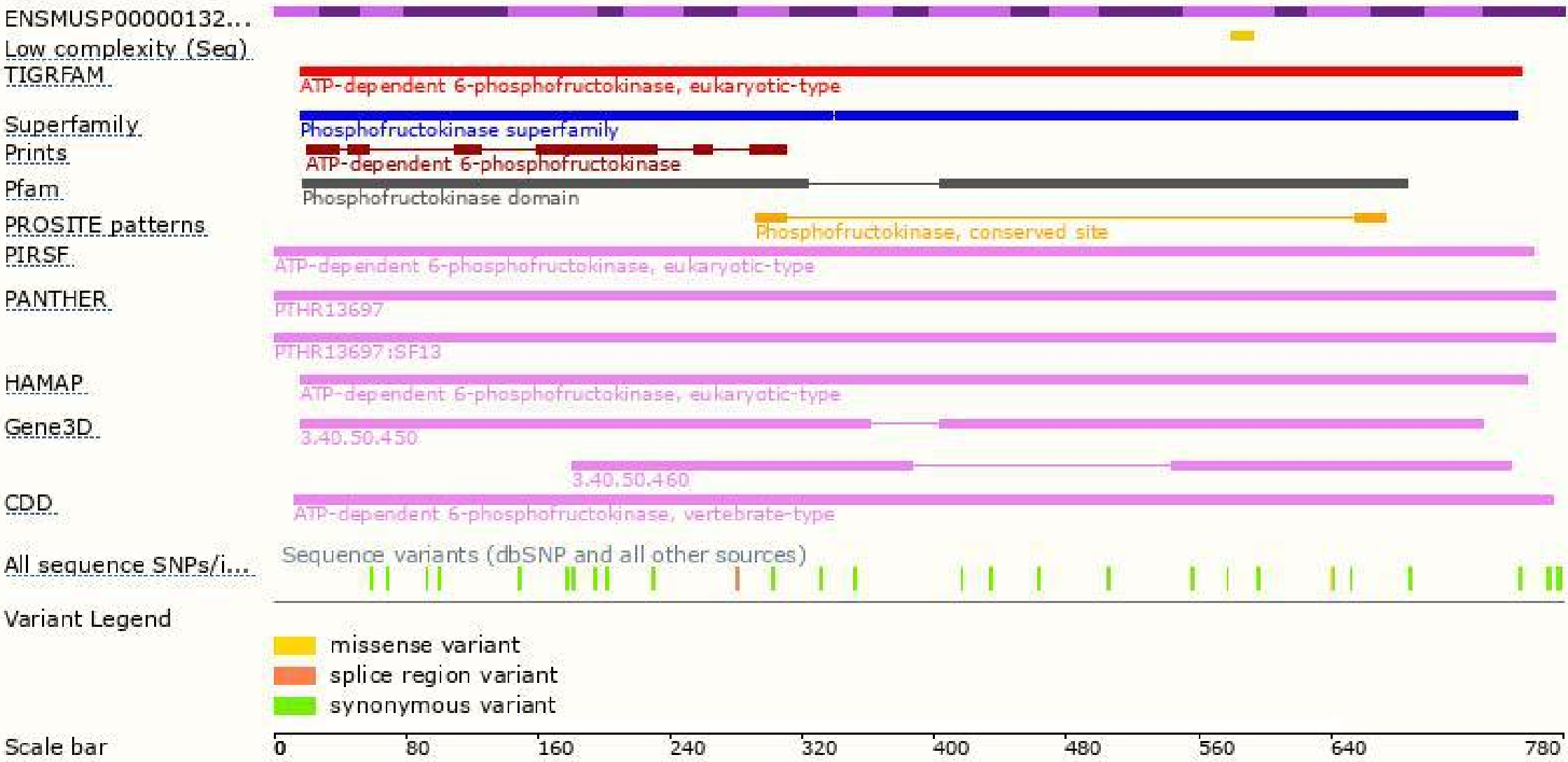


# Genomic location distribution

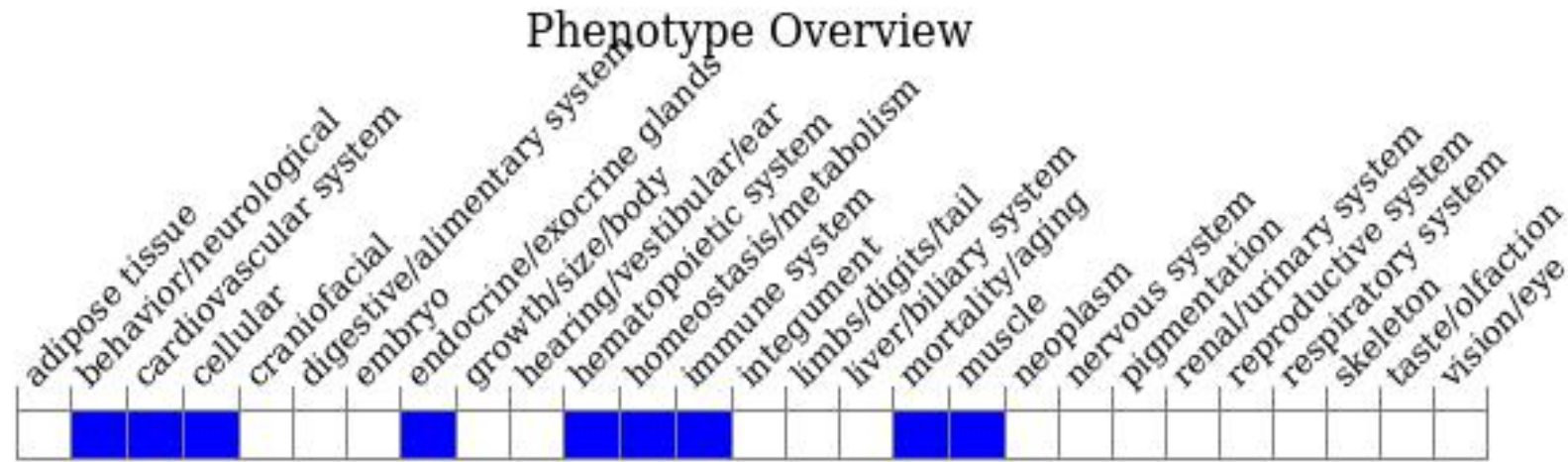




# Protein domain



# Mouse phenotype description(MGI )



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

According to the existing MGI data, mice homozygous for a gene trapped allele exhibit abnormal glucose homeostasis. Mice homozygous for a knock-out allele exhibit premature death, exercise intolerance, abnormal glucose homeostasis, cardiomegaly, splenomegaly, and abnormal muscle morphology and physiology.

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

Tel: 025-5864 1534

