Pfkfb2 Cas9-KO Strategy

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Project Overview

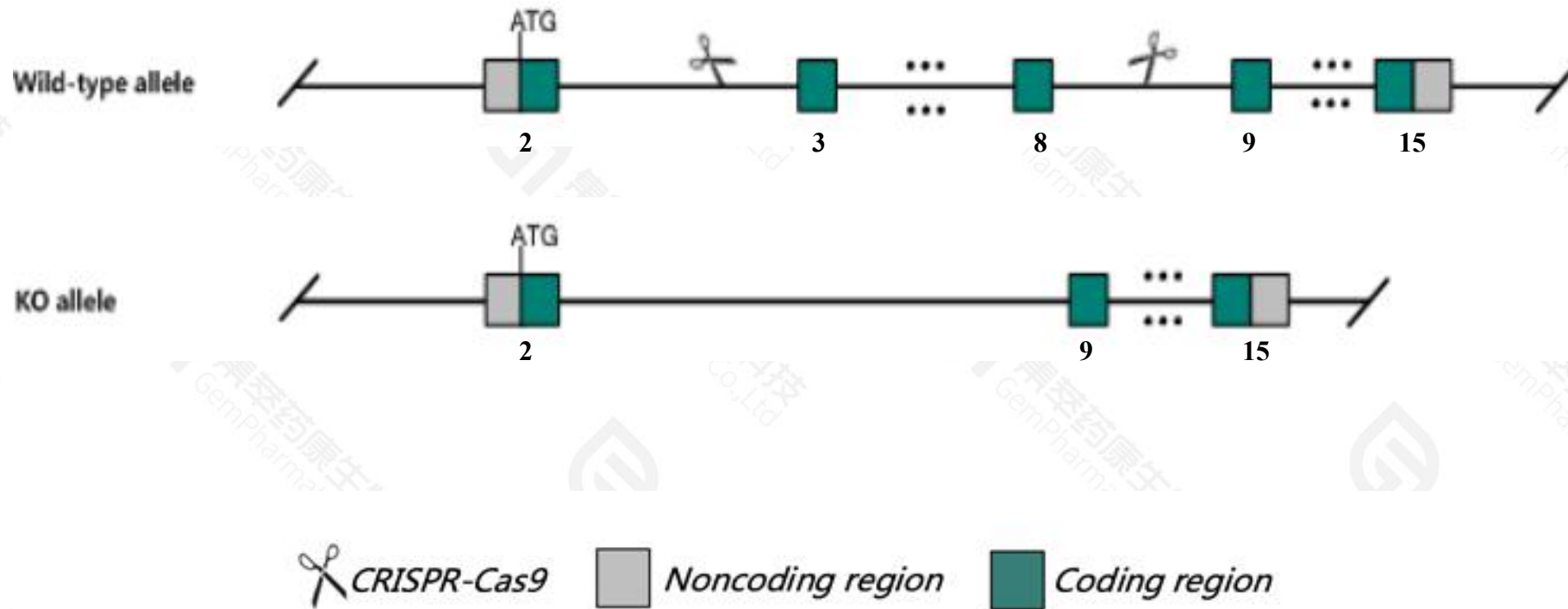|	
Project Name	***Pfkfb2***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR-Cas9 technology to edit the *Pfkfb2* gene. The schematic diagram is as follows:

Technical routes

➤ The *Pfkfb2* gene has 13 transcripts. According to the structure of *Pfkfb2* gene, exon3-exon8 of *Pfkfb2-202*(ENSMUST00000066863.13) transcript is recommended as the knockout region.The region contains 547bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR-Cas9 technology to modify *Pfkfb2* gene. The brief process is as follows: CRISPR-Cas9 system 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 partial sequence of Gm29427 will be deleted.

➢ The *Pfkfb2* gene is located on the Chr1. 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.

Pfkfb2 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2 [Mus musculus (house mouse)]

Gene ID: 18640, updated on 13-Mar-2020

▲ Summary

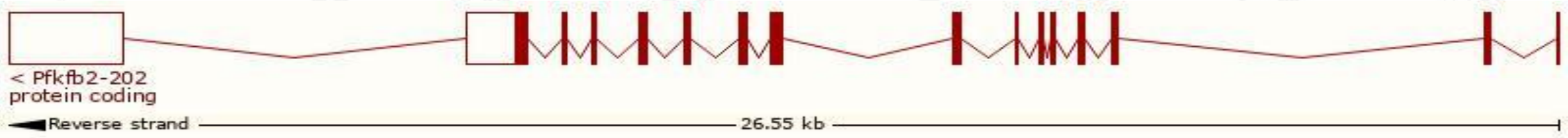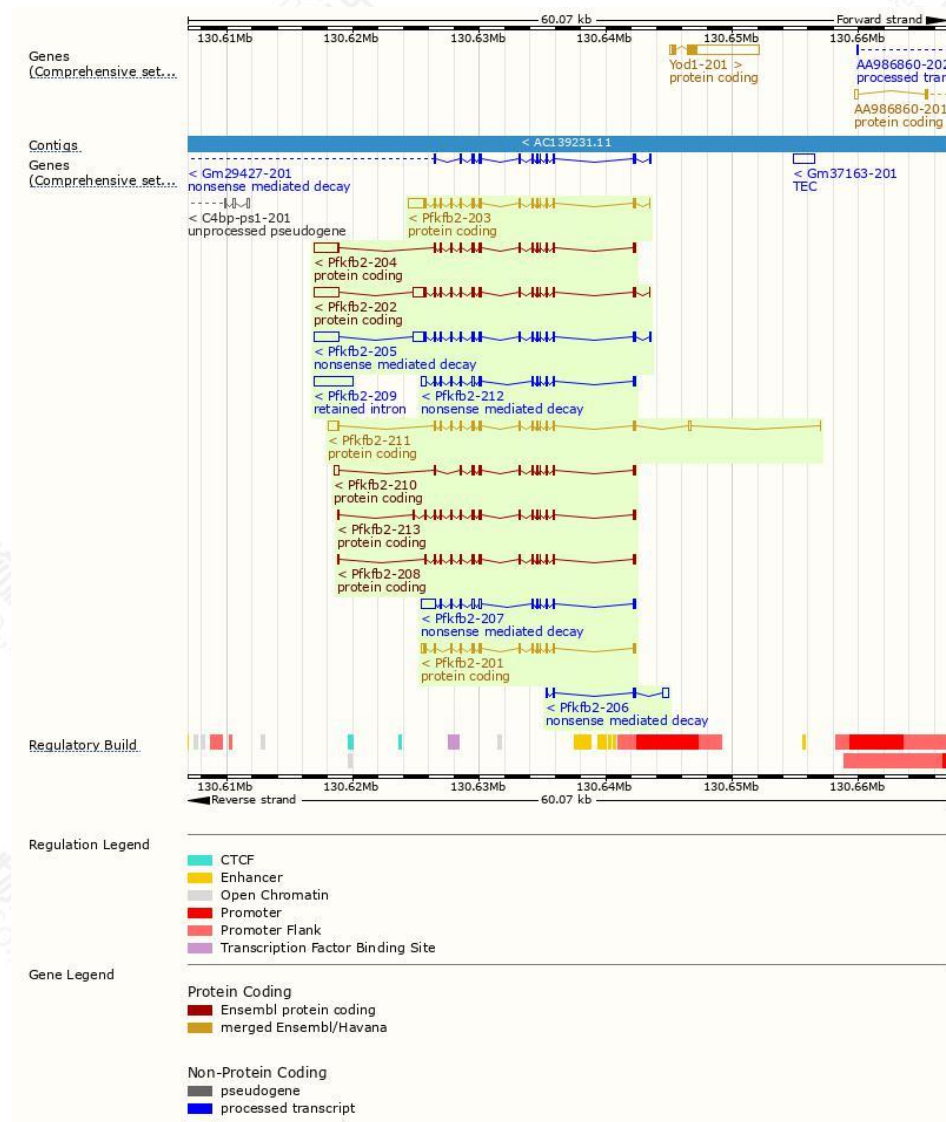|	
Official Symbol	Pfkfb2 provided by MGI
Official Full Name	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2 provided by MGI
Primary source	MGI:MGI:107815
See related	Ensembl:ENSMUSG00000026409
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	4930568D07Rik
Expression	Ubiquitous expression in heart adult (RPKM 6.3), frontal lobe adult (RPKM 5.3) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 13 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pfkfb2-202	ENSMUST00000066863.12	4425	518aa	Protein coding	CCDS35701	Q6GTL7	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 P3
Pfkfb2-204	ENSMUST00000171479.7	3342	473aa	Protein coding	CCDS48350	B2Z893	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 ALT2
Pfkfb2-203	ENSMUST00000169659.7	2849	518aa	Protein coding	CCDS35701	Q6GTL7	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 P3
Pfkfb2-211	ENSMUST00000189534.6	2454	473aa	Protein coding	CCDS48350	B2Z893	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 ALT2
Pfkfb2-201	ENSMUST00000050406.10	1701	497aa	Protein coding	CCDS48349	B2Z892	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 ALT2
Pfkfb2-213	ENSMUST00000191347.6	1759	556aa	Protein coding	-	A0A087WRM7	TSL:5 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 ALT2
Pfkfb2-208	ENSMUST00000187089.6	1690	533aa	Protein coding	-	A0A087WRG0	TSL:5 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 ALT2
Pfkfb2-210	ENSMUST00000189167.6	1558	374aa	Protein coding	-	B2Z894	TSL:1 GENCODE basic
Pfkfb2-205	ENSMUST00000185233.6	4505	518aa	Nonsense mediated decay	-	Q6GTL7	TSL:1
Pfkfb2-207	ENSMUST00000186867.6	2223	55aa	Nonsense mediated decay	-	A0A087WRW5	TSL:1
Pfkfb2-212	ENSMUST00000191301.1	1639	196aa	Nonsense mediated decay	-	B2Z891	TSL:1
Pfkfb2-206	ENSMUST00000186777.1	768	55aa	Nonsense mediated decay	-	A0A087WRW5	TSL:5
Pfkfb2-209	ENSMUST00000187522.1	3057	No protein	Retained intron	-	-	TSL:NA

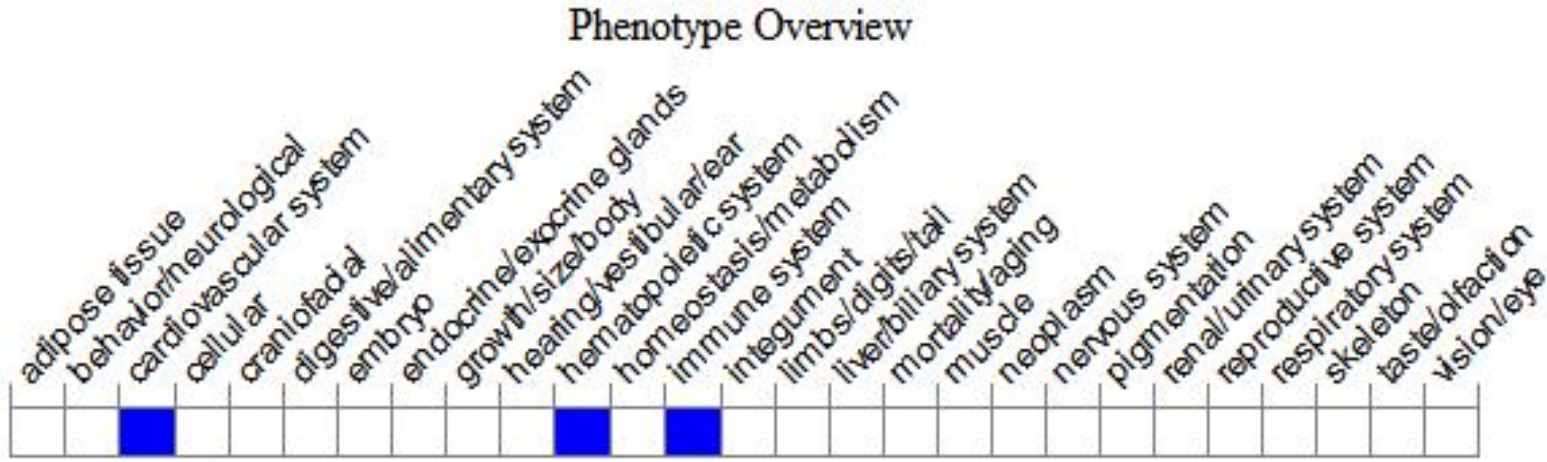The strategy is based on the design of *Pfkfb2-202* transcript,the transcription is shown below:

< Pfkfb2-202
protein coding

◄— Reverse strand

|———————————————————— 26.55 kb ————————————————————|

Genomic location distribution

Protein domain

ENSMUSP00000066...		
MobiDB lite		
Superfamily	P-loop containing nucleoside triphosphate hydrolase	Histidine phosphatase superfamily
SMART		Histidine phosphatase superfamily, clade-1
Prints	Fructose-2,6-bisphosphatase	
Pfam	6-phosphofructo-2-kinase	Histidine phosphatase superfamily, clade-1
PROSITE patterns		Phosphoglycerate/bisphosphoglycerate mutase, active site
PIRSF	PIRSF000709	
PANTHER	PTHR10606:SF48	
	Fructose-2,6-bisphosphatase	
Gene3D	3.40.50.300	Histidine phosphatase superfamily
CDD		Histidine phosphatase superfamily, clade-1
All sequence SNPs/i...	Sequence variants (dbSNP and all other sources)	

Variant Legend

🟨 missense variant
🟩 synonymous variant

Scale bar   0   60   120   180   240   300   360   420   518

Mouse phenotype description(MGI)

Phenotype Overview

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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