

Atp5f1b Cas9-KO Strategy

Designer: Jing Chen

Reviewer: Xingkai Xiao

Design Date: 2024-4-19

Overview

Target Gene Name

• Atp5f1b

Project Type

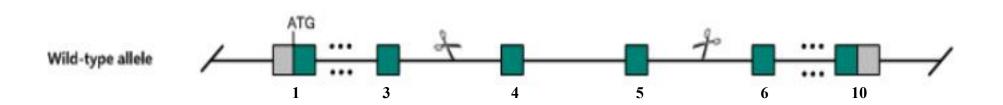
• Cas9-KO

Genetic Background

• C57BL/6JGpt



Strain Strategy





Schematic representation of CRISPR-Cas9 engineering used to edit the Atp5flb gene.

GemPharmatech

Technical Information

- The *Atp5f1b* gene has 9 transcripts. According to the structure of *Atp5f1b* gene, exon4-exon5 of *Atp5f1b*-201 (ENSMUST0000026459.6) transcript is recommended as the knockout region. The region contains 307bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Atp5f1b* gene. The brief process is as follows: gRNAs were transcribed in vitro. Cas9 and gRNAs 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.



Gene Information

Atp5f1b ATP synthase F1 subunit beta [Mus musculus (house mouse)]

Gene ID: 11947, updated on 5-Mar-2024

Summary	*	?
Official Symbol	Atp5f1b provided by MGI	
Official Full Name	ATP synthase F1 subunit beta provided by MGI	
Primary source	<u>MGI:MGI:107801</u>	
See related	Ensembl:ENSMUSG0000025393 AllianceGenome:MGI:107801	
	protein coding	
RefSeq status	VALIDATED	
Organism	Mus musculus	
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Murinae; Murinae; Mus; Mus	
Also known as	Atp5b	
	Predicted to enable several functions, including adenyl ribonucleotide binding activity; angiostatin binding activity; and proton-transporting ATP ase activity, rotational mechanism. Predicted to contribute to ATP hydrolysis activational proton-transporting ATP synthase activity, rotational mechanism. Acts upstream of or within cellular response to interleukin-7; lipid metabolic process; and negative regulation of cell adhesion involved in substrate-bouncell migration. Located in mitochondrion. Is expressed in several structures, including alimentary system; central nervous system; eye; heart; and integumental system. Orthologous to human ATP5F1B (ATP synthase F1 subunit beta). [provided by Alliance of Genome Resources, Apr 2022]	
Expression	Ubiquitous expression in heart adult (RPKM 1957.4), kidney adult (RPKM 1315.7) and 28 other tissues See more	
Orthologs	human all	
a design of the second s	Try the new <u>Gene table</u> Try the new <u>Transcript table</u>	
enomic context	*	6
ation: 10 D3; 10 76.	5.39 cM See Atp5f1b in Genome Data V	ie

Exon count: 10

GemPharmatech[™]

Annotation release	Status	Assembly	Chr	Location
RS_2024_02	current	GRCm39 (GCF_000001635.27)	10	NC_000076.7 (127919176127926257)
108.20200622	previous assembly	GRCm38.p6 (GCF_000001635.26)	10	NC_000076.6 (128083307128090388)

https://www.ncbi.nlm.nih.gov/gene/11947

Transcript Information

emPharmatech

The gene has 9 transcripts, all transcripts are shown below:

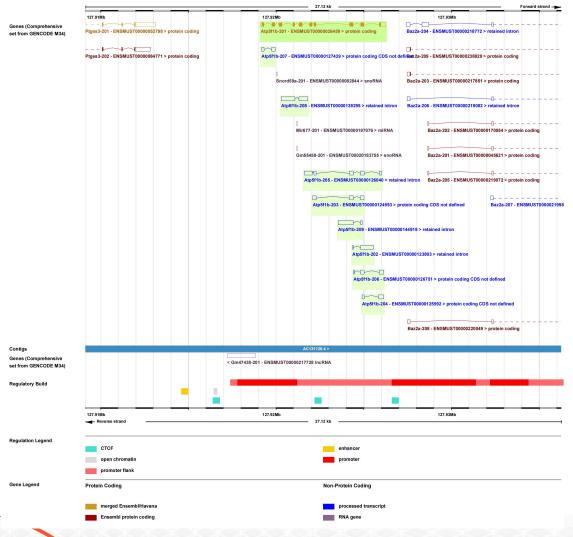
Transcript ID	Name 🍦	bp 🍦	Protein 🝦	Biotype 🝦	CCDS	UniProt Match 🖕	Flags
ENSMUST0000026459.6	Atp5f1b-201	1916	<u>529aa</u>	Protein coding	<u>CCDS24259</u>	P56480	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1
ENSMUST00000123803.2	Atp5f1b-202	768	No protein	Retained intron		0.731	TSL:1
ENSMUST00000124993.2	Atp5f1b-203	600	No protein	Protein coding CDS not defined		-	TSL:2
ENSMUST00000125992.2	Atp5f1b-204	399	No protein	Protein coding CDS not defined		127	TSL:3
ENSMUST00000126040.8	Atp5f1b-205	1131	No protein	Retained intron		120	TSL:2
ENSMUST00000126751.2	Atp5f1b-206	478	No protein	Protein coding CDS not defined		17.5	TSL:2
ENSMUST00000127439.2	Atp5f1b-207	343	No protein	Protein coding CDS not defined		-	TSL:2
ENSMUST00000139295.3	Atp5f1b-208	1288	No protein	Retained intron		121	TSL:2
ENSMUST00000144918.2	Atp5f1b-209	948	No protein	Retained intron			TSL:1

The strategy is based on the design of *Atp5f1b*-201 transcript, the transcription is shown below:



Source: https://www.ensembl.org

Genomic Information



GemPharmatech

Source: : https://www.ensembl.org

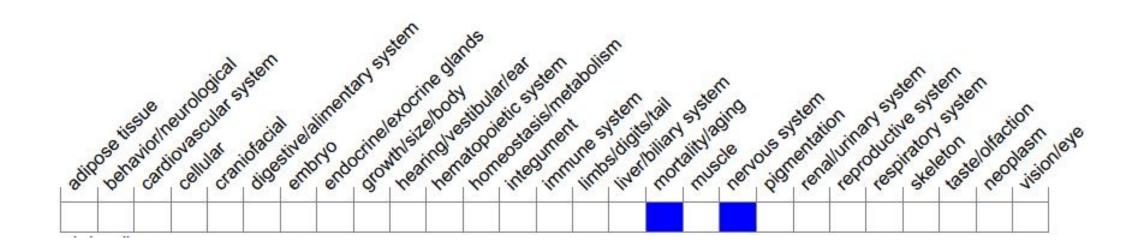
Protein Information



Source: : https://www.ensembl.org

GemPharmatech[™]

Mouse Phenotype Information (MGI)



Source: https://www.informatics.jax.org

emPharmatech

Important Information

- According to MGI, mice homozygous for *Atp5f1b* knockout exhibit embryonic lethality.
- The knockout region is about 0.5 kb away from the 3' of the *Snord59a* gene, 1.7 kb away from the 5' of the *Gm47438* gene, 5.7 kb away from the 5' of the *Baz2a* gene, which may affect the regulation of this gene.
- A part of amino acid sequence (162 aa) will still remain at the N-terminal of Atp5f1b-201.
- The knockout region contains the *Mir677* and *Gm55458* gene, which are knocked out together.
- *Atp5f1b* is located on Chr10. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

GemPharmatech