

# Atp5f1b Cas9-CKO Strategy

Designer: Jing Chen

Reviewer: Xingkai Xiao

Design Date: 2024-4-19

# Overview

## Target Gene Name

- Atp5f1b

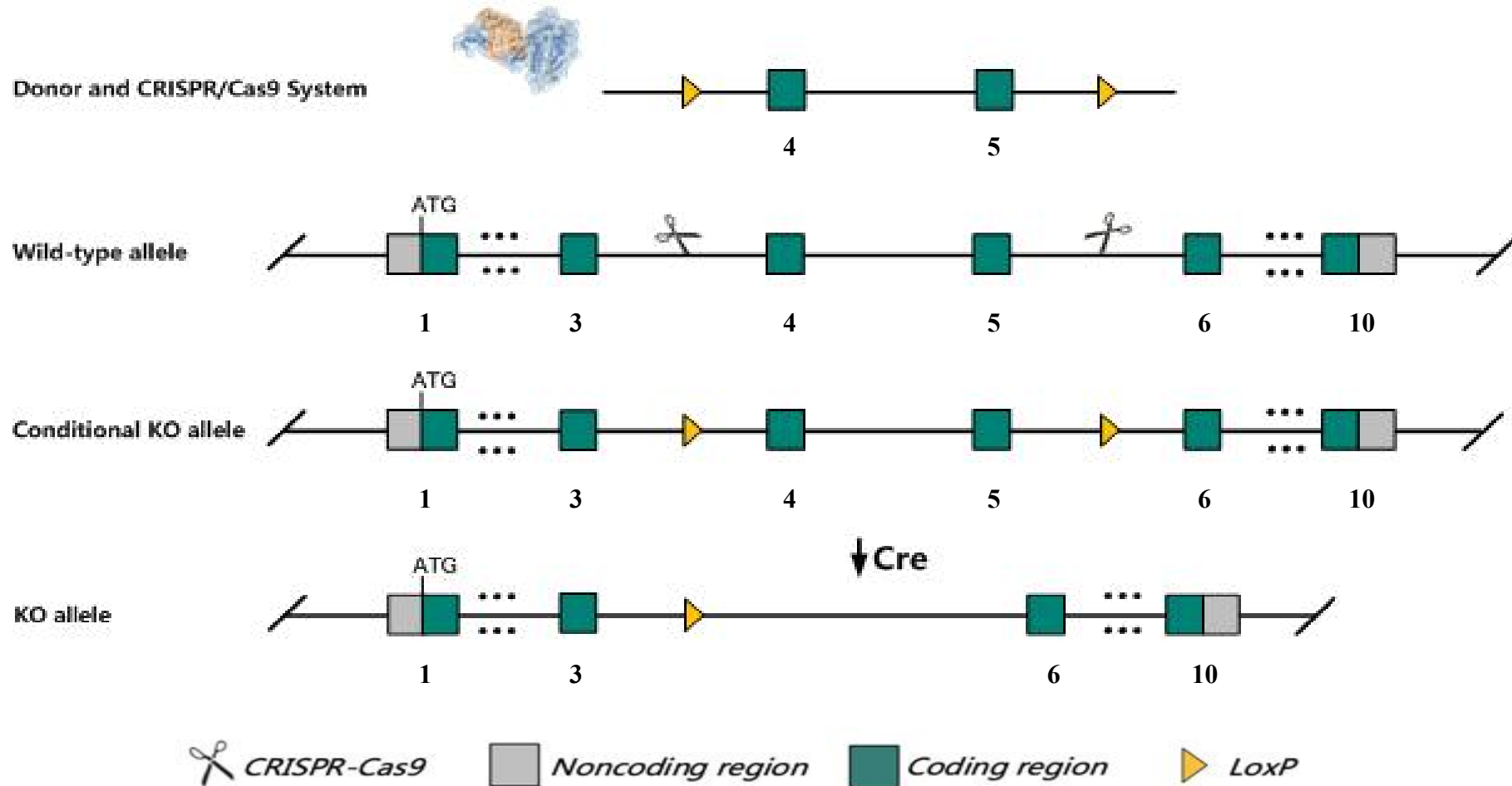
## Project Type

- Cas9-CKO

## Genetic Background

- C57BL/6JGpt

# Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Atp5f1b* gene.

# Technical Information

- The *Atp5f1b* gene has 9 transcripts. According to the structure of *Atp5f1b* gene, exon4-exon5 of *Atp5f1b*-201 (ENSMUST00000026459.6) transcript is recommended as the knockout region. The region contains 307 bp 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: CRISPR-Cas9 system 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 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.
- The flox mice will be 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.

# Gene Information

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

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

 Download Datasets

### Summary

**Official Symbol** Atp5f1b provided by MGI  
**Official Full Name** ATP synthase F1 subunit beta provided by MGI  
**Primary source** [MGI:MGI:107801](#)  
**See related** [Ensembl:ENSMUSG00000025393](#) [AllianceGenome:MGI:107801](#)  
**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** Atp5b  
**Summary** Predicted to enable several functions, including adenylyl ribonucleotide binding activity; angiotensin binding activity; and proton-transporting ATPase activity, rotational mechanism. Predicted to contribute to ATP hydrolysis activity and 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-bound cell 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](#)  
**NEW** Try the new [Gene table](#)  
Try the new [Transcript table](#)

### Genomic context

**Location:** 10 D3; 10 76.39 cM

See Atp5f1b in [Genome Data Viewer](#)

**Exon count:** 10

Annotation release	Status	Assembly	Chr	Location
RS_2024_02	current	GRCm39 ( <a href="#">GCF_000001635.27</a> )	10	NC_000076.7 (127919176..127926257)
108.20200622	previous assembly	GRCm38.p6 ( <a href="#">GCF_000001635.26</a> )	10	NC_000076.6 (128083307..128090388)

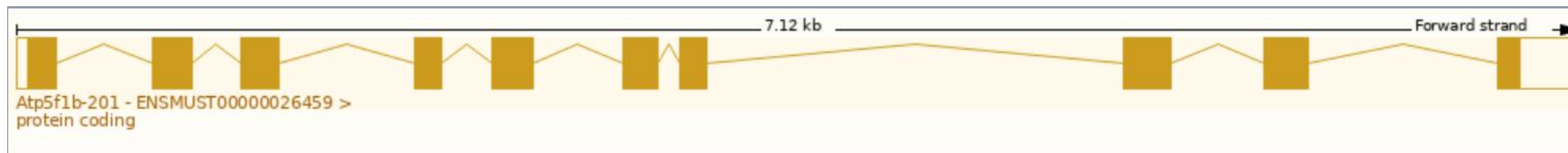
<https://www.ncbi.nlm.nih.gov/gene/11947>

# Transcript Information

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

Transcript ID ▲	Name ▲	bp ▲	Protein ▲	Biotype ▲	CCDS ▲	UniProt Match ▲	Flags ▲
<a href="#">ENSMUST00000026459.6</a>	Atp5f1b-201	1916	<a href="#">529aa</a>	Protein coding	<a href="#">CCDS24259</a>	<a href="#">P56480</a>	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1
<a href="#">ENSMUST000000123803.2</a>	Atp5f1b-202	768	No protein	Retained intron		-	TSL:1
<a href="#">ENSMUST000000124993.2</a>	Atp5f1b-203	600	No protein	Protein coding CDS not defined		-	TSL:2
<a href="#">ENSMUST000000125992.2</a>	Atp5f1b-204	399	No protein	Protein coding CDS not defined		-	TSL:3
<a href="#">ENSMUST000000126040.8</a>	Atp5f1b-205	1131	No protein	Retained intron		-	TSL:2
<a href="#">ENSMUST000000126751.2</a>	Atp5f1b-206	478	No protein	Protein coding CDS not defined		-	TSL:2
<a href="#">ENSMUST000000127439.2</a>	Atp5f1b-207	343	No protein	Protein coding CDS not defined		-	TSL:2
<a href="#">ENSMUST000000139295.3</a>	Atp5f1b-208	1288	No protein	Retained intron		-	TSL:2
<a href="#">ENSMUST000000144918.2</a>	Atp5f1b-209	948	No protein	Retained intron		-	TSL:1

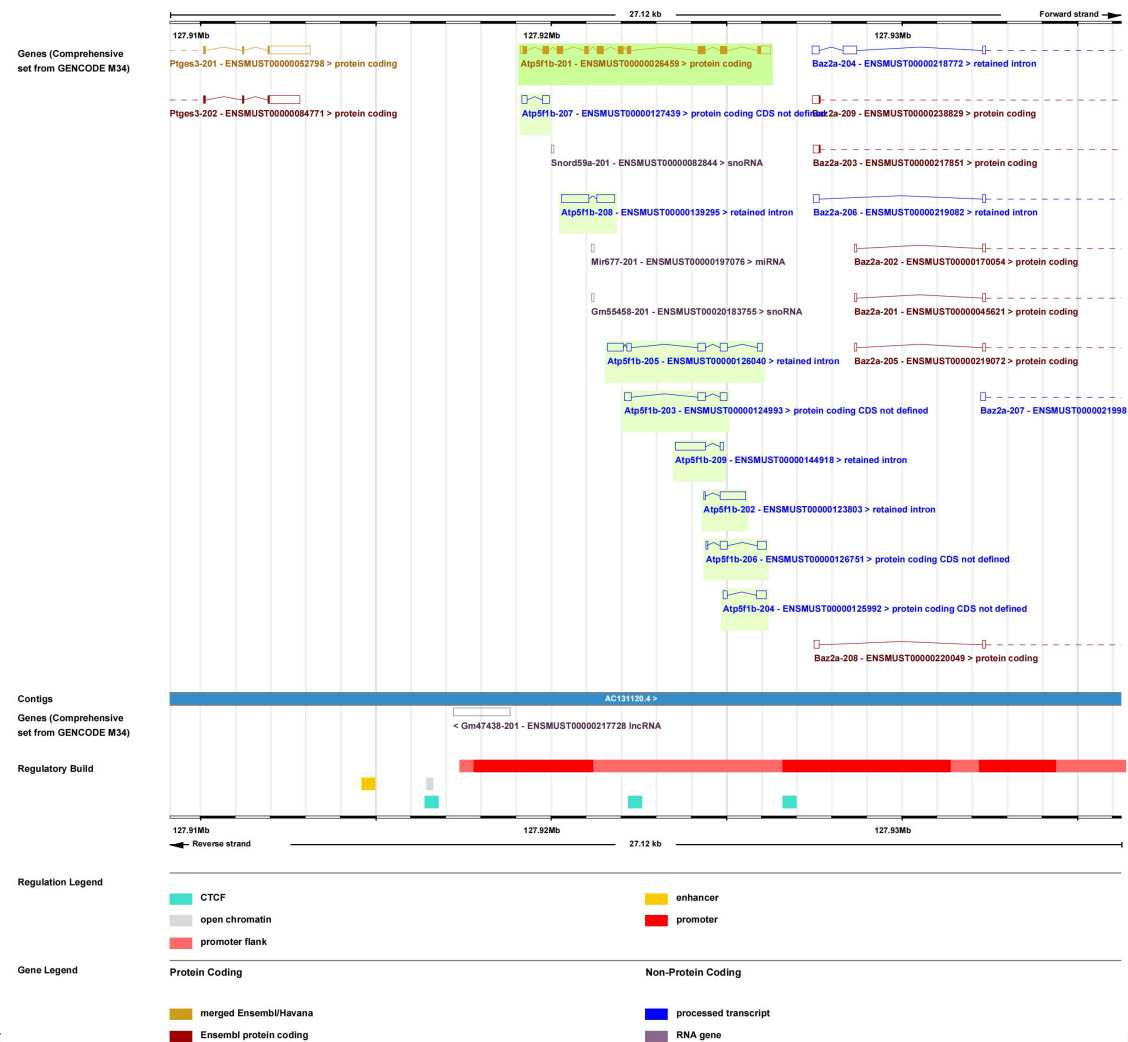
The strategy is based on the design of *Atp5f1b*-201 transcript, the transcription is shown below:



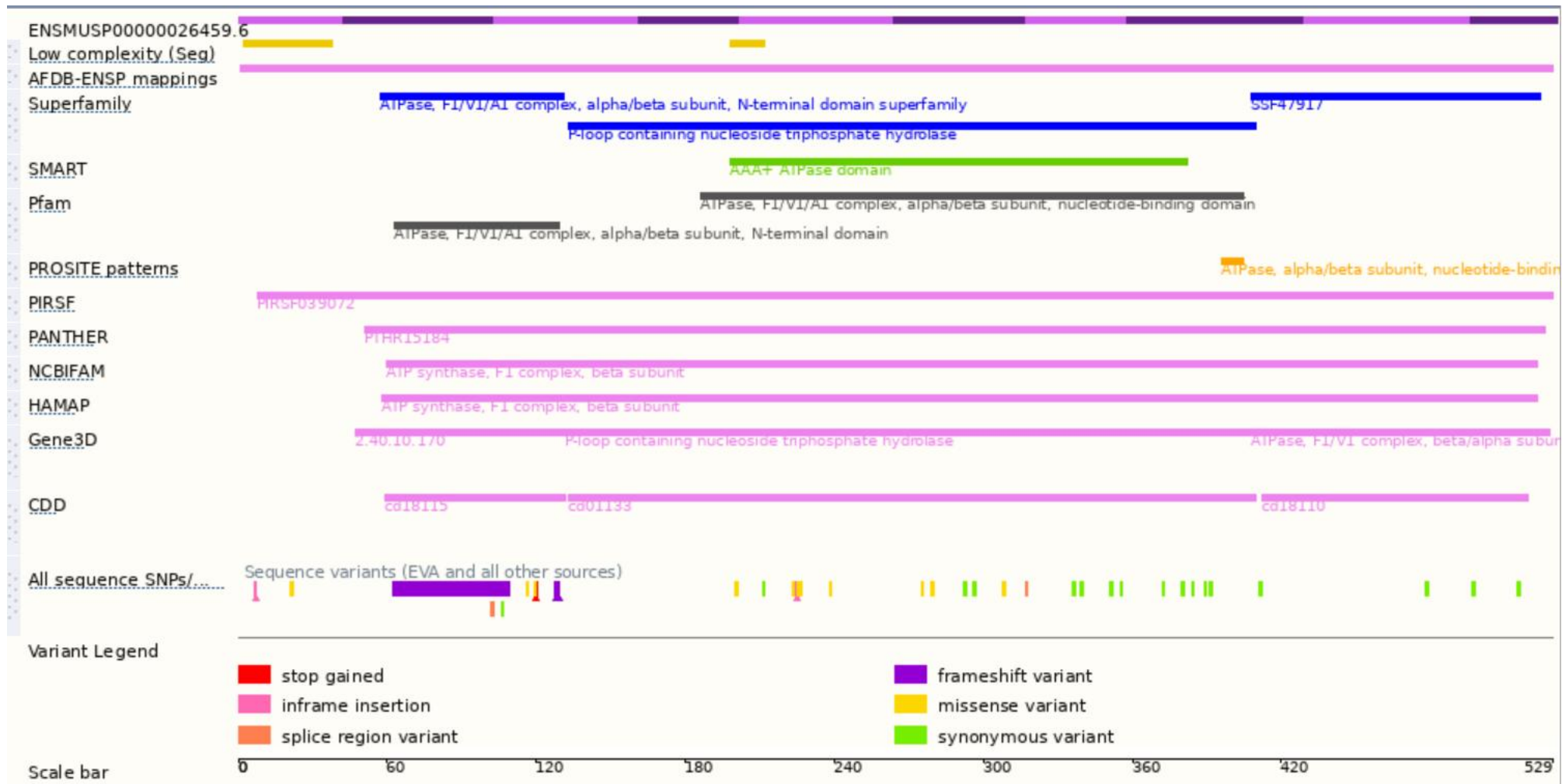
Source: <https://www.ensembl.org>



# Genomic Information

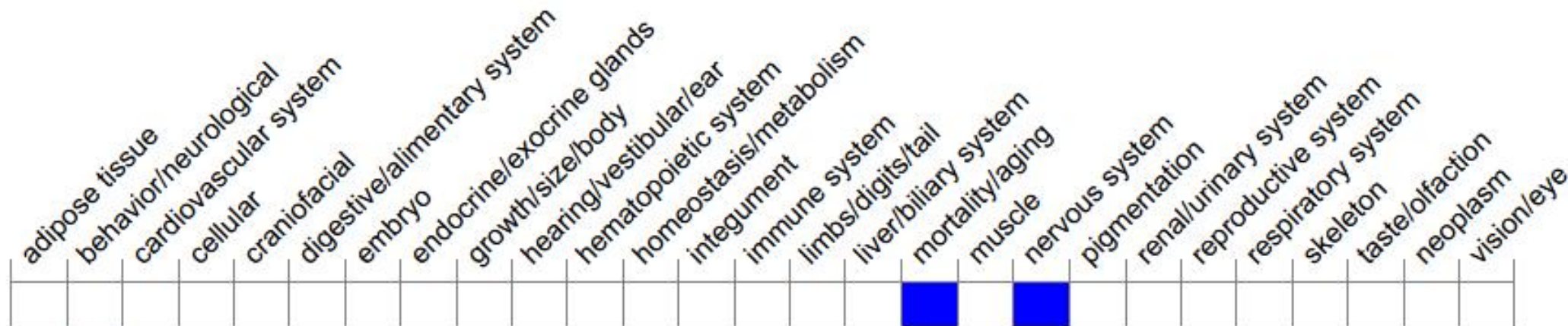


# Protein Information





# Mouse Phenotype Information (MGI)



# Important Information

- According to MGI, mice homozygous for *Atp5f1b* knockout exhibit embryonic lethality.
- A part of amino acid sequence (162 aa) will still remain at the N-terminal of *Atp5f1b*-201.
- The intron 3-4 of *Atp5f1b*-201 is 619 bp, The intron 5-6 of *Atp5f1b*-201 is 411 bp, the loxp insertion may affect the regulation of this gene.
- 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.
- 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 risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.