

Atp5h Cas9-KO Strategy

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Reviewer: DaoHua Xu

Design Date: 2023-03-24

Overview

Target Gene Name

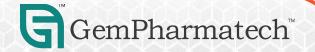
• Atp5h

Project Type

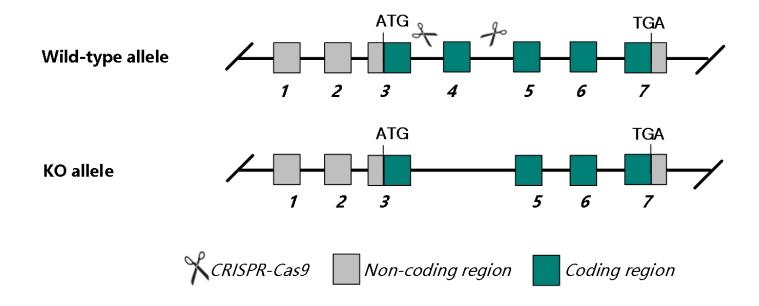
• Cas9-KO

Genetic Background

• C57BL/6JGpt



Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the Atp5h gene.



Technical Information

- The *Atp5h* gene has 8 transcripts. According to the structure of *Atp5h* gene, exon 4 of *Atp5h*-203(ENSMUST00000106537.8) transcript is recommended as the knockout region. The region contains contains 97bp coding sequences. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Atp5h* 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

Atp5h ATP synthase, H+ transporting, mitochondrial F0 complex, subunit D [Mus musculus (house mouse)]

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Gene ID: 71679, updated on 15-Mar-2023



Official Symbol Atp5h provided by MGI

Official Full Name ATP synthase, H+ transporting, mitochondrial F0 complex, subunit D provided by MGI

Primary source MGI:MGI:1918929

See related Ensembl: ENSMUSG00000034566 Alliance Genome: MGI: 1918929

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; Muridae; Murinae;

Mus; Mus

Also known as Atp5pd; 0610009D10Rik

Summary Predicted to enable proton transmembrane transporter activity. Predicted to contribute to proton-transporting ATP synthase activity, rotational mechanism. Predicted to be

involved in mitochondrial ATP synthesis coupled proton transport. Predicted to act upstream of or within ion transport. Located in mitochondrion. Is expressed in several structures, including alimentary system; integumental system; nervous system; respiratory system; and sensory organ. Orthologous to human ATP5PD (ATP synthase

peripheral stalk subunit d). [provided by Alliance of Genome Resources, Apr 2022]

Expression Ubiquitous expression in heart adult (RPKM 94.5), CNS E11.5 (RPKM 81.5) and 25 other tissues See more

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Human an

Try the new Gene table

Try the new Transcript table

Source: https://www.ncbi.nlm.nih.gov/

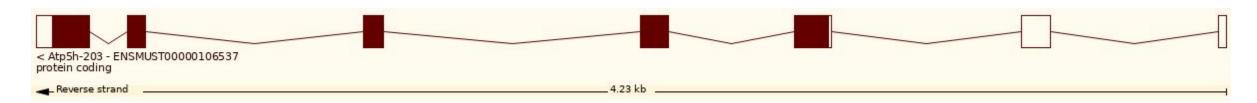


Transcript Information

The gene has 8 transcripts, all transcripts are shown below:

| Show/hide columns (1 hidden) | | | | | | | Filter |
|------------------------------|-----------|------|--------------|--------------------------------|-------------|---------------|-------------------------------------------------|
| Transcript ID | Name A | bp 🛊 | Protein 🌲 | Biotype | CCDS | UniProt Match | Flags |
| ENSMUST00000043931.9 | Atp5h-201 | 625 | <u>161aa</u> | Protein coding | CCDS25633 ₺ | Q9DCX2 ₺ | GENCODE basic APPRIS P1 TSL:1 |
| ENSMUST00000073791.10 | Atp5h-202 | 659 | <u>161aa</u> | Protein coding | CCDS25633 ₺ | Q9DCX2₺ | GENCODE basic APPRIS P1 TSL:5 |
| ENSMUST00000106537.8 | Atp5h-203 | 682 | <u>161aa</u> | Protein coding | CCDS25633₺ | Q9DCX2₽ | Ensembl Canonical GENCODE basic APPRIS P1 TSL:5 |
| ENSMUST00000137563.8 | Atp5h-204 | 510 | No protein | Protein coding CDS not defined | | | TSL:2 |
| ENSMUST00000137754.2 | Atp5h-205 | 468 | <u>138aa</u> | Protein coding | | B1ASE2₺ | TSL:3 CDS 3' incomplete |
| ENSMUST00000138779.2 | Atp5h-206 | 857 | No protein | Protein coding CDS not defined | | | TSL2 |
| ENSMUST00000141474.8 | Atp5h-207 | 420 | No protein | Protein coding CDS not defined | | ¥ | TSL:2 |
| ENSMUST00000180072.8 | Atp5h-208 | 486 | <u>161aa</u> | Protein coding | CCDS25633 ₺ | Q9DCX2₺ | GENCODE basic APPRIS P1 TSL:2 |

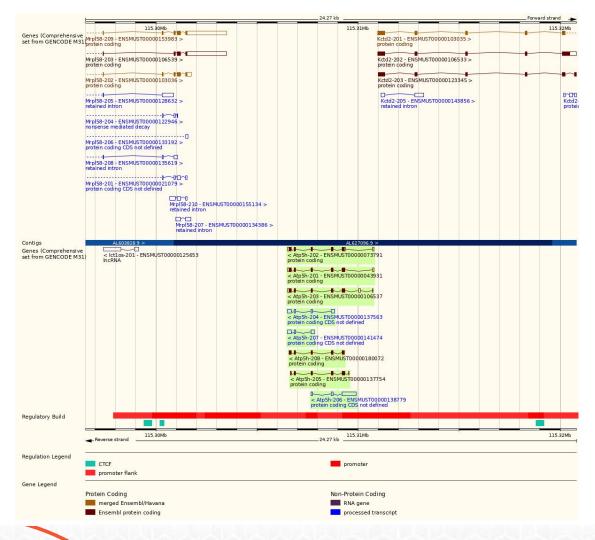
The strategy is based on the design of *Atp5h*-203 transcript, the transcription is shown below:



Source: https://www.ensembl.org



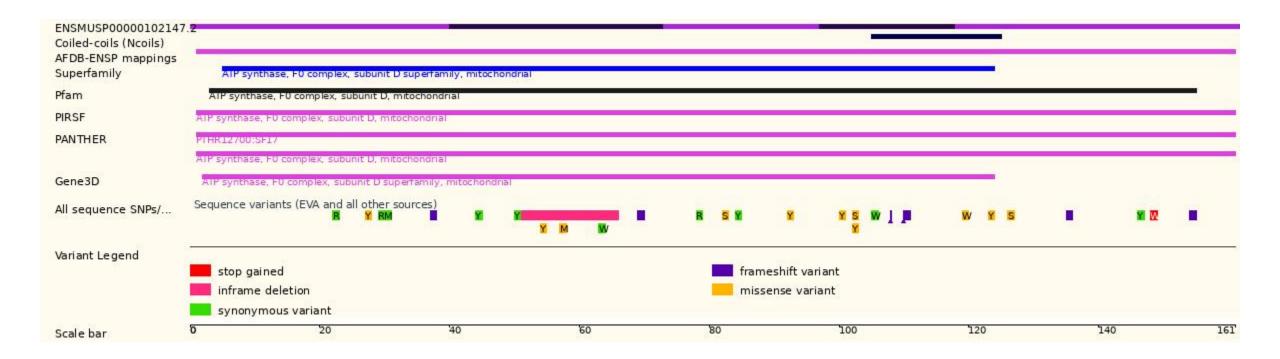
Genomic Information





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

Protein Information





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

Important Information

- The effect of *Kctd2* gene is unknown.
- The effect of this strategy on the Atp5h -207 transcript is unknown.
- *Atp5h* is located on Chr 11. 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.

