

Atp5h Cas9-CKO Strategy

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Reviewer: DaoHua Xu

Design Date: 2023-03-24

Overview

Target Gene Name

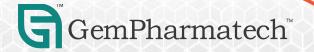
• Atp5h

Project Type

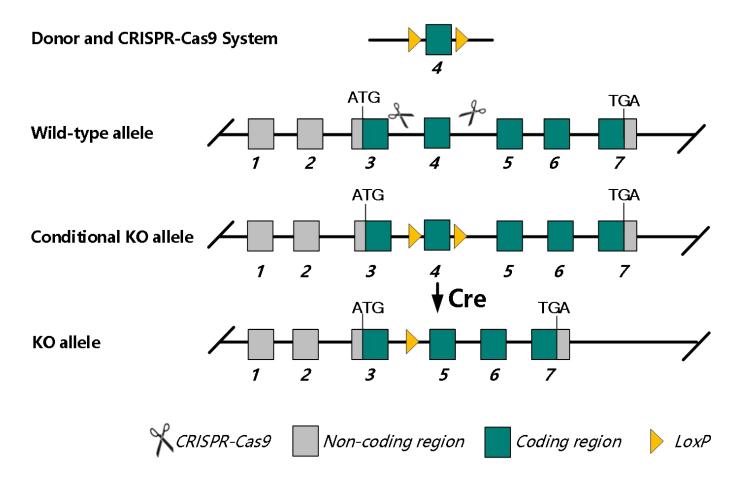
• Cas9-CKO

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 (ENSMUST0000106537.8) 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: 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

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

≜ Summary

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 AllianceGenome: 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; 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

Orthologs human all

Try the new Gene table

Try the new Transcript table

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

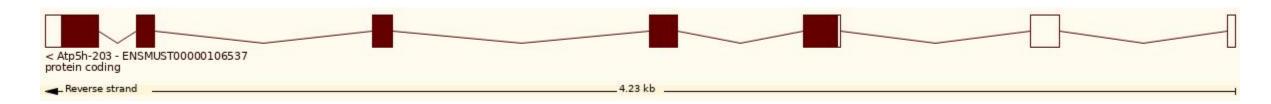


Transcript Information

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

Transcript ID	Name A	bp 🛔	Protein A	Biotype	CCDS A	UniProt Match	Flags
ENSMUST00000043931.9	Atp5h-201	625		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
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			TSL:2
ENSMUST00000141474.8	Atp5h-207	420	No protein	Protein coding CDS not defined		2	TSL:2
ENSMUST00000180072.8	Atp5h-208	486	<u>161aa</u>	Protein coding	CCDS25633 ₽	Q9DCX2	GENCODE basic APPRIS P1 TSL:2

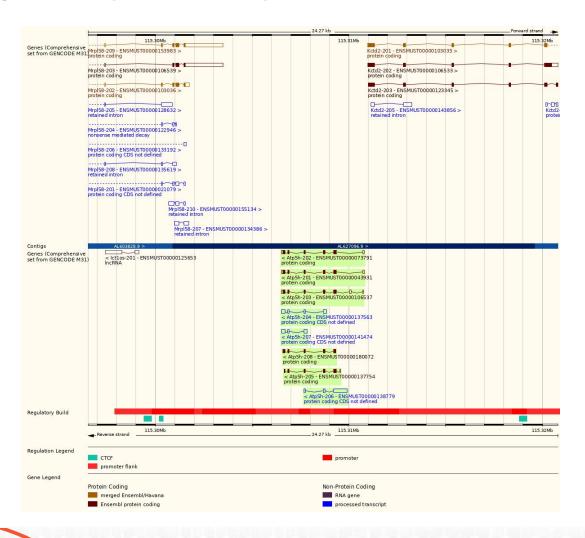
The strategy is based on the design of *Atp5h*-203 transcript, the transcription is shown below:

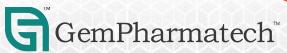


Source: https://www.ensembl.org



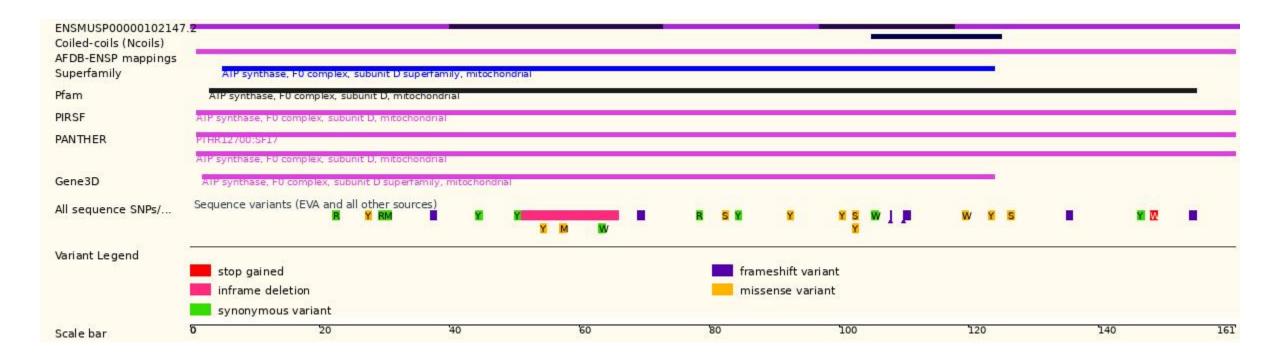
Genomic Information





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

Protein Information





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

Important Information

- A loxp site is about 2kb from the 5 'end of *Kctd2* gene, which may affect the function of the gene.
- 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 risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

