

Atp8a2 Cas9-CKO Strategy

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Project Overview

Project Name

Atp8a2

Project type

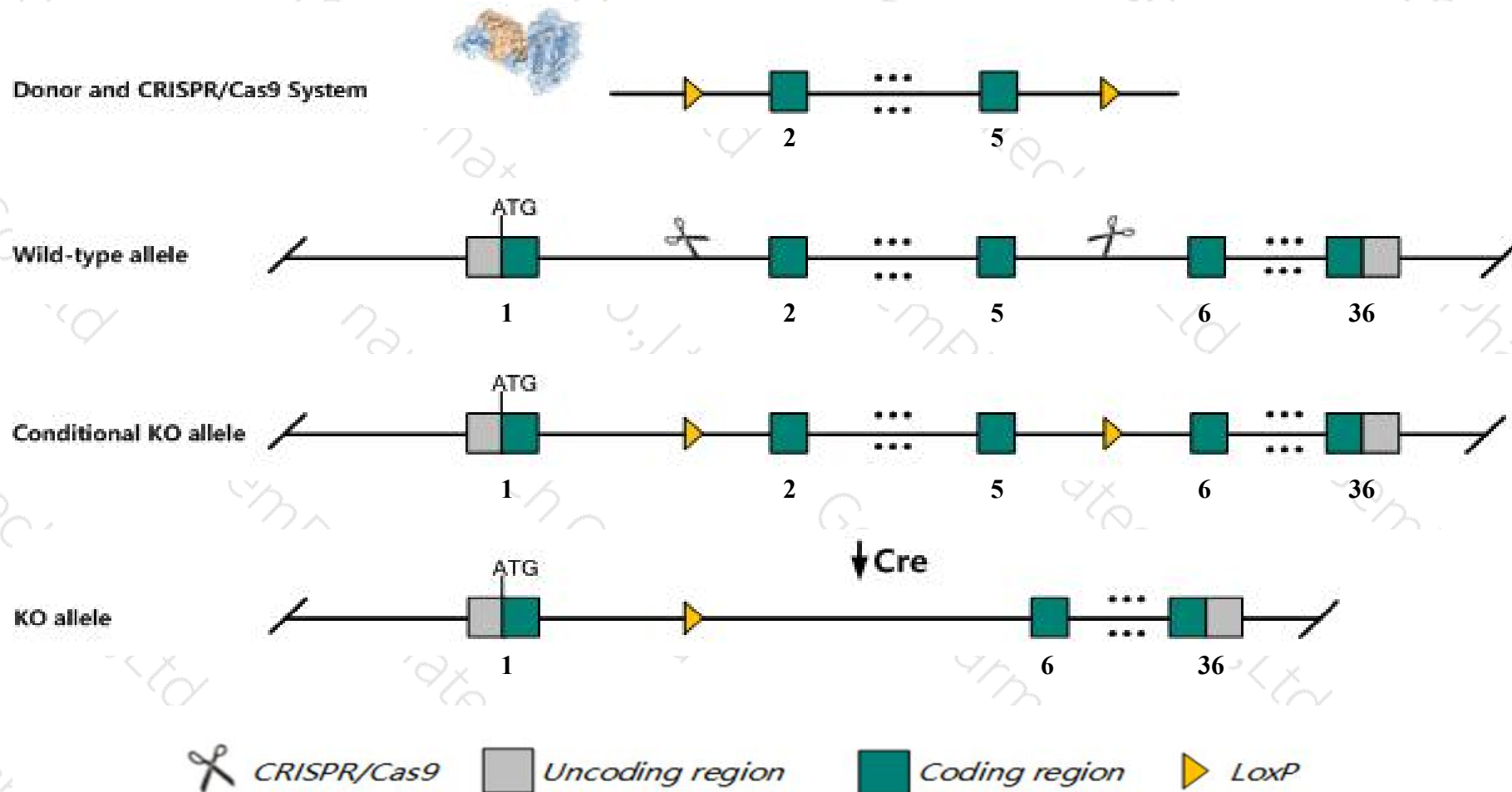
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Atp8a2* gene. The schematic diagram is as follows:



- The *Atp8a2* gene has 6 transcripts. According to the structure of *Atp8a2* gene, exon2-exon5 of *Atp8a2-201* (ENSMUST00000080368.12) transcript is recommended as the knockout region. The region contains 286bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Atp8a2* 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 sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.
- 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.

- According to the existing MGI data, mice homozygotes for spontaneous mutations have abnormal gait and tremors, with axonal degeneration in central and peripheral neurons. symptoms progress to immobility and death by 1-month of age. heterozygotes show subtle locomotor abnormalities and are hyporesponsive to tail pinching. mice also show shortening of the retina outer segment.
- Transcript *Atp8a2-203 and Atp8a2-205* may not be affected.
- The *Atp8a2* gene is located on the Chr14. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)

Atp8a2 ATPase, aminophospholipid transporter-like, class I, type 8A, member 2 [Mus musculus (house mouse)]

Gene ID: 50769, updated on 13-Mar-2020

Summary



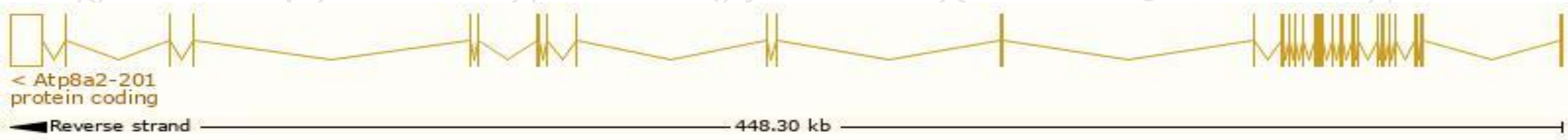
Official Symbol	Atp8a2 provided by MGI
Official Full Name	ATPase, aminophospholipid transporter-like, class I, type 8A, member 2 provided by MGI
Primary source	MGI:MGI:1354710
See related	Ensembl:ENSMUSG000000021983
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI415030, Atpc1b, agil, wl
Expression	Broad expression in colon adult (RPKM 3.2), testis adult (RPKM 2.3) and 18 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

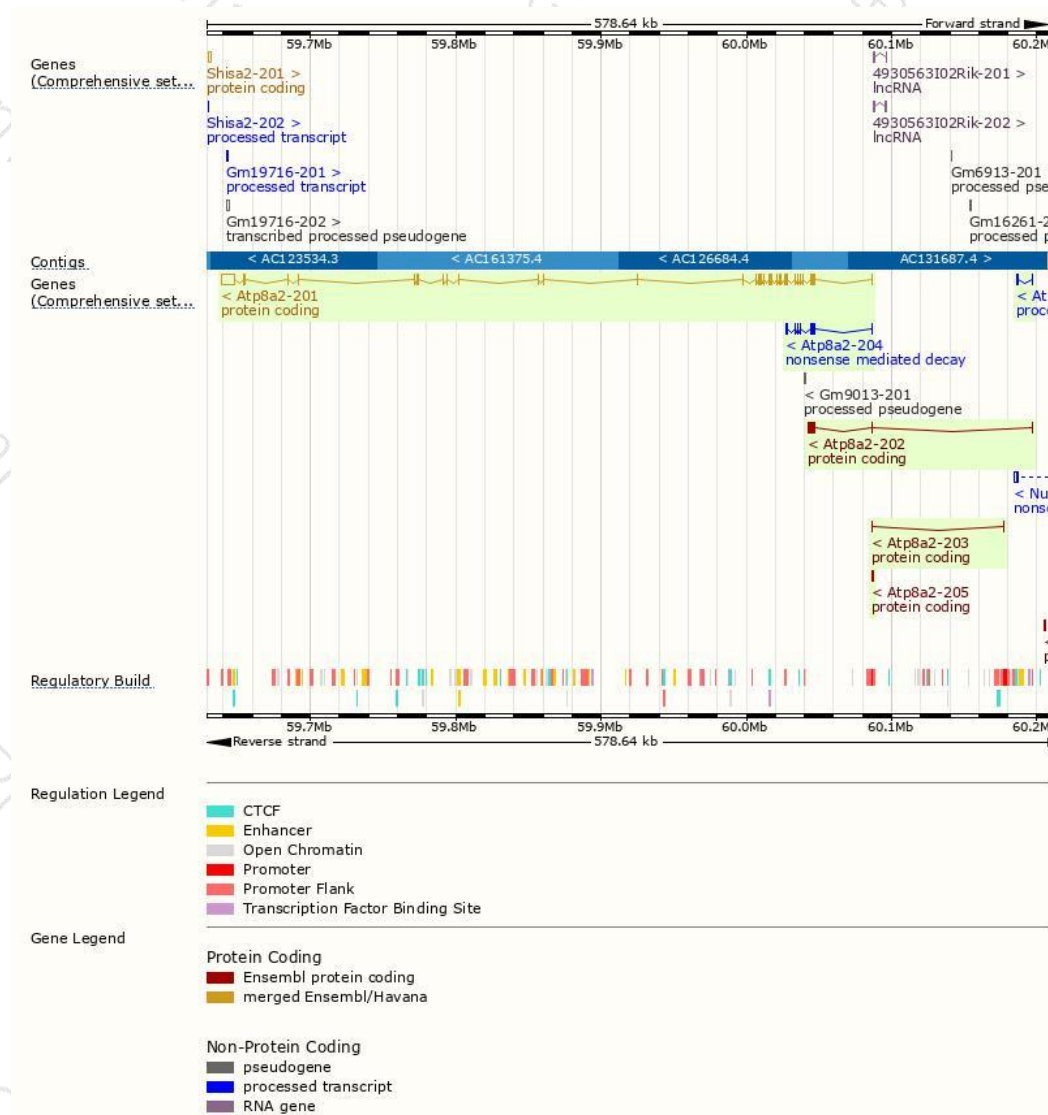
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Atp8a2-201	ENSMUST00000080368.12	12886	1148aa	Protein coding	CCDS27174	P98200	TSL:1 GENCODE basic APPRIS P1
Atp8a2-202	ENSMUST00000131670.2	1725	160aa	Protein coding	-	E9Q8X4	TSL:2 GENCODE basic
Atp8a2-203	ENSMUST00000140924.2	373	73aa	Protein coding	-	D3Z2Z6	CDS 3' incomplete TSL:3
Atp8a2-205	ENSMUST00000149414.1	363	14aa	Protein coding	-	D3Z1A2	CDS 3' incomplete TSL:3
Atp8a2-204	ENSMUST00000145071.7	1499	129aa	Nonsense mediated decay	-	D6RG72	TSL:1
Atp8a2-206	ENSMUST00000152688.1	399	No protein	Processed transcript	-	-	TSL:3

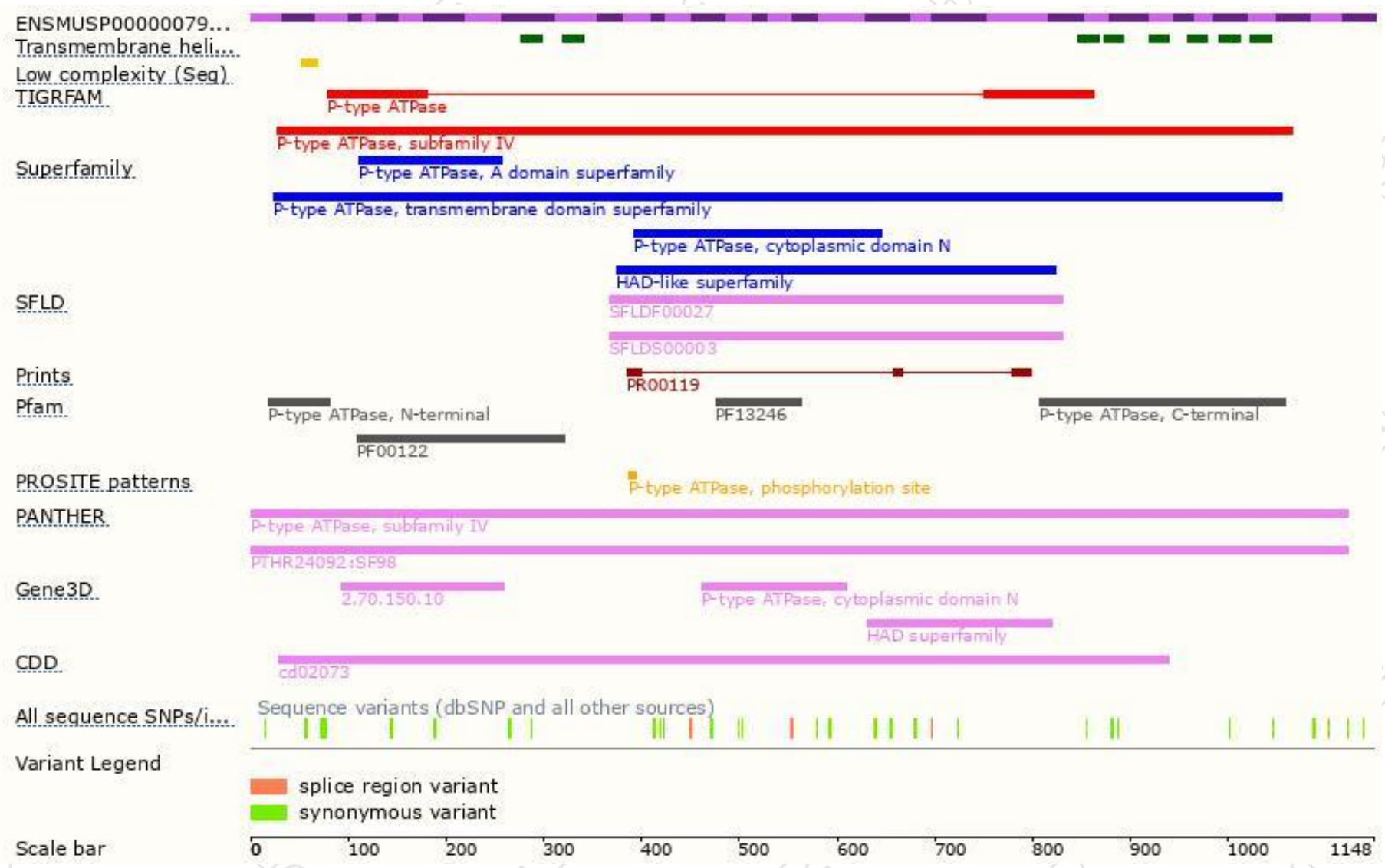
The strategy is based on the design of *Atp8a2-201* transcript,the transcription is shown below:



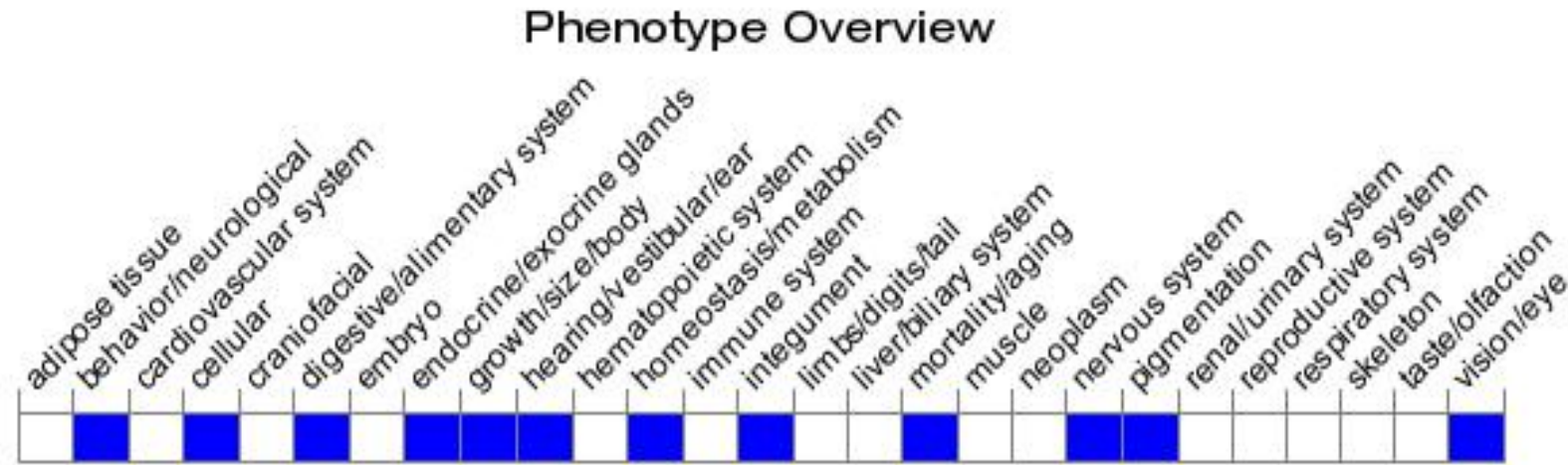
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(<http://www.informatics.jax.org/>).

According to the existing MGI data, mice homozygotes for spontaneous mutations have abnormal gait and tremors, with axonal degeneration in central and peripheral neurons. Symptoms progress to immobility and death by 1-month of age.

Heterozygotes show subtle locomotor abnormalities and are hyporesponsive to tail pinching. Mice also show shortening of the retina outer segment.

If you have any questions, you are welcome to inquire.

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