

# Atp8b2 Cas9-CKO Strategy

Designer: Daohua Xu

**Design Date:** 2019-7-18

# **Project Overview**



**Project Name** 

Atp8b2

**Project type** 

Cas9-CKO

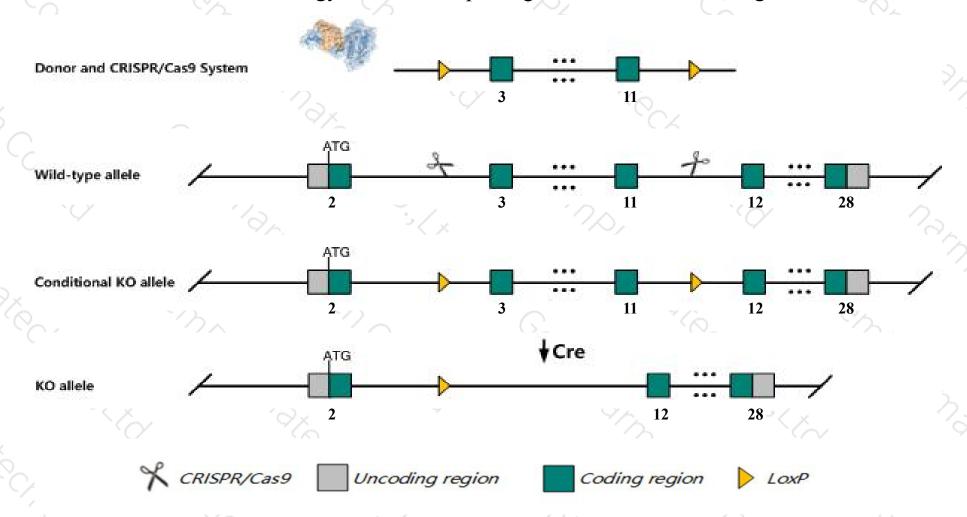
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Atp8b2* gene. The schematic diagram is as follows:



### Technical routes



- The *Atp8b2* gene has 17 transcripts. According to the structure of *Atp8b2* gene, exon3-exon11 of *Atp8b2-210* (ENSMUST00000168276.7) transcript is recommended as the knockout region. The region contains 806bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Atp8b2* 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.

### **Notice**



- Transcript Atp8b2-206 and Atp8b2-217 may not be affected.
- ➤ The *Atp8b2* gene is located on the Chr3. 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)



#### Atp8b2 ATPase, class I, type 8B, member 2 [Mus musculus (house mouse)]

Gene ID: 54667, updated on 16-Feb-2019

#### Summary

☆ ?

Official Symbol Atp8b2 provided by MGI

Official Full Name ATPase, class I, type 8B, member 2 provided by MGI

Primary source MGI:MGI:1859660

See related Ensembl: ENSMUSG00000060671

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 Id

Expression Ubiquitous expression in ovary adult (RPKM 20.9), limb E14.5 (RPKM 18.2) and 27 other tissuesSee more

Orthologs human all

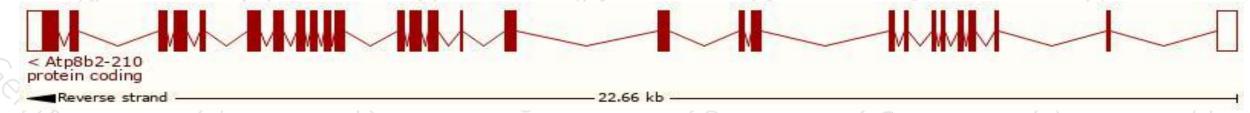
# Transcript information (Ensembl)



#### The gene has 17 transcripts, all transcripts are shown below:

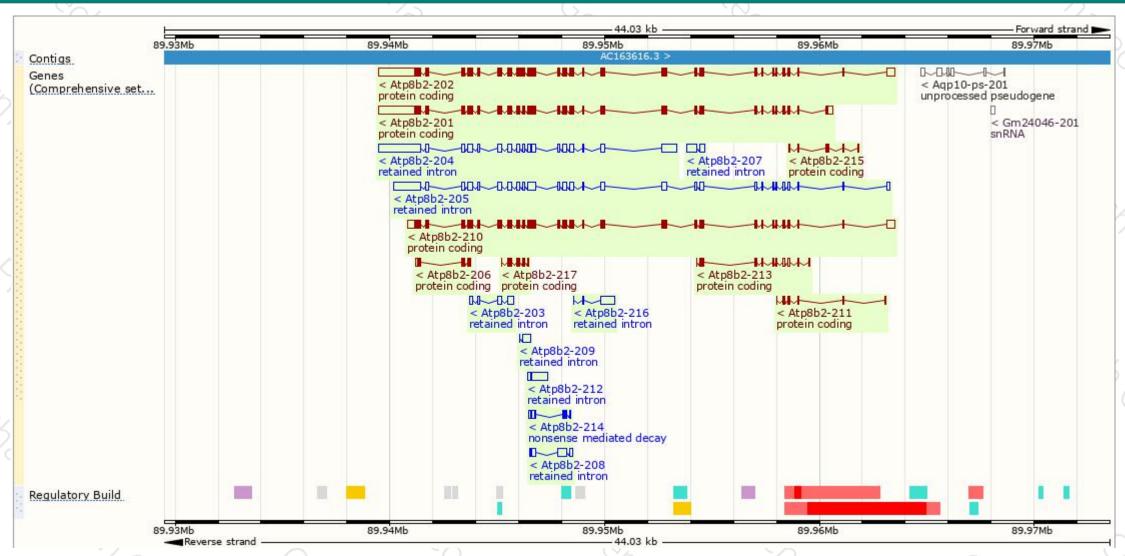
/ \			_	~ / / 1 =			
Name 🍦	Transcript ID	bp 🌲	Protein	Biotype	CCDS	UniProt 🌲	Flags
Atp8b2-210	ENSMUST00000168276.7	4265	1190aa	Protein coding	CCDS38497 ₽	E9QAL4®	TSL:5 GENCODE basic APPRIS P2
Atp8b2-202	ENSMUST00000107396.7	5705	<u>1214aa</u>	Protein coding		D3YXQ5₽	TSL:5 GENCODE basic
Atp8b2-201	ENSMUST00000069805.13	5559	<u>1209aa</u>	Protein coding	-	P98199₽	TSL:5 GENCODE basic APPRIS ALT1
Atp8b2-213	ENSMUST00000170696.1	785	<u>128aa</u>	Protein coding	100	E9Q114₽	CDS 3' incomplete TSL:3
Atp8b2-206	ENSMUST00000166502.1	518	<u>145aa</u>	Protein coding	100	F6WT01₽	CDS 5' incomplete TSL:2
tp8b2-217	ENSMUST00000171941.1	507	<u>169aa</u>	Protein coding		F6QLG1函	CDS 5' and 3' incomplete TSL:3
Atp8b2-211	ENSMUST00000168880.7	459	<u>112aa</u>	Protein coding		E9Q8R2₽	CDS 3' incomplete TSL:5
tp8b2-215	ENSMUST00000171422.1	360	<u>72aa</u>	Protein coding	951	E9Q491₽	CDS 3' incomplete   TSL:3
tp8b2-214	ENSMUST00000170739.1	447	86aa	Nonsense mediated decay	15-0	F6SPT4₽	CDS 5' incomplete   TSL:5
htp8b2-204	ENSMUST00000163354.7	4894	No protein	Retained intron			TSL:2
htp8b2-205	ENSMUST00000166347.7	4814	No protein	Retained intron	-	-	TSL:2
Atp8b2-212	ENSMUST00000170324.1	831	No protein	Retained intron	100		TSL:3
tp8b2-208	ENSMUST00000167257.1	756	No protein	Retained intron	100	29	TSL:5
Atp8b2-203	ENSMUST00000163152.1	736	No protein	Retained intron		2	TSL:2
tp8b2-216	ENSMUST00000171818.1	697	No protein	Retained intron			TSL:2
Atp8b2-207	ENSMUST00000166705.1	652	No protein	Retained intron	951	- 1	TSL:3
Atp8b2-209	ENSMUST00000167442.1	403	No protein	Retained intron	19-9		TSL:3

The strategy is based on the design of Atp8b2-210 transcript, The transcription is shown below



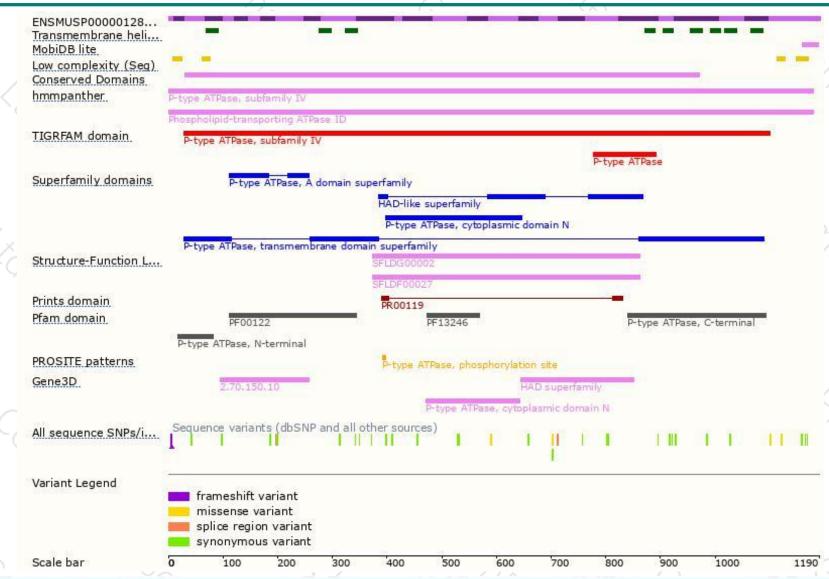
### Genomic location distribution





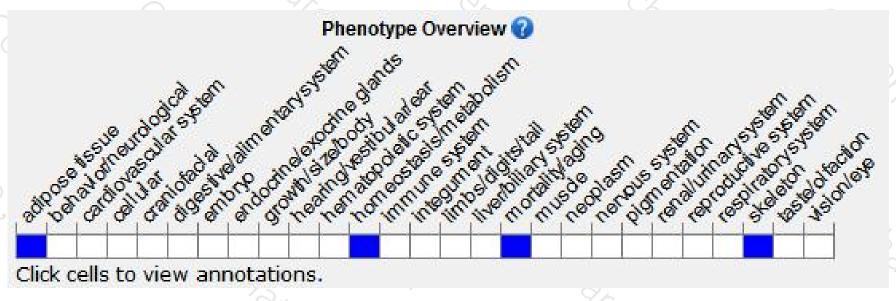
### Protein domain





# Mouse phenotype description(MGI)





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



If you have any questions, you are welcome to inquire. Tel: 400-9660890





