

# Atp2a2 Cas9-CKO Strategy

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**Design Date:** 2019-12-25

# **Project Overview**



**Project Name** 

Atp2a2

**Project type** 

Cas9-CKO

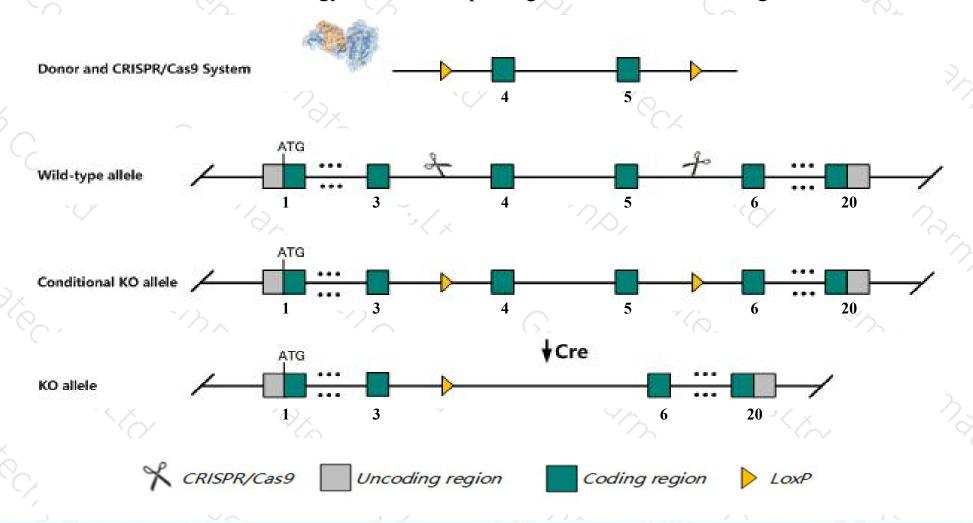
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Atp2a2 gene. The schematic diagram is as follows:



### Technical routes



- The *Atp2a2* gene has 5 transcripts. According to the structure of *Atp2a2* gene, exon4-exon5 of *Atp2a2-201* (ENSMUST00000031423.9) transcript is recommended as the knockout region. The region contains 244bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Atp2a2* 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**



- ➤ According to the existing MGI data, Targeted homozygous mutants are embryonic lethal while heterozygotes show reduced blood pressure and mildly impaired cardiac contractility and relaxation. Aged heterozygotes for one targeted mutation develop squamous cell tumors of the forestomach, esophagus, oral mucosa, tongue, and skin.
- ➤ The KO region contains functional region of the *Gm43360* gene. Knockout the region may affect the function of *Gm43360* gene.
- The Atp2a2 gene is located on the Chr5. 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)



#### Atp2a2 ATPase, Ca++ transporting, cardiac muscle, slow twitch 2 [Mus musculus (house mouse)]

Gene ID: 11938, updated on 7-Apr-2019

#### Summary

↑ ?

Official Symbol Atp2a2 provided by MGI

Official Full Name ATPase, Ca++ transporting, cardiac muscle, slow twitch 2 provided by MGI

Primary source MGI:MGI:88110

See related Ensembl:ENSMUSG00000029467

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 9530097L16Rik, D5Wsu150e, SERCA2, SERCA2B, Serca2a, mKlAA4195

Expression Biased expression in heart adult (RPKM 1140.8), bladder adult (RPKM 124.3) and 3 other tissuesSee more

Orthologs human all

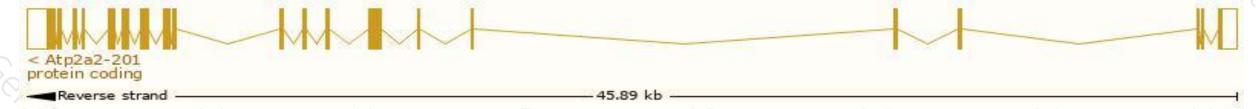
# Transcript information (Ensembl)



The gene has 5 transcripts, all transcripts are shown below:

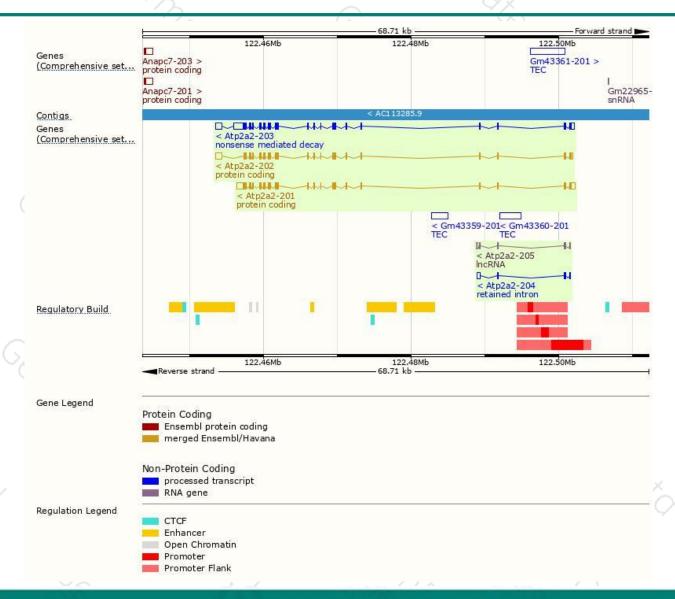
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Atp2a2-201	ENSMUST00000031423.9	4486	<u>1044aa</u>	Protein coding	CCDS57378	<u>055143</u>	TSL:1 GENCODE basic APPRIS P4
Atp2a2-202	ENSMUST00000177974.7	3984	<u>998aa</u>	Protein coding	CCDS57379	055143	TSL:1 GENCODE basic APPRIS ALT1
Atp2a2-203	ENSMUST00000179939.7	5674	1044aa	Nonsense mediated decay	-	055143	TSL:1
Atp2a2-205	ENSMUST00000197415.4	615	No protein	Processed transcript	-	3528	TSL:3
Atp2a2-204	ENSMUST00000196490.1	698	No protein	Retained intron		121	TSL:2

The strategy is based on the design of Atp2a2-201 transcript, The transcription is shown below



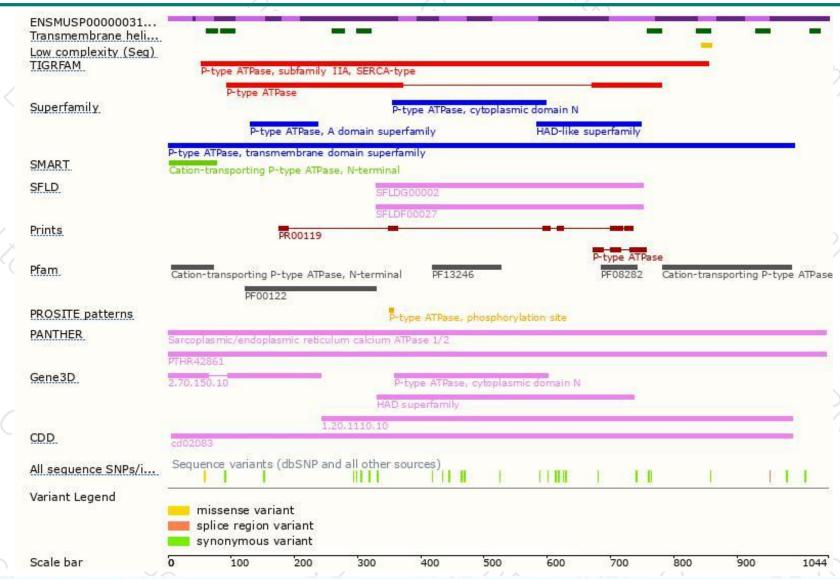
### Genomic location distribution





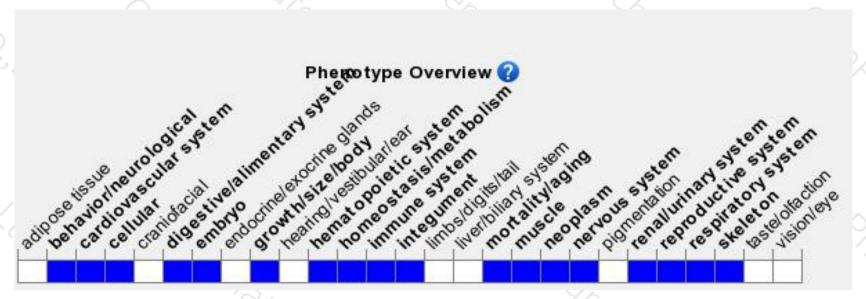
### Protein domain





# 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, Targeted homozygous mutants are embryonic lethal while heterozygotes show reduced blood pressure and mildly impaired cardiac contractility and relaxation. Aged heterozygotes for one targeted mutation develop squamous cell tumors of the forestomach, esophagus, oral mucosa, tongue, and skin.



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





