Atp2a1 Cas9-CKO Strategy

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Reviewer: Xiaojing Li

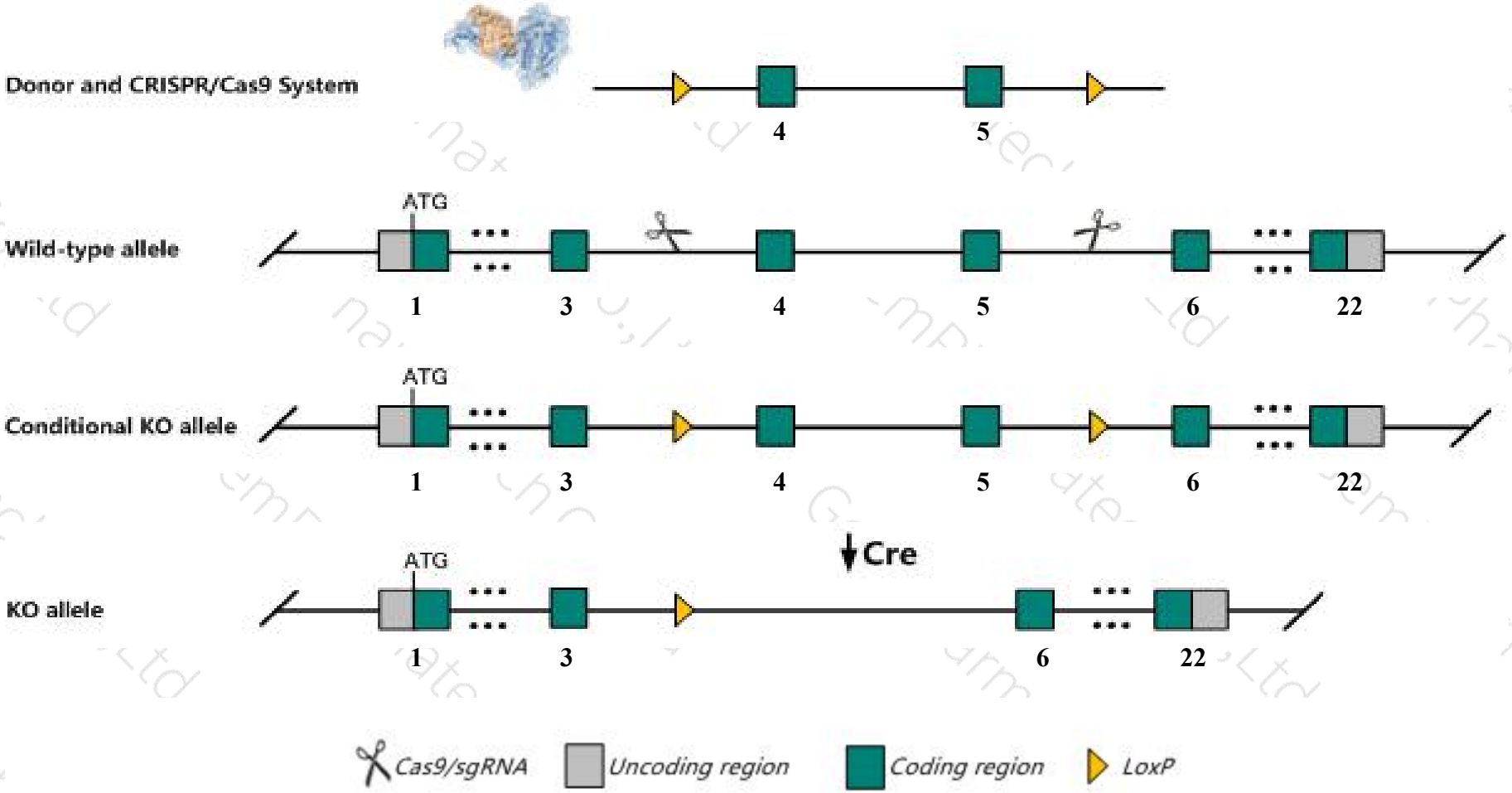
Design Date: 2018-11-5

Project Overview

Project Name	***Atp2a1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Atp2a1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Atp2a1* gene has 2 transcripts. According to the structure of *Atp2a1* gene, exon4-exon5 of *Atp2a1-201*(ENSMUST00000032974.12) 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 *Atp2a1* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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,homozygous mutation of this gene results in perinatal lethality. Mutant neonates display respiratory distress, progressive cyanosis, and die within 30 minutes-2 hours after birth. Lung tissues and the diaphragm muscle show aberrant morphology.

➢ The *Atp2a1* gene is located on the Chr7. 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）

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Atp2a1 ATPase, Ca++ transporting, cardiac muscle, fast twitch 1 [Mus musculus (house mouse)]

Gene ID: 11937, updated on 13-Mar-2020

Summary

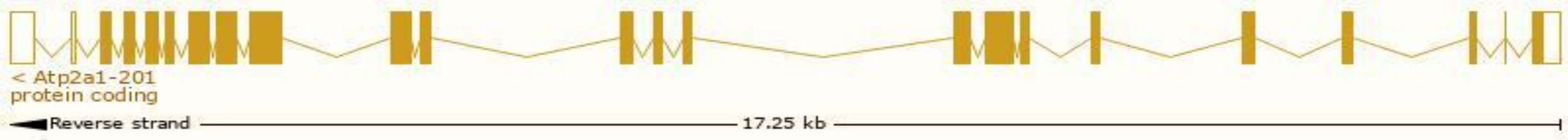
Official Symbol	Atp2a1 provided by MGI
Official Full Name	ATPase, Ca++ transporting, cardiac muscle, fast twitch 1 provided byMGI
Primary source	MGI:MGI:105058
See related	Ensembl:ENSMUSG00000030730
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	SERCA1
Expression	Restricted expression toward mammary gland adult (RPKM 780.9)See more
Orthologs	human all

Transcript information（Ensembl）

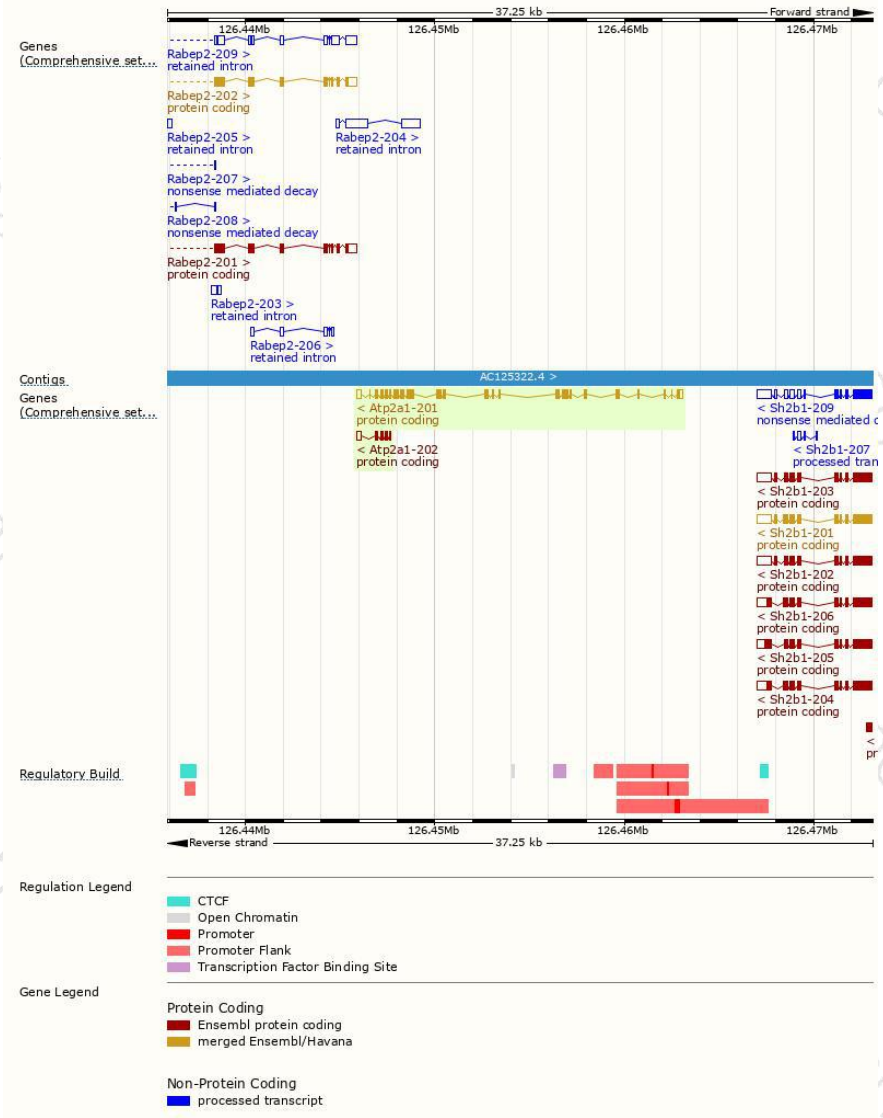
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Atp2a1-201	ENSMUST00000032974.12	3477	994aa	Protein coding	CCDS40125	Q8R429	TSL:1 GENCODE basic APPRIS P1
Atp2a1-202	ENSMUST00000146973.1	681	146aa	Protein coding	-	F6RQN3	CDS 5' incomplete TSL:2

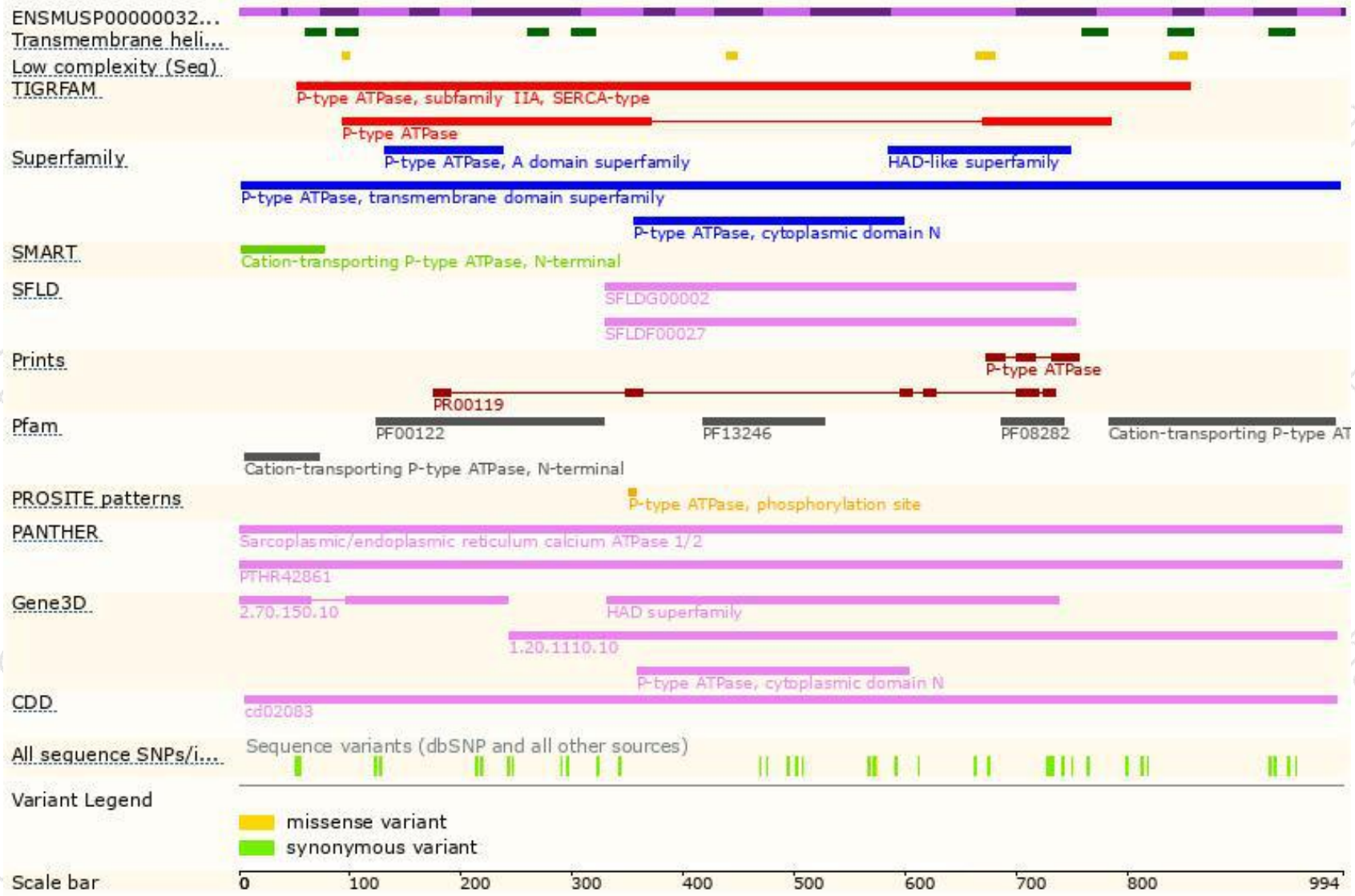
The strategy is based on the design of *Atp2a1-201* transcript,the transcription is shown below:



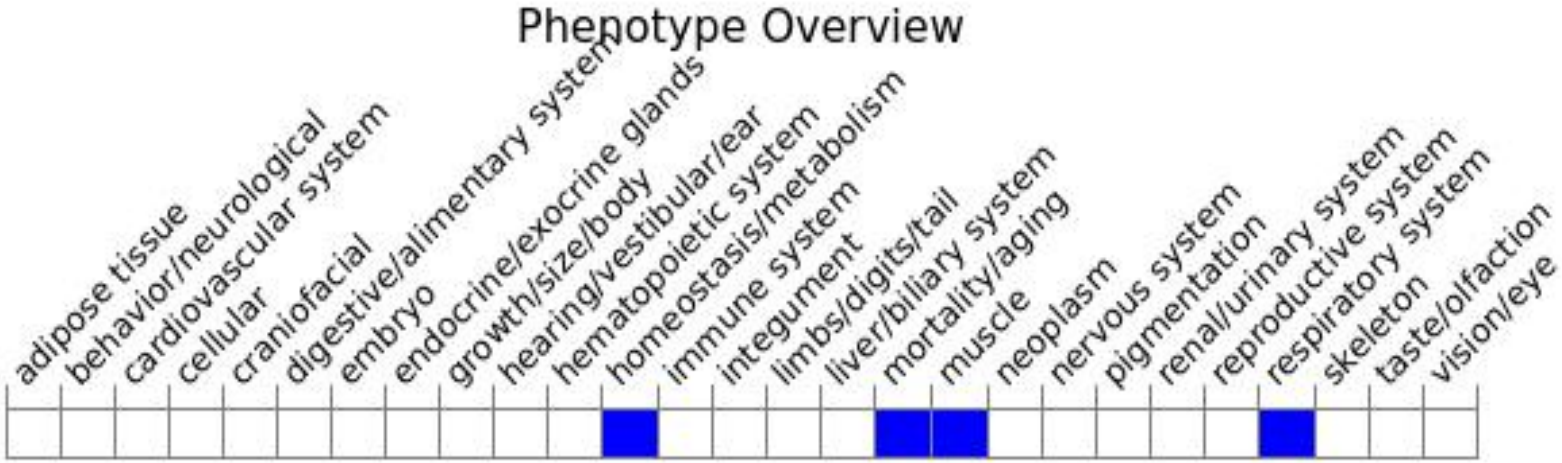
Genomic location distribution



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/).

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

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