

Atg9a Cas9-CKO Strategy

Designer: Huimin Su

Reviewer: Ruiuri Zhang

Design Date: 2020-5-27

Project Overview

Project Name

Atg9a

Project type

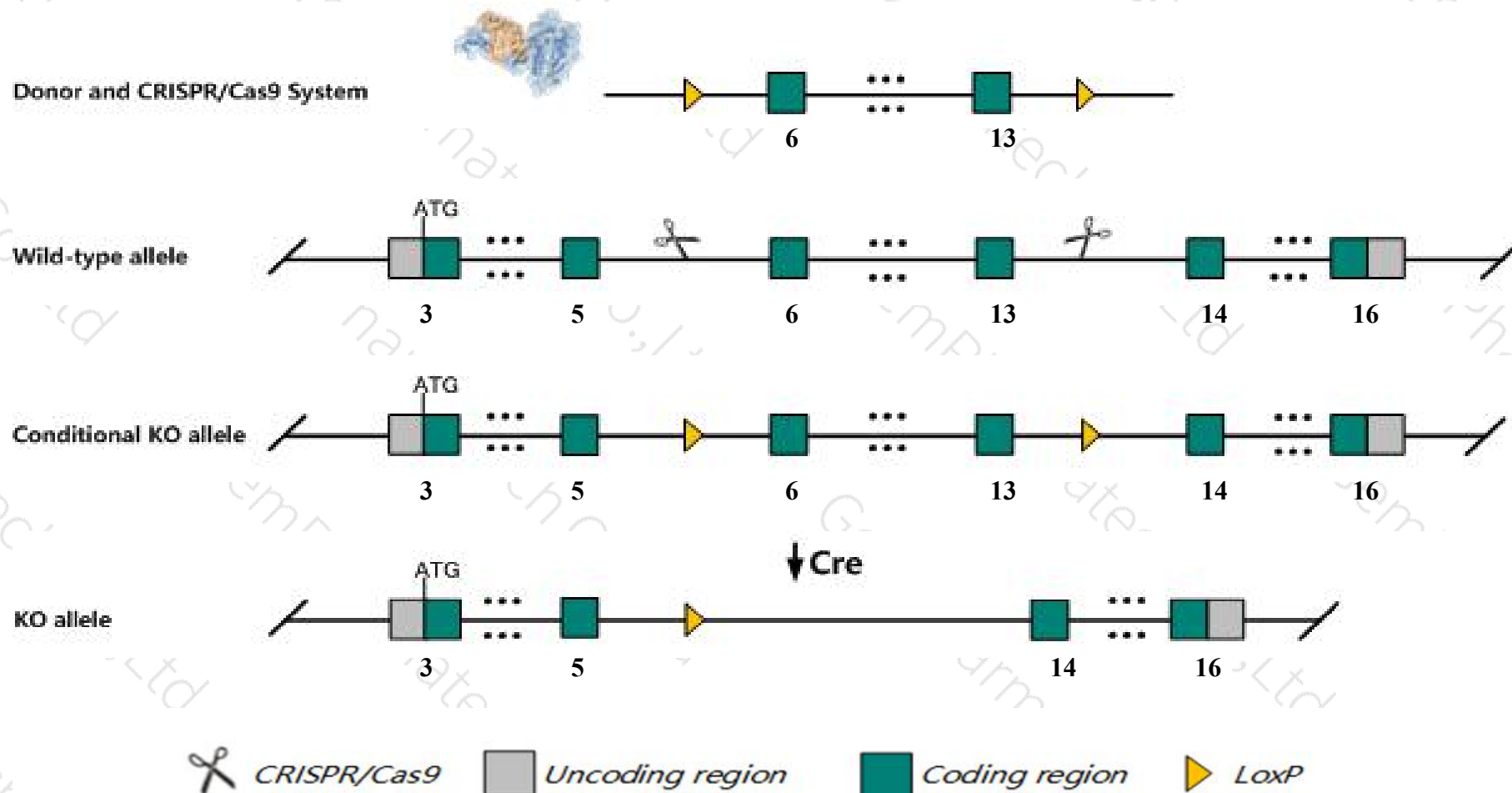
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Atg9a* gene. The schematic diagram is as follows:



- The *Atg9a* gene has 10 transcripts. According to the structure of *Atg9a* gene, exon6-exon13 of *Atg9a*-208 (ENSMUST00000189702.6) transcript is recommended as the knockout region. The region contains 1933bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Atg9a* 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 homozygous for a null mutation all die within 1 day of birth and display impaired autophagy.
- Transcript *Atg9a-202* is incomplete, so the effect on it is unknown.
- The *Atg9a* gene is located on the Chr1. 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)

Atg9a autophagy related 9A [*Mus musculus* (house mouse)]

Gene ID: 245860, updated on 31-Mar-2020

Summary

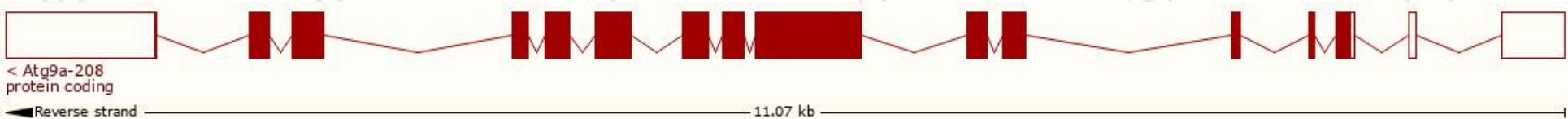
Official Symbol	Atg9a provided by MGI
Official Full Name	autophagy related 9A provided by MGI
Primary source	MGI:MGI:2138446
See related	Ensembl:ENSMUSG00000033124
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Atg9; Apg9l1; Atg9l1; AU019532
Expression	Ubiquitous expression in testis adult (RPKM 166.6), duodenum adult (RPKM 40.8) and 26 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

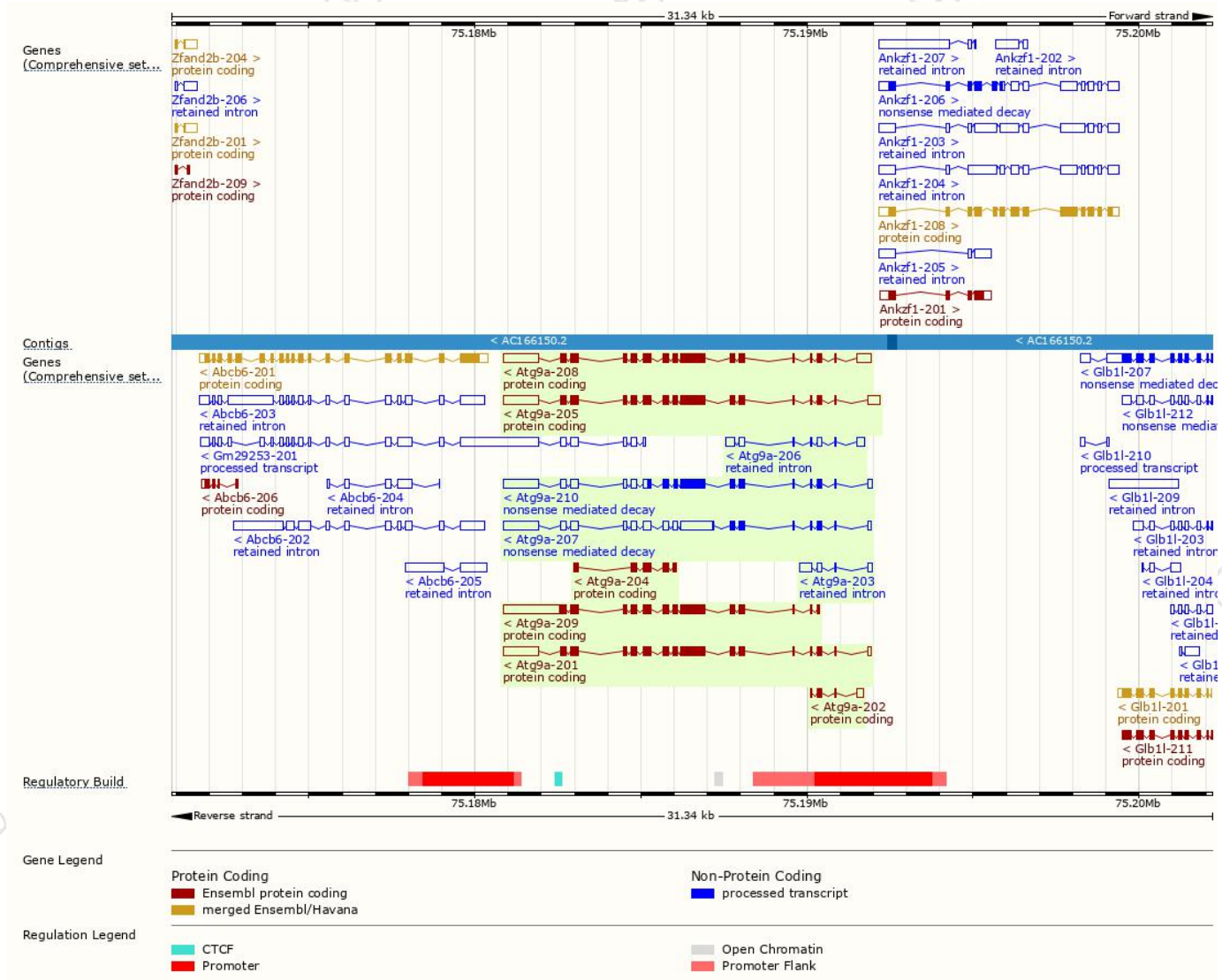
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Atg9a-208	ENSMUST00000189702.6	4110	839aa	Protein coding	CCDS35622	Q3ZQA4	TSL:5 GENCODE basic APPRIS P1
Atg9a-205	ENSMUST00000188347.6	4041	839aa	Protein coding	CCDS35622	Q3ZQA4	TSL:5 GENCODE basic APPRIS P1
Atg9a-201	ENSMUST00000040689.14	3764	839aa	Protein coding	CCDS35622	Q3ZQA4	TSL:1 GENCODE basic APPRIS P1
Atg9a-209	ENSMUST00000189820.6	4218	847aa	Protein coding	-	A0A087WP33	CDS 5' incomplete TSL:2
Atg9a-204	ENSMUST00000187785.1	832	278aa	Protein coding	-	A0A087WPL8	CDS 5' and 3' incomplete TSL:3
Atg9a-202	ENSMUST00000186744.1	457	47aa	Protein coding	-	A0A087WRG3	CDS 3' incomplete TSL:3
Atg9a-207	ENSMUST00000189665.6	4013	176aa	Nonsense mediated decay	-	A0A087WQ26	TSL:1
Atg9a-210	ENSMUST00000239085.1	3741	551aa	Nonsense mediated decay	-	Q68FE2	-
Atg9a-206	ENSMUST00000188430.6	967	No protein	Retained intron	-	-	TSL:1
Atg9a-203	ENSMUST00000187601.6	716	No protein	Retained intron	-	-	TSL:3

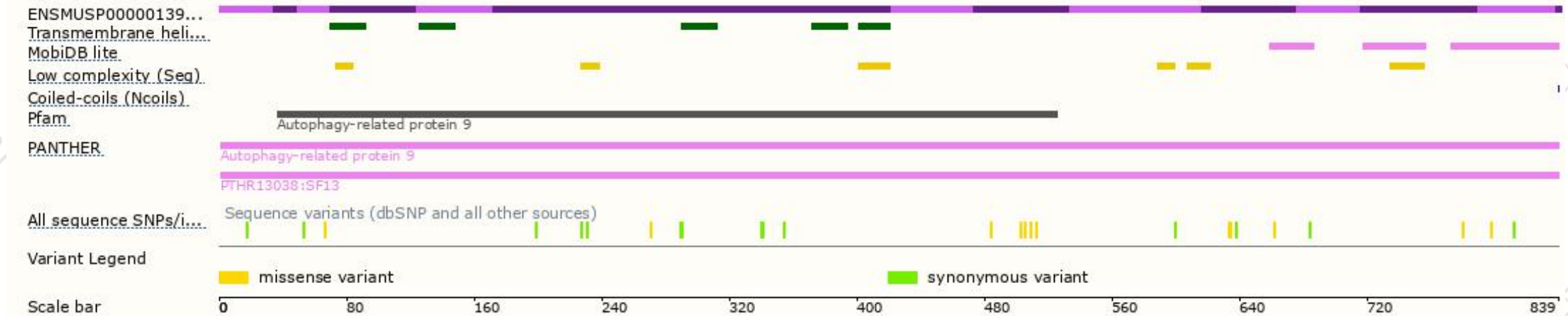
The strategy is based on the design of *Atg9a-208* transcript,the transcription is shown below:



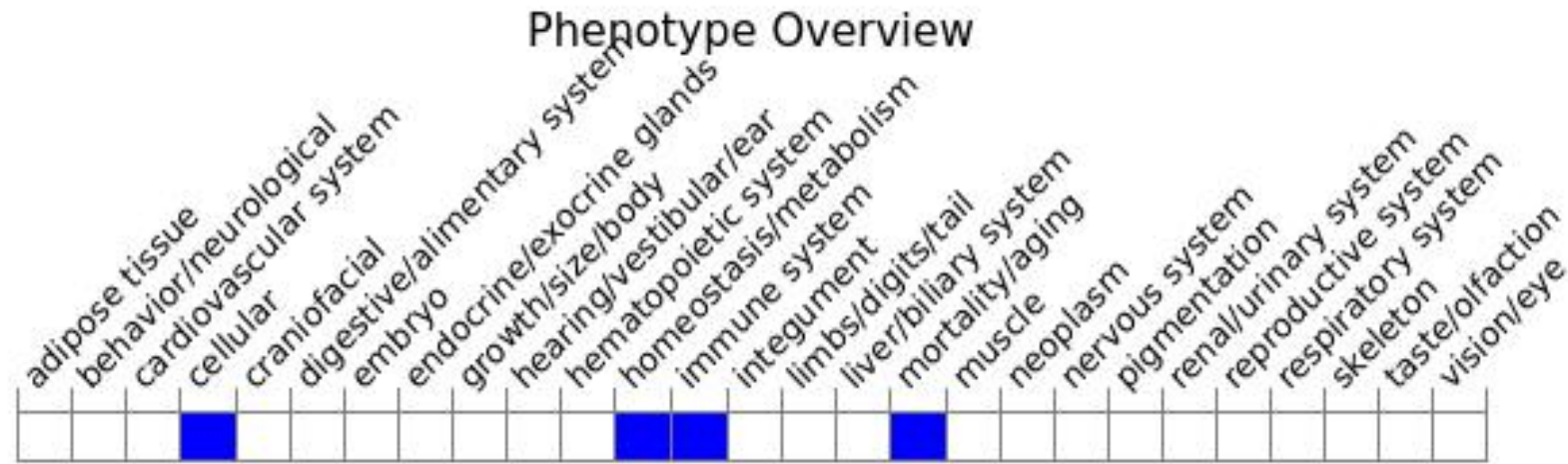
Genomic location distribution



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 homozygous for a null mutation all die within 1 day of birth and display impaired autophagy.

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

Tel: 400-9660890

