

Atg4d Cas9-CKO Strategy

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Design Date: 2019-8-1

Project Overview



Project Name

Atg4d

Project type

Cas9-CKO

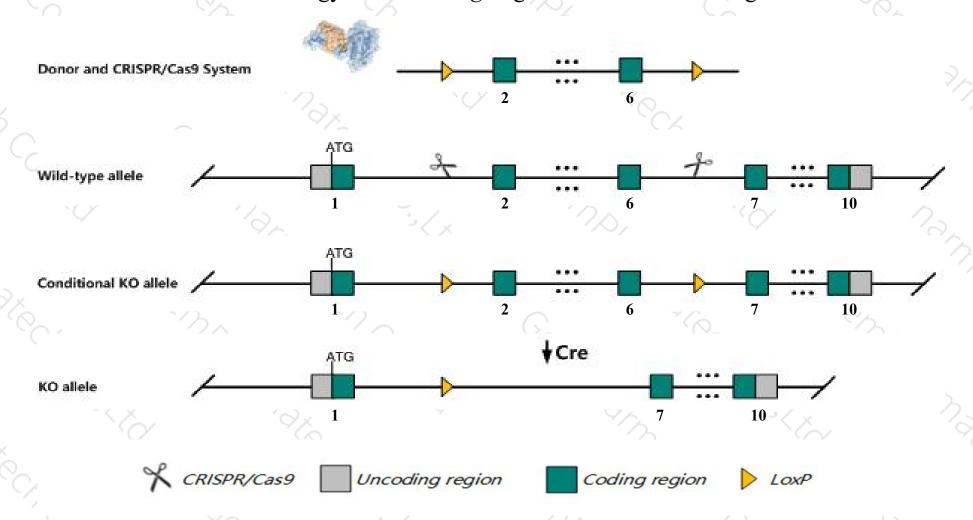
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Atg4d gene. The schematic diagram is as follows:



Technical routes



- The *Atg4d* gene has 5 transcripts. According to the structure of *Atg4d* gene, exon2-exon6 of *Atg4d-201* (ENSMUST0000065005.4) transcript is recommended as the knockout region. The region contains 731bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Atg4d* 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



- > The *Atg4d* gene is located on the Chr9. 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)



Atg4d autophagy related 4D, cysteine peptidase [Mus musculus (house mouse)]

Gene ID: 235040, updated on 4-Mar-2019

Summary

☆ ?

Official Symbol Atg4d provided by MGI

Official Full Name autophagy related 4D, cysteine peptidase provided by MGI

Primary source MGI:MGI:2444308

See related Ensembl:ENSMUSG00000002820

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 9830134P12Rik, APG4-D, Apg4d, Atg4dl, Autl4

Expression Ubiquitous expression in duodenum adult (RPKM 44.0), colon adult (RPKM 34.3) and 28 other tissuesSee more

Orthologs <u>human</u> all

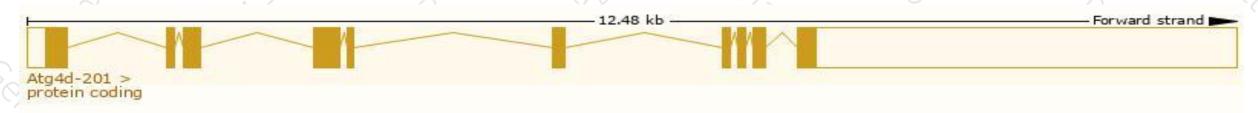
Transcript information (Ensembl)



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

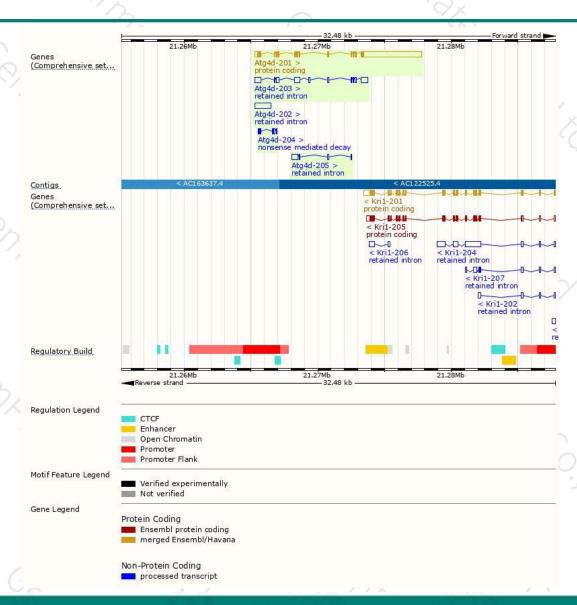
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Atg4d-201	ENSMUST00000065005.4	5951	<u>474aa</u>	Protein coding	CCDS22899	Q8BGV9	TSL:1 GENCODE basic APPRIS P1
Atg4d-204	ENSMUST00000217269.1	445	<u>100aa</u>	Nonsense mediated decay	1 8	A0A1L1STB3	CDS 5' incomplete TSL:2
Atg4d-203	ENSMUST00000216826.1	2123	No protein	Retained intron	10	=	TSL:2
Atg4d-202	ENSMUST00000215767.1	1204	No protein	Retained intron	20	20	TSL:NA
Atg4d-205	ENSMUST00000217591.1	697	No protein	Retained intron	₹å	5	TSL:5

The strategy is based on the design of Atg4d-201 transcript, The transcription is shown below



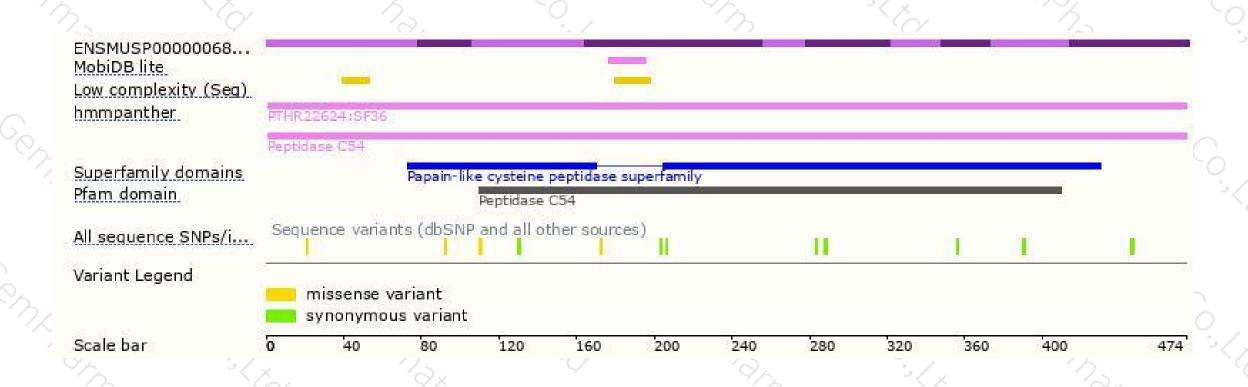
Genomic location distribution





Protein domain







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





