

Ruben Cas9-CKO Strategy

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Reviewer:

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Design Date:

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Project Overview



Project Name

Rubcn

Project type

Cas9-CKO

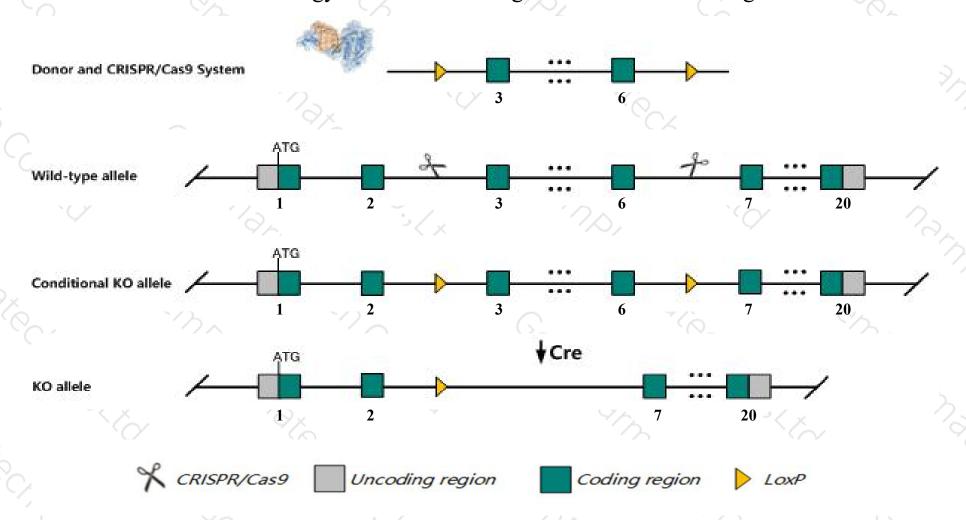
Strain background

C57BL/6JGpt

Conditional Knockout strategy



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



Technical routes



- The *Rubcn* gene has 9 transcripts. According to the structure of *Rubcn* gene, exon3-exon6 of *Rubcn-202* (ENSMUST00000089684.9) transcript is recommended as the knockout region. The region contains 511bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rubcn* 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, Homozygous knockout affects the functioning of LAP (LC3-associated phagocytosis)-engaged phagosomes.
- The *Rubcn* gene is located on the Chr16. 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)



Rubcn RUN domain and cysteine-rich domain containing, Beclin 1-interacting protein [Mus musculus (house mouse)]

Gene ID: 100502698, updated on 31-Jan-2019

Summary



Official Symbol Rubon provided by MGI

Official Full Name RUN domain and cysteine-rich domain containing, Beclin 1-interacting protein provided by MGI

Primary source MGI:MGI:1915160

See related Ensembl:ENSMUSG00000035629

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 1700021K19Rik, 5330403K09, Rubicon, mKIAA0226

Expression Ubiquitous expression in spleen adult (RPKM 22.1), testis adult (RPKM 17.2) and 28 other tissuesSee more

Orthologs <u>human</u> all

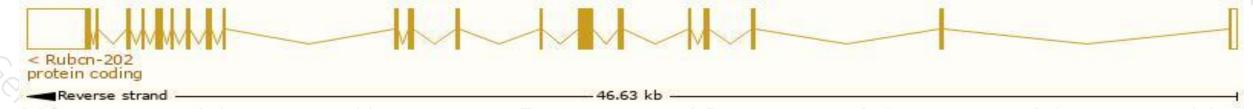
Transcript information (Ensembl)



The gene has 9 transcripts, all transcripts are shown below:

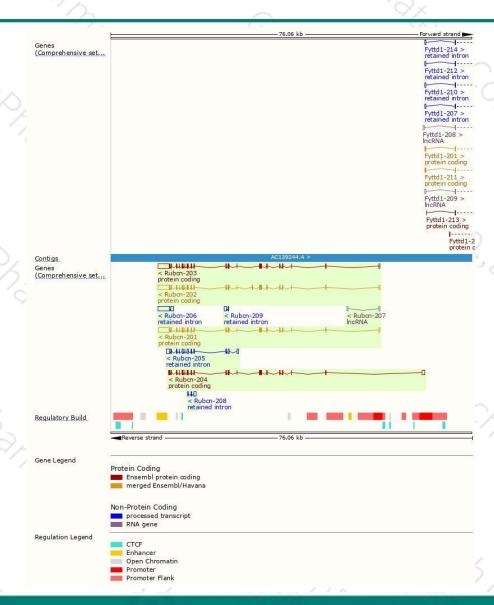
Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
ENSMUST00000089684.9	5311	956aa	Protein coding	CCDS49832	Q80U62	TSL:1 GENCODE basic APPRIS ALT2
ENSMUST00000040986.14	5257	941aa	Protein coding	CCDS28124	Q80U62	TSL:1 GENCODE basic APPRIS P3
ENSMUST00000115105.8	5255	<u>927aa</u>	Protein coding	8	Q80U62	TSL:5 GENCODE basic APPRIS ALT2
ENSMUST00000119810.1	3371	880aa	Protein coding	10	D3Z7B1	TSL:5 GENCODE basic APPRIS ALT2
ENSMUST00000140229.1	2982	No protein	Retained intron	-	150	TSL:1
ENSMUST00000135480.7	2301	No protein	Retained intron	* .	6.00	TSL:1
ENSMUST00000152920.1	664	No protein	Retained intron	Si .	140	TSL:3
ENSMUST00000153556.1	653	No protein	Retained intron	10	V20	TSL:3
ENSMUST00000149621.1	404	No protein	IncRNA	8	1753	TSL:3
Ē	ENSMUST00000135480.7 ENSMUST00000152920.1 ENSMUST00000153556.1	ENSMUST00000135480.7 2301 ENSMUST00000152920.1 664 ENSMUST00000153556.1 653	ENSMUST00000135480.7 2301 No protein ENSMUST00000152920.1 664 No protein ENSMUST00000153556.1 653 No protein	ENSMUST00000135480.7 2301 No protein Retained intron ENSMUST00000152920.1 664 No protein Retained intron ENSMUST00000153556.1 653 No protein Retained intron	ENSMUST00000135480.7 2301 No protein Retained intron - ENSMUST00000152920.1 664 No protein Retained intron - ENSMUST00000153556.1 653 No protein Retained intron -	ENSMUST00000135480.7 2301 No protein Retained intron - - ENSMUST00000152920.1 664 No protein Retained intron - - ENSMUST00000153556.1 653 No protein Retained intron - -

The strategy is based on the design of Ruben-202 transcript, The transcription is shown below



Genomic location distribution





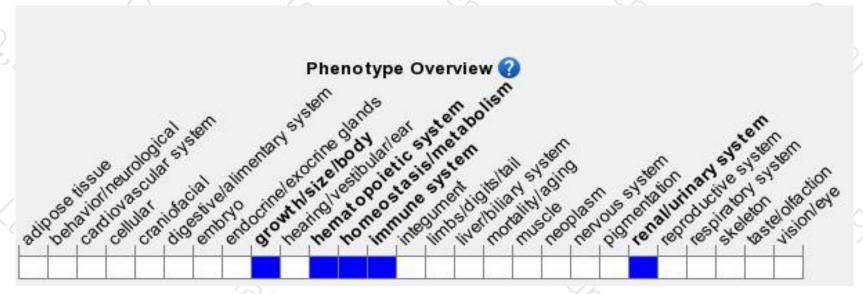
Protein domain





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, Homozygous knockout affects the functioning of LAP (LC3-associated phagocytosis)-engaged phagosomes.



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





