

Atp10b Cas9-CKO Strategy

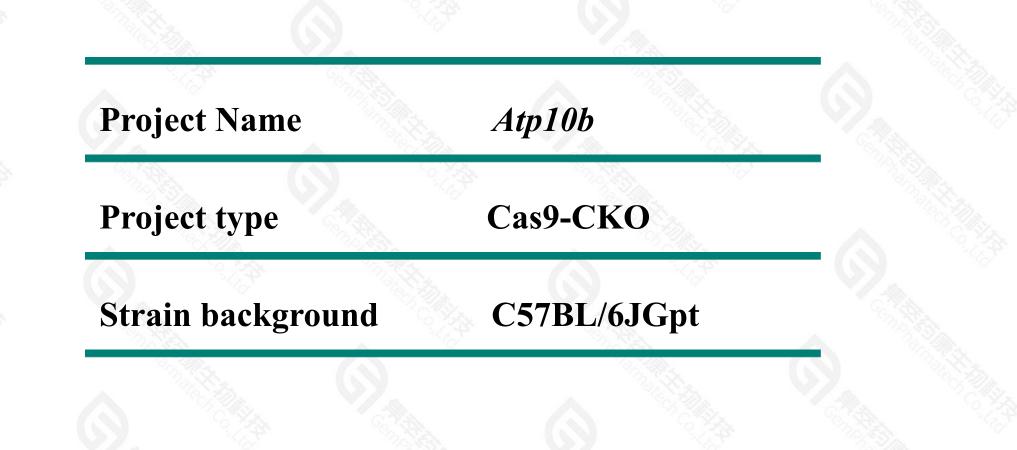
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Reviewer: Xueting Zhang

Design Date: 2021-3-23

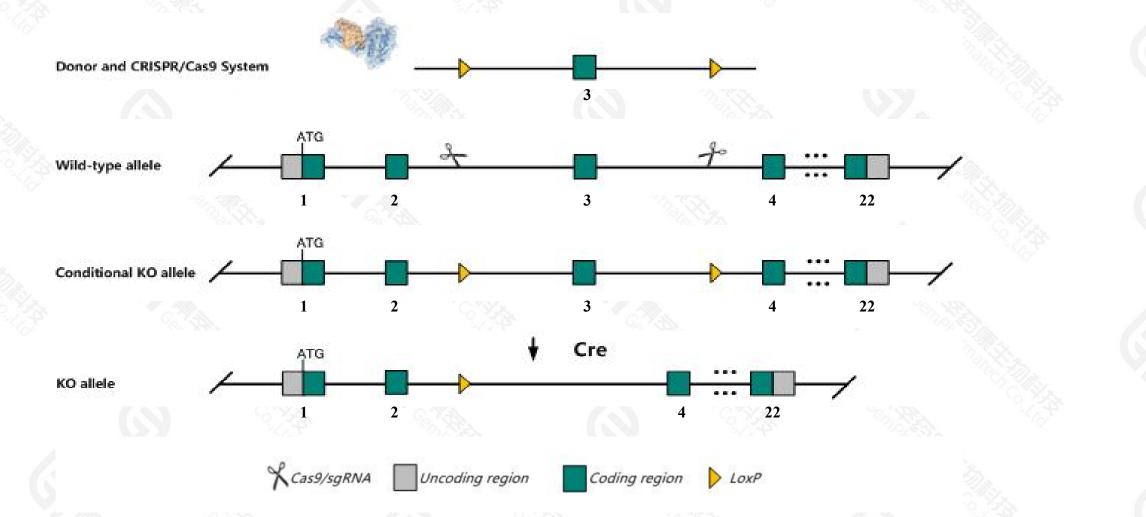
Project Overview





Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Atp10b* gene. The schematic diagram is as follows:



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Technical routes



The *Atp10b* gene has 3 transcripts. According to the structure of *Atp10b* gene, exon3 of *Atp10b*-201(ENSMUST00000077659.6) transcript is recommended as the knockout region. The region contains 205bp coding sequence.
Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Atp10b* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor 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.



- > The Atp10b gene is located on the Chr11. 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.

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Gene information (NCBI)

Atp10b ATPase, class V, type 10B [Mus musculus (house mouse)]

Gene ID: 319767, updated on 17-Dec-2020

Summary

Official Symbol	Atp10b provided by MGI
Official Full Name	ATPase, class V, type 10B provided by MGI
Primary source	MGI:MGI:2442688
See related	Ensembl:ENSMUSG00000055415
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;
	Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	5930426013Rik, 9030605H24Rik
Expression	Biased expression in genital fat pad adult (RPKM 11.0), large intestine adult (RPKM 9.6) and 6 other tissuesSee more
Orthologs	human all



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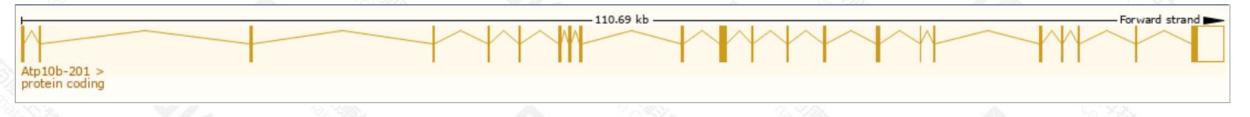
Transcript information (Ensembl)



The gene has 3 transcripts, all transcripts are shown below:

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Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Atp10b-201	ENSMUST0000077659.6	6825	<u>1474aa</u>	Protein coding	CCD536129		TSL:5 , GENCODE basic , APPRIS P2 ,	
Atp10b-203	ENSMUST00000238415.2	6949	<u>1446aa</u>	Protein coding	-		APPRIS ALT2,	
Atp10b-202	ENSMUST00000148911.2	3477	No protein	Processed transcript	120		TSL:1,	

The strategy is based on the design of *Atp10b-201* transcript, the transcription is shown below:



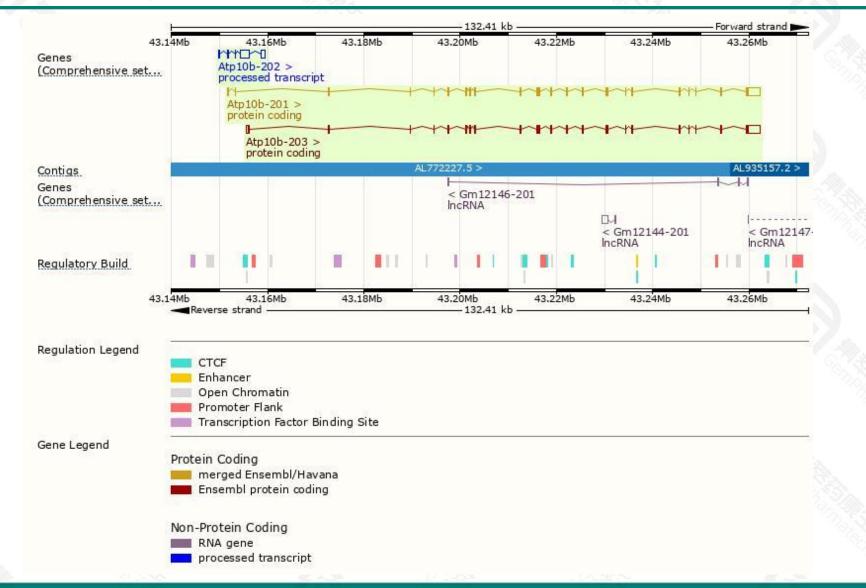
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Genomic location distribution





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Protein domain







If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



