

Arl8a Cas9-CKO Strategy

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Reviewer: Longyun Hu

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Project Overview



Project Name Arl8a

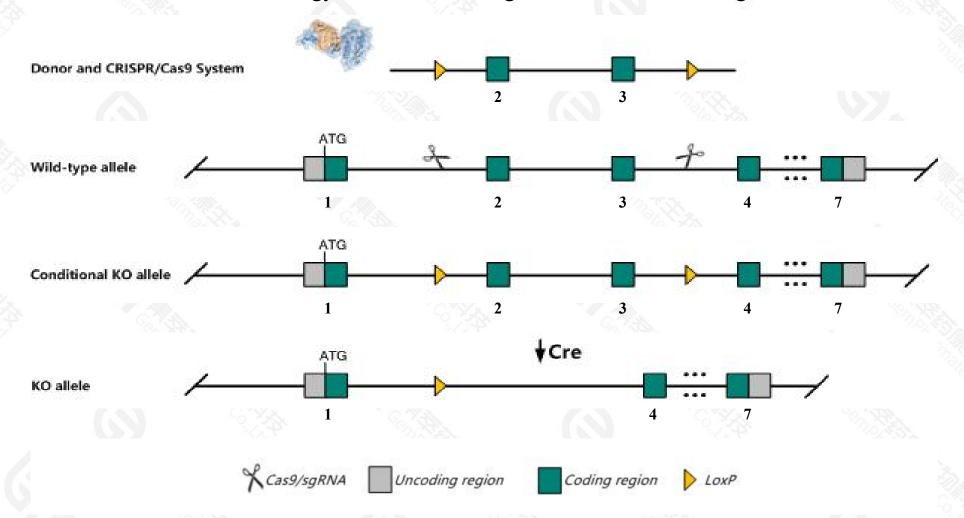
Project type Cas9-CKO

Strain background C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Arl8a* gene. The schematic diagram is as follows:



Technical routes



- The *Arl8a* gene has 4 transcripts. According to the structure of *Arl8a* gene, exon2-exon3 of *Arl8a-201*(ENSMUST00000027684.11) transcript is recommended as the knockout region. The region contains 155bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Arl8a* 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.

Notice



- > *Gm15445-201* gene will be destroyed.
- > The floxed region is near to the N-terminal of $5S_rRNA.111-201$ gene, this strategy may influence the regulatory function of the N-terminal of $5S_rRNA.111-201$ gene.
- The *Arl8a* 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)



Arl8a ADP-ribosylation factor-like 8A [Mus musculus (house mouse)]

Gene ID: 68724, updated on 17-Feb-2021

Summary

☆ ?

Official Symbol Arl8a provided by MGI

Official Full Name ADP-ribosylation factor-like 8A provided byMGI

Primary source MGI:MGI:1915974

See related Ensembl:ENSMUSG00000026426

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 1110033P22Rik, Arl, Arl10b, gie2

Expression Ubiquitous expression in CNS E18 (RPKM 133.7), whole brain E14.5 (RPKM 99.2) and 25 other tissuesSee more

Orthologs <u>human all</u>

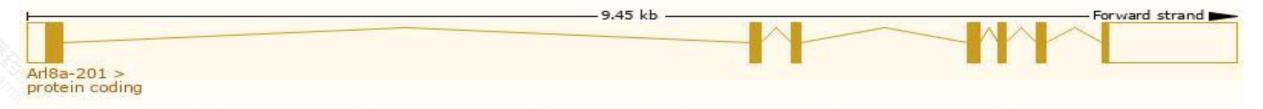
Transcript information (Ensembl)



The gene has 4 transcripts, all transcripts are shown below:

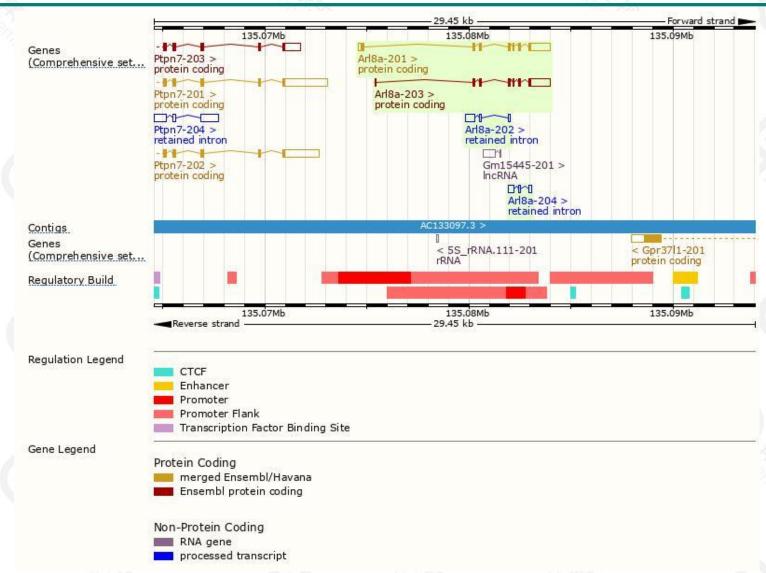
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Arl8a-201	ENSMUST00000027684.11	1713	186aa	Protein coding	CCDS15316		TSL:1 , GENCODE basic , APPRIS P1 ,
Arl8a-203	ENSMUST00000125774.2	1495	<u>165aa</u>	Protein coding			CDS 5' incomplete , TSL:3 ,
Arl8a-202	ENSMUST00000123344.2	664	No protein	Retained intron			TSL:2,
Arl8a-204	ENSMUST00000141177.2	472	No protein	Retained intron	-		TSL:2,

The strategy is based on the design of *Arl8a-201* transcript, the transcription is shown below:



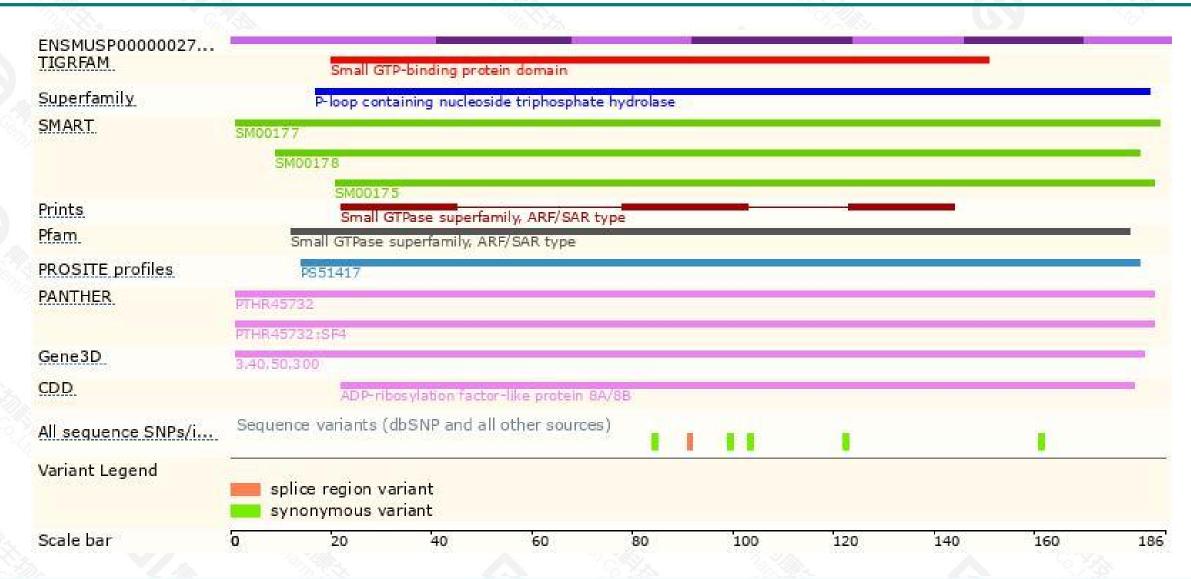
Genomic location distribution





Protein domain







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

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