

# Arl13b Cas9-CKO Strategy

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# **Project Overview**



Project Name Arl13b

Project type Cas9-CKO

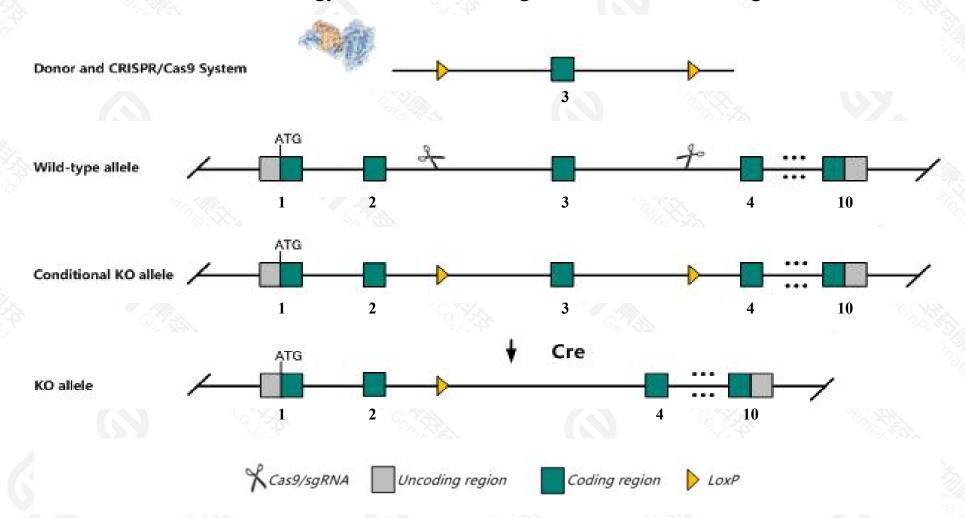
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Arl13b* gene. The schematic diagram is as follows:



### **Technical routes**



- ➤ The Arl13b gene has 4 transcripts. According to the structure of Arl13b gene, exon3 of Arl13b201(ENSMUST00000089289.6) transcript is recommended as the knockout region. The region contains 250bp coding sequence.

  Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Arl13b* 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**



- > According to the existing MGI data, mice homozygous for an ENU-induced mutation die at E13-E14 exhibiting left-right randomization and absence of the floor plate in the caudal spinal cord.
- The Arl13b 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)



#### Arl13b ADP-ribosylation factor-like 13B [Mus musculus (house mouse)]

Gene ID: 68146, updated on 23-Feb-2021

#### Summary

☆ ?

Official Symbol Arl13b provided by MGI

Official Full Name ADP-ribosylation factor-like 13B provided by MGI

Primary source MGI:MGI:1915396

See related Ensembl: ENSMUSG00000022911

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 A530097K21Rik, A930014M17Rik, Ar, Arl2l1, C530009C10Rik, h, hnn

Expression Ubiquitous expression in limb E14.5 (RPKM 4.6), testis adult (RPKM 3.9) and 27 other tissuesSee more

Orthologs <u>human all</u>

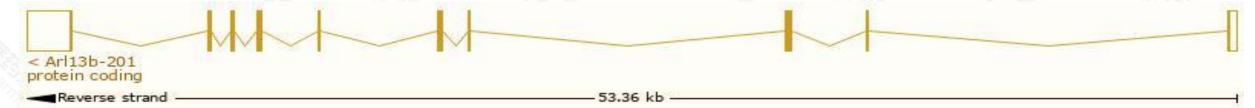
# Transcript information (Ensembl)



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

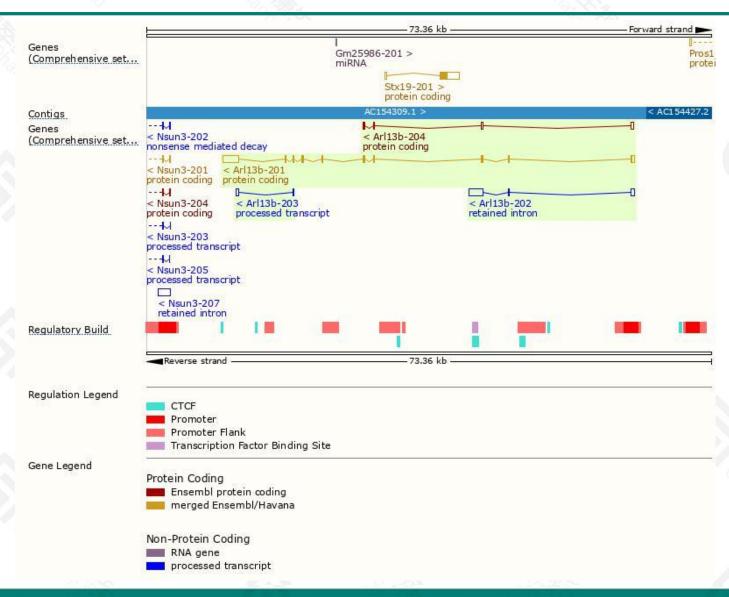
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Arl13b-201	ENSMUST00000089289.6	3528	427aa	Protein coding	CCDS28261		TSL:5 , GENCODE basic , APPRIS P1
Arl13b-204	ENSMUST00000232561.2	867	<u>126aa</u>	Protein coding	3-		CDS 3' incomplete ,
Arl13b-203	ENSMUST00000141665.2	473	No protein	Processed transcript	1-		TSL:3,
Arl13b-202	ENSMUST00000132902.2	2236	No protein	Retained intron	-		TSL:2,

The strategy is based on the design of Arl13b-201 transcript, the transcription is shown below:



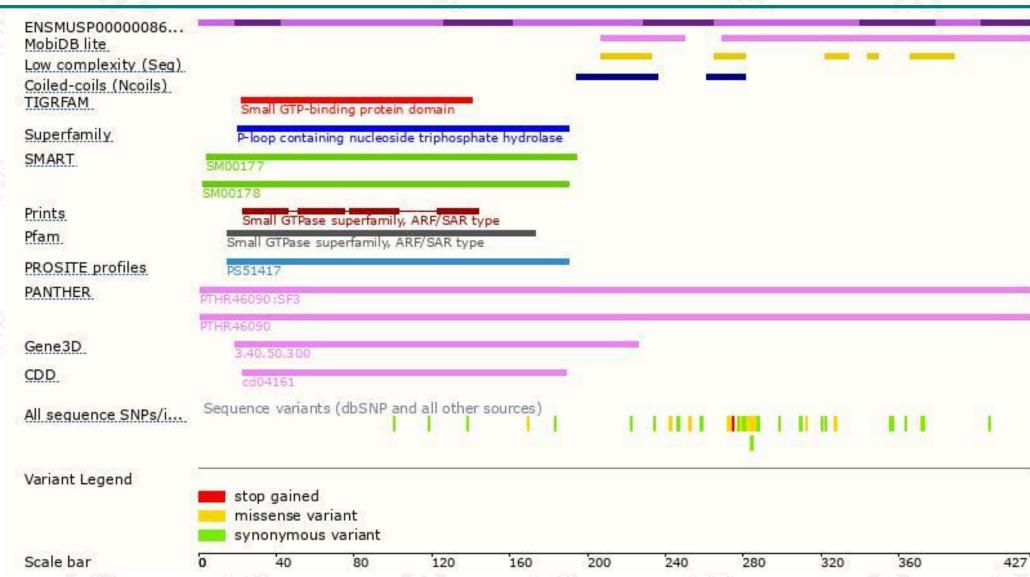
### Genomic location distribution





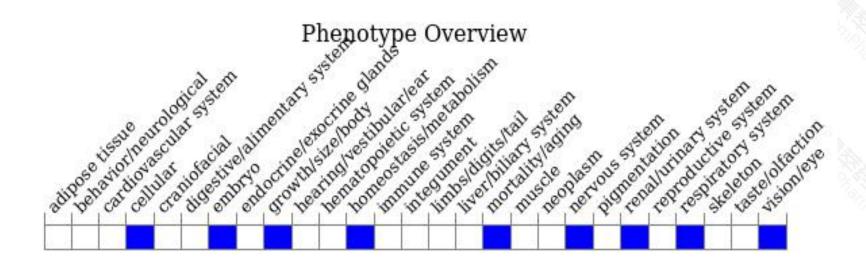
### Protein domain





# 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 an ENU-induced mutation die at E13-E14 exhibiting left-right randomization and absence of the floor plate in the caudal spinal cord.



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

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