

# Bin1 Cas9-CKO Strategy

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**Reviewer:** Huan Fan

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## **Project Overview**



**Project Name** 

Bin1

**Project type** 

Cas9-CKO

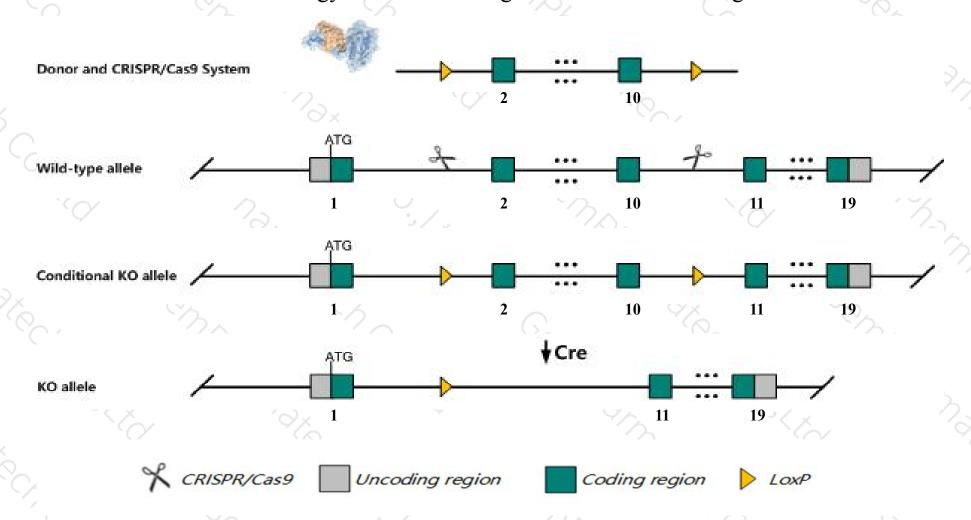
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Bin1* gene. The schematic diagram is as follows:



### Technical routes



- ➤ The *Bin1* gene has 6 transcripts. According to the structure of *Bin1* gene, exon2-exon10 of *Bin1-201* (ENSMUST00000025239.8) transcript is recommended as the knockout region. The region contains 773bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Bin1* 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 mutation of this gene results in thickened ventricular walls, densely packed myocardiocytes, and disorganization of myofibrils. mutant animals die shortly after birth.
- ➤ The *Bin1* gene is located on the Chr18. 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)



#### Bin1 bridging integrator 1 [Mus musculus (house mouse)]

Gene ID: 30948, updated on 13-Mar-2020

#### Summary

☆ ?

Official Symbol Bin1 provided by MGI

Official Full Name bridging integrator 1 provided by MGI

Primary source MGI:MGI:108092

See related Ensembl:ENSMUSG00000024381

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 ALP-1, Amphl, BRAMP-2, SH3P9

Expression Broad expression in cerebellum adult (RPKM 72.8), cortex adult (RPKM 65.7) and 25 other tissuesSee more

Orthologs <u>human</u> all

## Transcript information (Ensembl)



The gene has 6 transcripts, all transcripts are shown below:

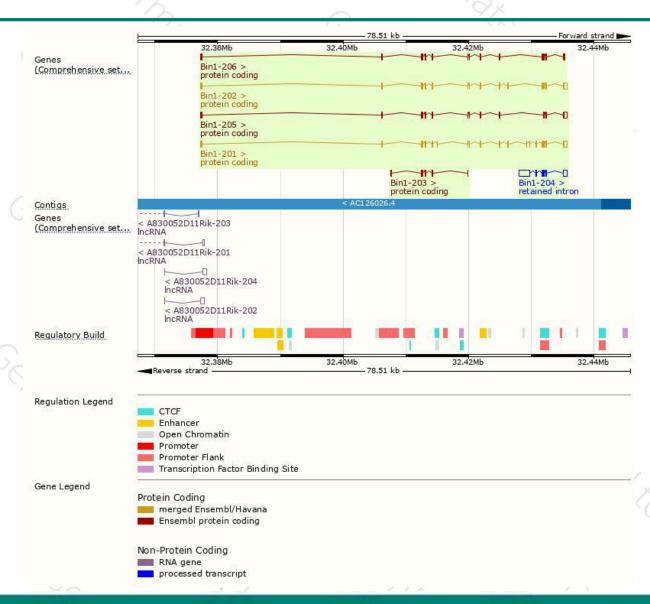
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Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags		
Bin1-201	ENSMUST00000025239.8	2406	588aa	Protein coding	CCDS29119	008539	TSL:2 GENCODE basic		
Bin1-202	ENSMUST00000091967.12	2091	<u>477aa</u>	Protein coding	CCDS37755	Q6P1B9	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P2		
Bin1-205	ENSMUST00000234496.1	1886	410aa	Protein coding	-	A0A3Q4EBK4	GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT1		
Bin1-206	ENSMUST00000234857.1	1530	<u>434aa</u>	Protein coding	72	008539	GENCODE basic		
Bin1-203	ENSMUST00000234024.1	539	120aa	Protein coding	-	A0A3Q4EBR8	CDS 3' incomplete		
Bin1-204	ENSMUST00000234373.1	2682	No protein	Retained intron	-	-			

The strategy is based on the design of *Bin1-201* transcript, the transcription is shown below



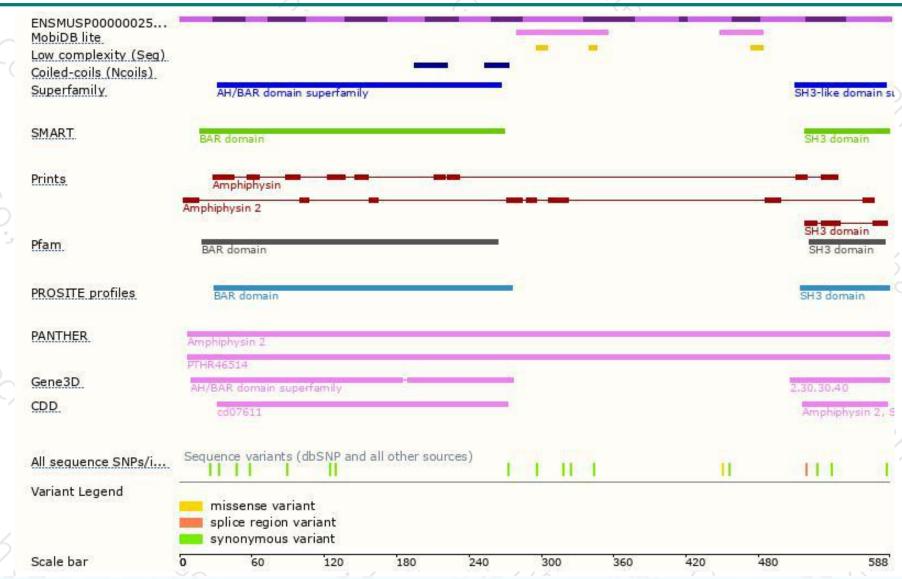
### Genomic location distribution





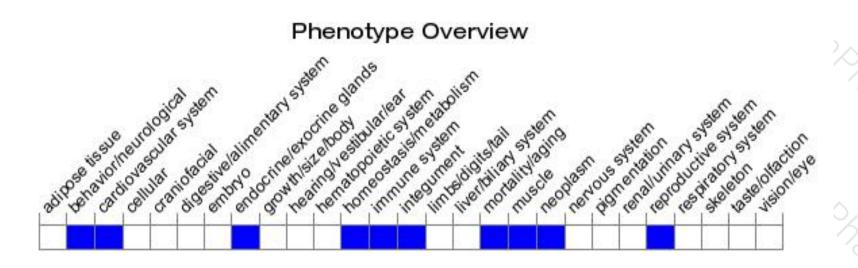
### Protein domain





## 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, homozygous mutation of this gene results in thickened ventricular walls, densely packed myocardiocytes, and disorganization of myofibrils. Mutant animals die shortly after birth.



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





