

# Myh13 Cas9-CKO Strategy

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Reviewer: Daohua Xu

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## **Project Overview**



**Project Name** 

Myh13

**Project type** 

Cas9-CKO

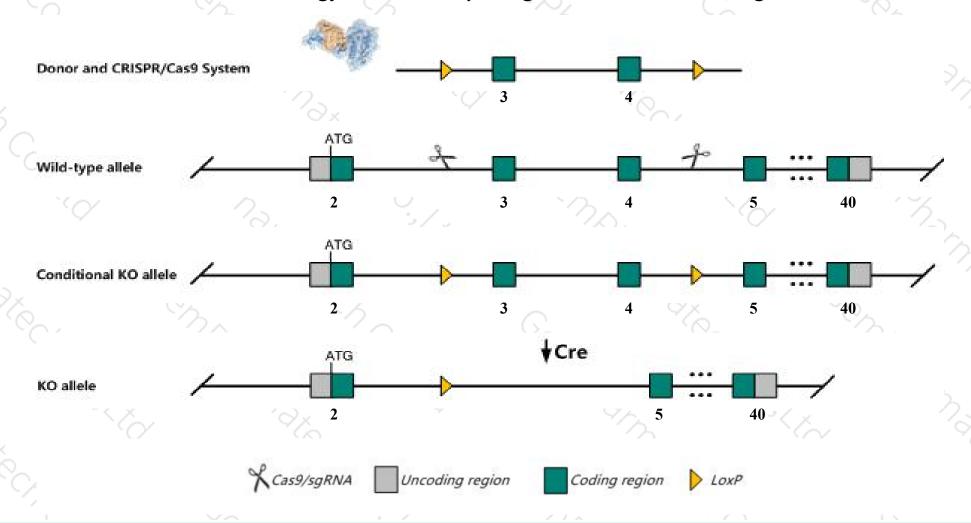
Strain background

C57BL/6JGpt

### Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Myh13* gene. The schematic diagram is as follows:



### Technical routes



- The *Myh13* gene has 4 transcripts. According to the structure of *Myh13* gene, exon3-exon4 of *Myh13*-202(ENSMUST00000108684.7) transcript is recommended as the knockout region. The region contains 301bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Myh13* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector 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**



- > The *Myh13* 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.
- ➤ The effect on transcript *Myh13*-204 is unknown.
- 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)



#### Myh13 myosin, heavy polypeptide 13, skeletal muscle [Mus musculus (house mouse)]

Gene ID: 544791, updated on 13-Mar-2020

#### Summary

☆ ?

Official Symbol Myh13 provided by MGI

Official Full Name myosin, heavy polypeptide 13, skeletal muscle provided by MGI

Primary source MGI:MGI:1339967

See related Ensembl:ENSMUSG00000060180

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 MyHC-eo

Expression Biased expression in mammary gland adult (RPKM 3.6), limb E14.5 (RPKM 1.6) and 3 other tissuesSee more

Orthologs <u>human all</u>

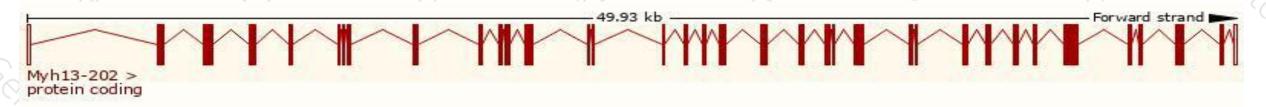
## Transcript information (Ensembl)



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

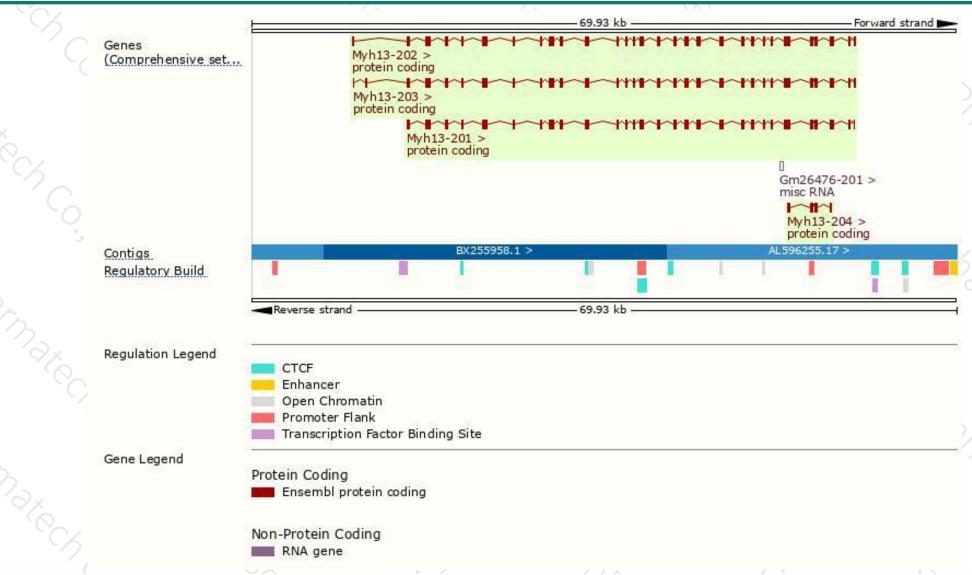
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Myh13-202	ENSMUST00000108684.7	6062	1938aa	Protein coding	CCDS36185	B1AR69	TSL:5 GENCODE basic APPRIS P1
Myh13-203	ENSMUST00000180845.7	5991	1938aa	Protein coding	CCDS36185	B1AR69	TSL:5 GENCODE basic APPRIS P1
Myh13-201	ENSMUST00000081911.11	5817	1938aa	Protein coding	CCDS36185	B1AR69	TSL:5 GENCODE basic APPRIS P1
Myh13-204	ENSMUST00000181027.1	668	223aa	Protein coding		MOQWLO	CDS 5' and 3' incomplete TSL:3

The strategy is based on the design of *Myh13-202* 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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