

Myo9b Cas9-CKO Strategy

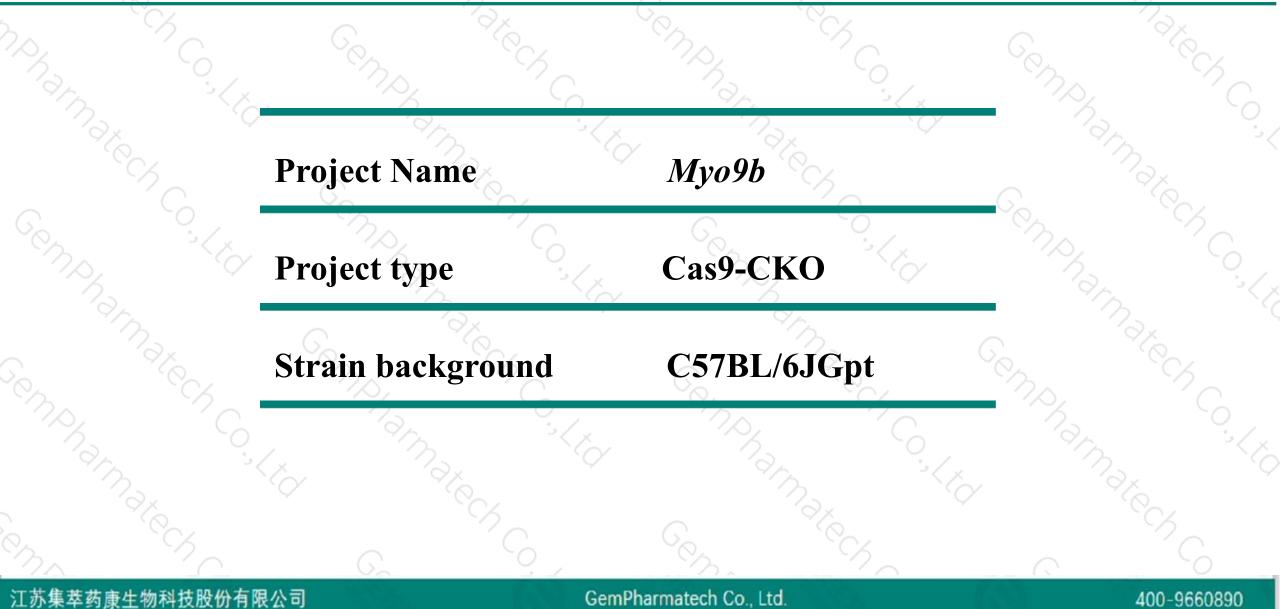
Designer: Reviewer:

Design Date:

Huan Wang Huan Fan 2020-3-6

Project Overview

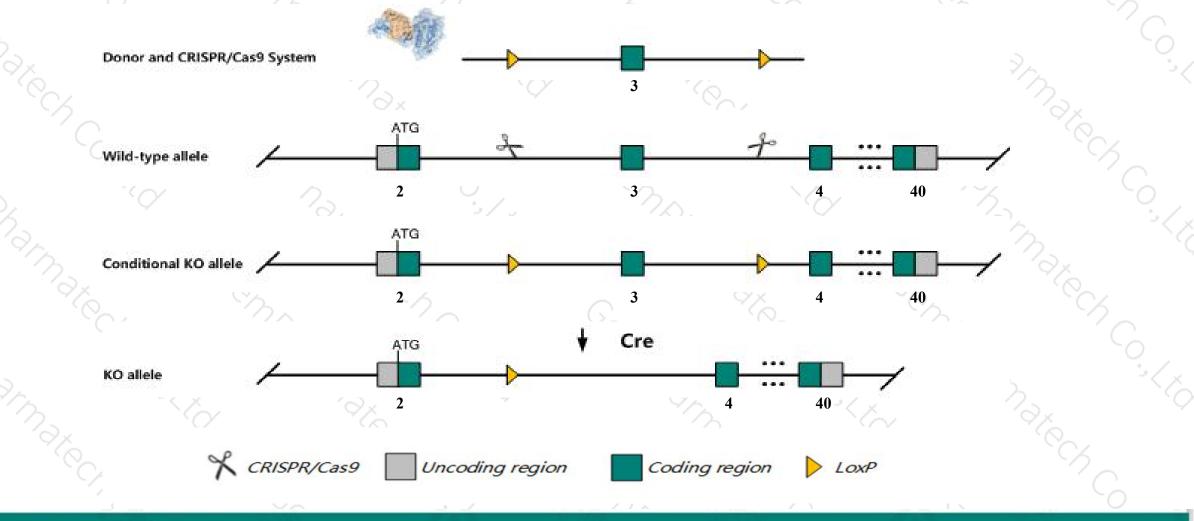




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Myo9b* gene. The schematic diagram is as follows:



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The Myo9b gene has 6 transcripts. According to the structure of Myo9b gene, exon3 of Myo9b-203 (ENSMUST00000170242.7) transcript is recommended as the knockout region. The region contains 95bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Myo9b* 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 null mutants breed normal, but shows defect in macrophage motility and chemotaxis.
- The *Myo9b* gene is located on the Chr8. 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)



× 1

Myo9b myosin IXb [Mus musculus (house mouse)]

Gene ID: 17925, updated on 31-Jan-2019

Summary

Official SymbolMyo9b provided by MGIOfficial Full Namemyosin IXb provided byMGIPrimary sourceMGI:MGI:106624See relatedEnsembl:ENSMUSG0000004677Gene typeprotein codingVALIDATEDVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
Muroidea; Muriae; Mus; MusExpressionUbiquitous expression in thymus adult (RPKM 15.8), spleen adult (RPKM 13.0) and 28 other tissues
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Transcript information (Ensembl)



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

| Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|----------------------|---|--|--|--|---|---|
| ENSMUST00000170242.7 | 7297 | <u>2128aa</u> | Protein coding | CCDS52580 | E9PZW8 | TSL:5 GENCODE basic APPRIS ALT2 |
| ENSMUST00000168839.8 | 7102 | <u>1975aa</u> | Protein coding | CCDS52581 | E9PWZ6 | TSL:5 GENCODE basic APPRIS ALT2 |
| ENSMUST00000071935.6 | 7082 | <u>1961aa</u> | Protein coding | CCDS40379 | E9QKV6 | TSL:5 GENCODE basic APPRIS P3 |
| ENSMUST00000212935.1 | 6316 | <u>1963aa</u> | Protein coding | <u>-</u> 2 | A0A1D5RLD1 | TSL:5 GENCODE basic APPRIS ALT2 |
| ENSMUST00000212412.1 | 3614 | <u>787aa</u> | Protein coding | | A0A1D5RLW4 | CDS 5' incomplete TSL:5 |
| ENSMUST00000212173.2 | 289 | No protein | IncRNA | | | TSL:1 |
| | ENSMUST00000170242.7 ENSMUST00000168839.8 ENSMUST0000071935.6 ENSMUST00000212935.1 ENSMUST00000212412.1 | ENSMUST00000170242.7 7297 ENSMUST00000168839.8 7102 ENSMUST0000071935.6 7082 ENSMUST00000212935.1 6316 ENSMUST00000212412.1 3614 | ENSMUST00000170242.7 7297 2128aa ENSMUST00000168839.8 7102 1975aa ENSMUST0000071935.6 7082 1961aa ENSMUST00000212935.1 6316 1963aa ENSMUST00000212412.1 3614 787aa | ENSMUST00000170242.772972128aaProtein codingENSMUST00000168839.871021975aaProtein codingENSMUST0000071935.670821961aaProtein codingENSMUST00000212935.163161963aaProtein codingENSMUST0000212412.13614787aaProtein codingENSMUST00000212173.2289No proteinIncRNA | ENSMUST00000170242.772972128aaProtein codingCCDS52580ENSMUST00000168839.871021975aaProtein codingCCDS52581ENSMUST0000071935.670821961aaProtein codingCCDS40379ENSMUST00000212935.163161963aaProtein codingCOS40379ENSMUST00000212412.13614787aaProtein coding | ENSMUST0000170242.772972128aaProtein codingCCDS52580E9PZW8ENSMUST0000168839.871021975aaProtein codingCCDS40379E9PWZ6ENSMUST0000071935.670821961aaProtein codingCCDS40379E9QKV6ENSMUST0000212935.163161963aaProtein codingA0A1D5RLD1ENSMUST0000212412.13614787aaProtein codingA0A1D5RLW4ENSMUST0000212173.2289No proteinIncRNA |

88.00 kb

The strategy is based on the design of Myo9b-203 transcript, The transcription is shown below

Myo9b-203 > protein coding

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400-9660890

Forward strand

Genomic location distribution

Genes

(Comprehensive set...

71.28Mb

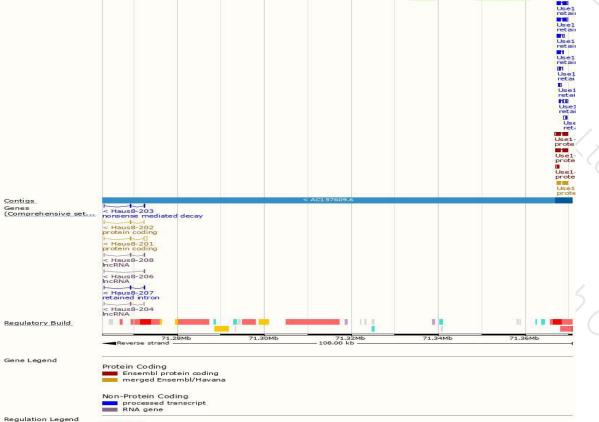
Myo9b-203 > protein coding

Myo9b-206 > protein coding Myo9b-201 > protein coding Myo9b-202 >

CTCE Enhancer Open Chromatin Promoter Promoter Flank

Transcription Factor Binding Site





08.00 kt

71.32

71.34Mb

Myo9b-204 >

71.30M

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Protein domain





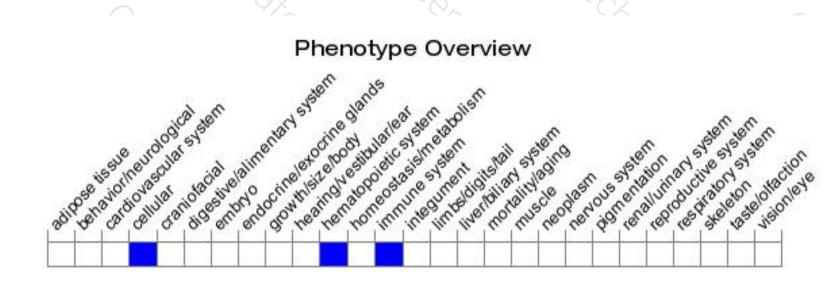


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Mouse phenotype description(MGI)





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 null mutants breed normal, but shows defect in macrophage motility and chemotaxis.



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



