Myo1b Cas9-CKO Strategy

Designer: Xueting Zhang
Reviewer:Yanhua Shen
Date:2020-02-25

Project Overview

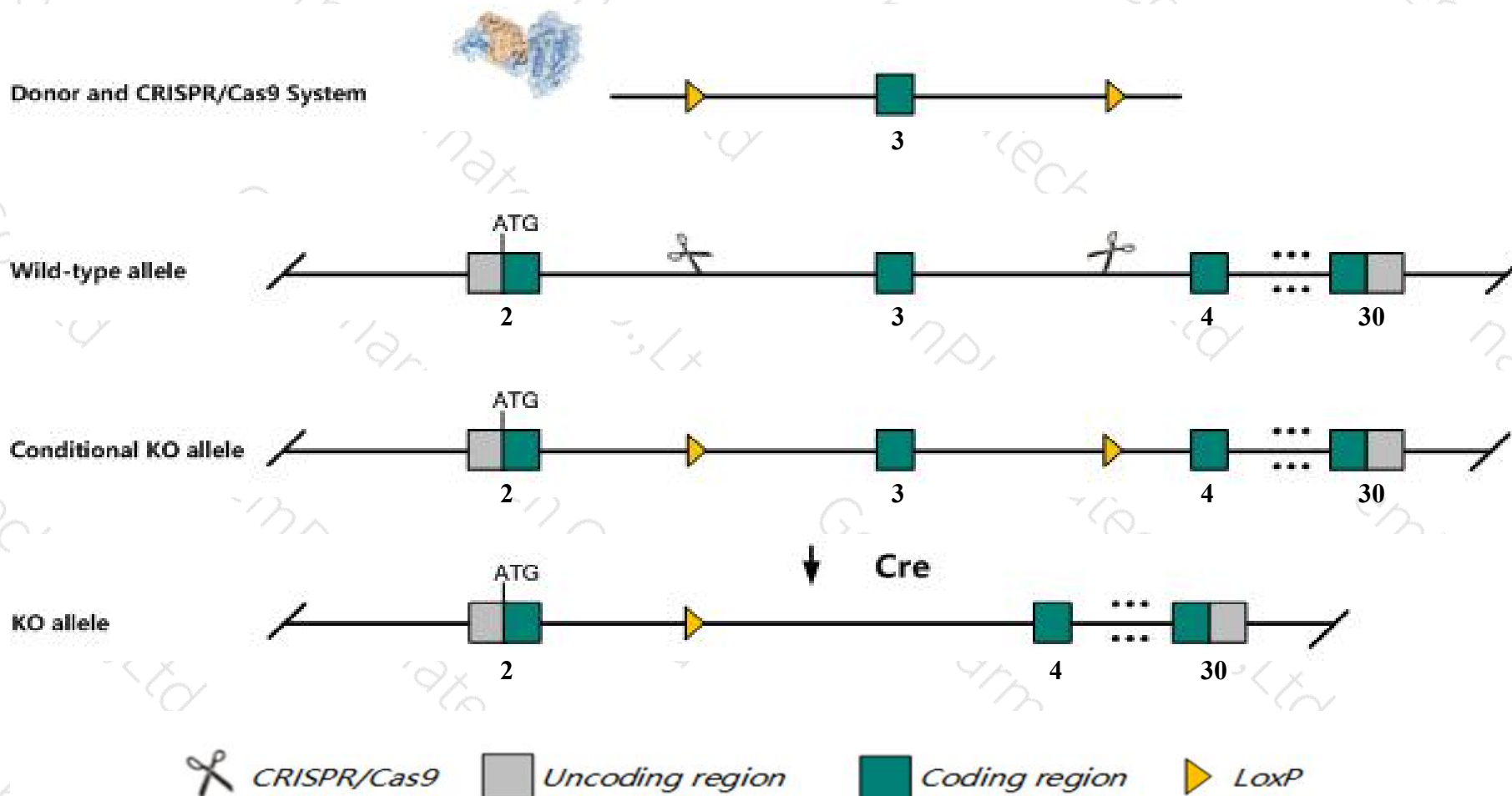|	
Project Name	***Myo1b***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Myo1b* gene. The schematic diagram is as follows:

Technical routes

➤ The *Myo1b* gene has 15 transcripts. According to the structure of *Myo1b* gene, exon3 of *Myo1b-202* (ENSMUST00000046390.13) transcript is recommended as the knockout region.The region contains 116bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Myo1b* 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

➢ Transcript *Myo1b*-205&207&208&209&210&211&212&214 may not be affected.

➢ The *Myo1b* 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）

Myo1b   myosin IB [*Mus musculus* (house mouse)]

Gene ID: 17912, updated on 18-Feb-2020

▲ Summary

Official Symbol	Myo1b provided by MGI
Official Full Name	myosin IB provided by MGI
Primary source	MGI:MGI:107752
See related	Ensembl:ENSMUSG00000018417
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	MMIa; MIH-L; AA406997
Expression	Broad expression in CNS E11.5 (RPKM 15.5), CNS E14 (RPKM 12.3) and 22 other tissues See more
Orthologs	human all

▲ Genomic context

Location: 1 C1.1; 1 26.58 cM

See Myo1b in Genome Data Viewer

Exon count: 34

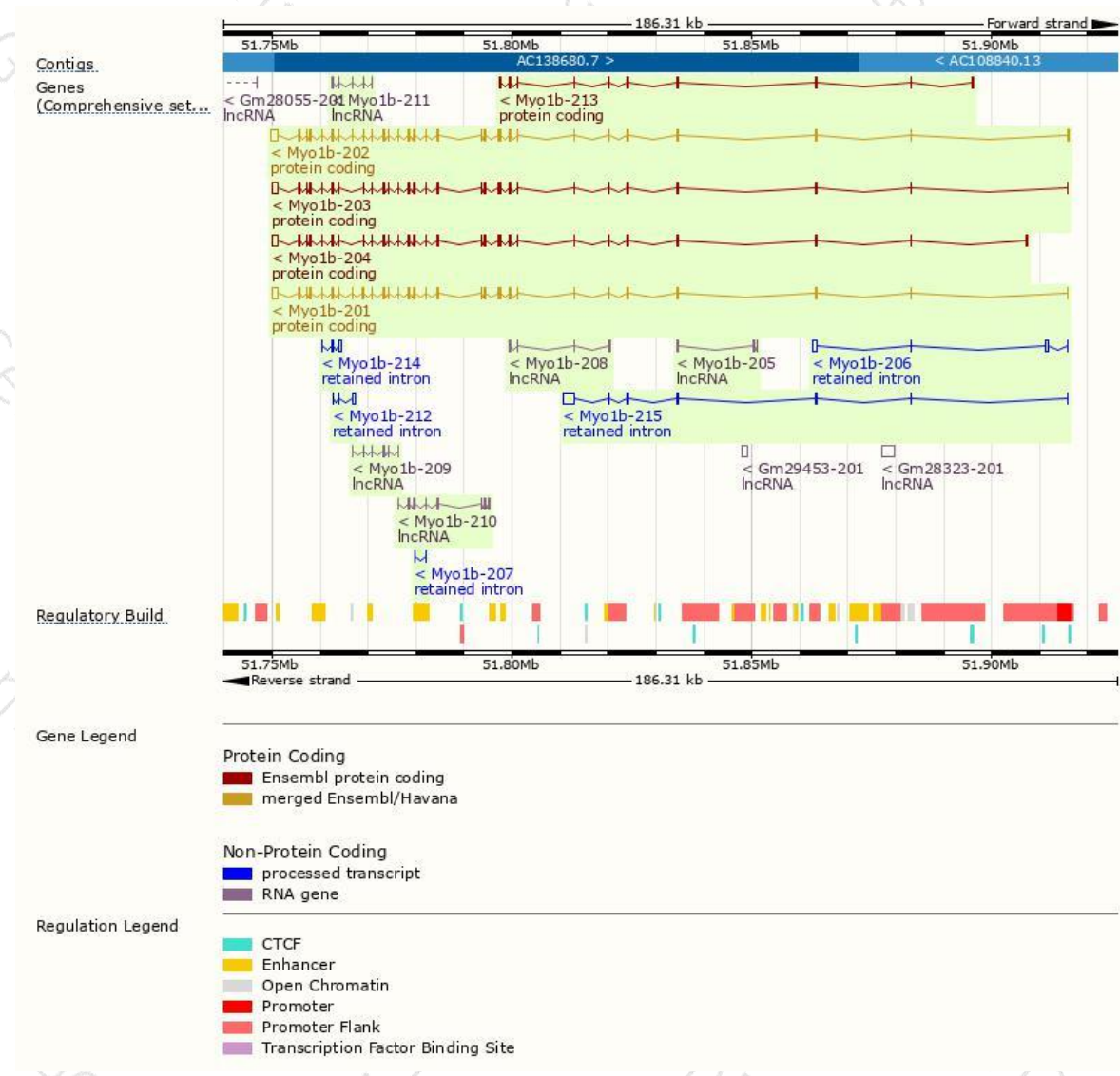| Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	1	NC_000067.6 (51749758..51916063, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	1	NC_000067.5 (51806609..51972818, complement)

Transcript information（Ensembl）

The gene has 15 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Myo1b-202	ENSMUST00000046390.13	5052	1107aa	Protein coding	CCDS14942	P46735	TSL:1 GENCODE basic APPRIS P3
Myo1b-201	ENSMUST00000018561.13	4767	1136aa	Protein coding	CCDS48254	Q7TQD7	TSL:1 GENCODE basic APPRIS ALT1
Myo1b-203	ENSMUST00000114537.8	4618	1078aa	Protein coding	CCDS69887	E9QNH6	TSL:5 GENCODE basic APPRIS ALT1
Myo1b-204	ENSMUST00000114541.7	4616	1084aa	Protein coding	-	E9Q580	TSL:5 GENCODE basic
Myo1b-213	ENSMUST00000144694.1	1244	299aa	Protein coding	-	D3YV80	CDS 3' incomplete TSL:5
Myo1b-215	ENSMUST00000151525.7	3045	No protein	Retained intron	-	-	TSL:1
Myo1b-206	ENSMUST00000126657.1	1558	No protein	Retained intron	-	-	TSL:1
Myo1b-214	ENSMUST00000145367.1	765	No protein	Retained intron	-	-	TSL:5
Myo1b-212	ENSMUST00000140930.1	683	No protein	Retained intron	-	-	TSL:3
Myo1b-207	ENSMUST00000126952.1	282	No protein	Retained intron	-	-	TSL:3
Myo1b-210	ENSMUST00000136701.1	969	No protein	lncRNA	-	-	TSL:1
Myo1b-209	ENSMUST00000136273.1	666	No protein	lncRNA	-	-	TSL:5
Myo1b-211	ENSMUST00000140731.7	448	No protein	lncRNA	-	-	TSL:2
Myo1b-208	ENSMUST00000135125.7	354	No protein	lncRNA	-	-	TSL:2
Myo1b-205	ENSMUST00000123299.1	280	No protein	lncRNA	-	-	TSL:5

The strategy is based on the design of *Myo1b-202* transcript,The transcription is shown below

< Myo1b-202
protein coding

Reverse strand   166.31 kb
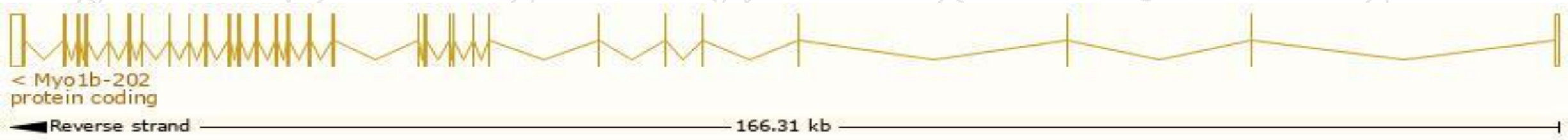
Genomic location distribution

Protein domain

ENSMUSP00000040...	
Low complexity (Seg)	
Superfamily	P-loop containing nucleoside triphosphate hydrolase
SMART	Myosin head, motor domain / IQ motif, EF-hand binding site
Prints	Myosin head, motor domain
Pfam	Myosin head, motor domain / Class I myosin tail homo / IQ motif, EF-hand binding site
PROSITE profiles	Myosin head, motor domain / IQ motif, EF-hand binding site / Class I myosin tail hom
PANTHER	PTHR13140 / PTHR13140:SF277
Gene3D	1.20.120.720 1.20.58.530 3.30.70.3240 1.20.5.190 / 1.10.10.820
CDD	Kinesin motor domain superfamily / Class I myosin, motor domain
All sequence SNPs/i...	Sequence variants (dbSNP and all other sources)

Variant Legend

- 🟨 missense variant
- 🟩 synonymous variant

Scale bar: 0 100 200 300 400 500 600 700 800 900 1000 1107

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

集萃药康生物科技
GemPharmatech Co.,Ltd