Myl1 Cas9-CKO Strategy

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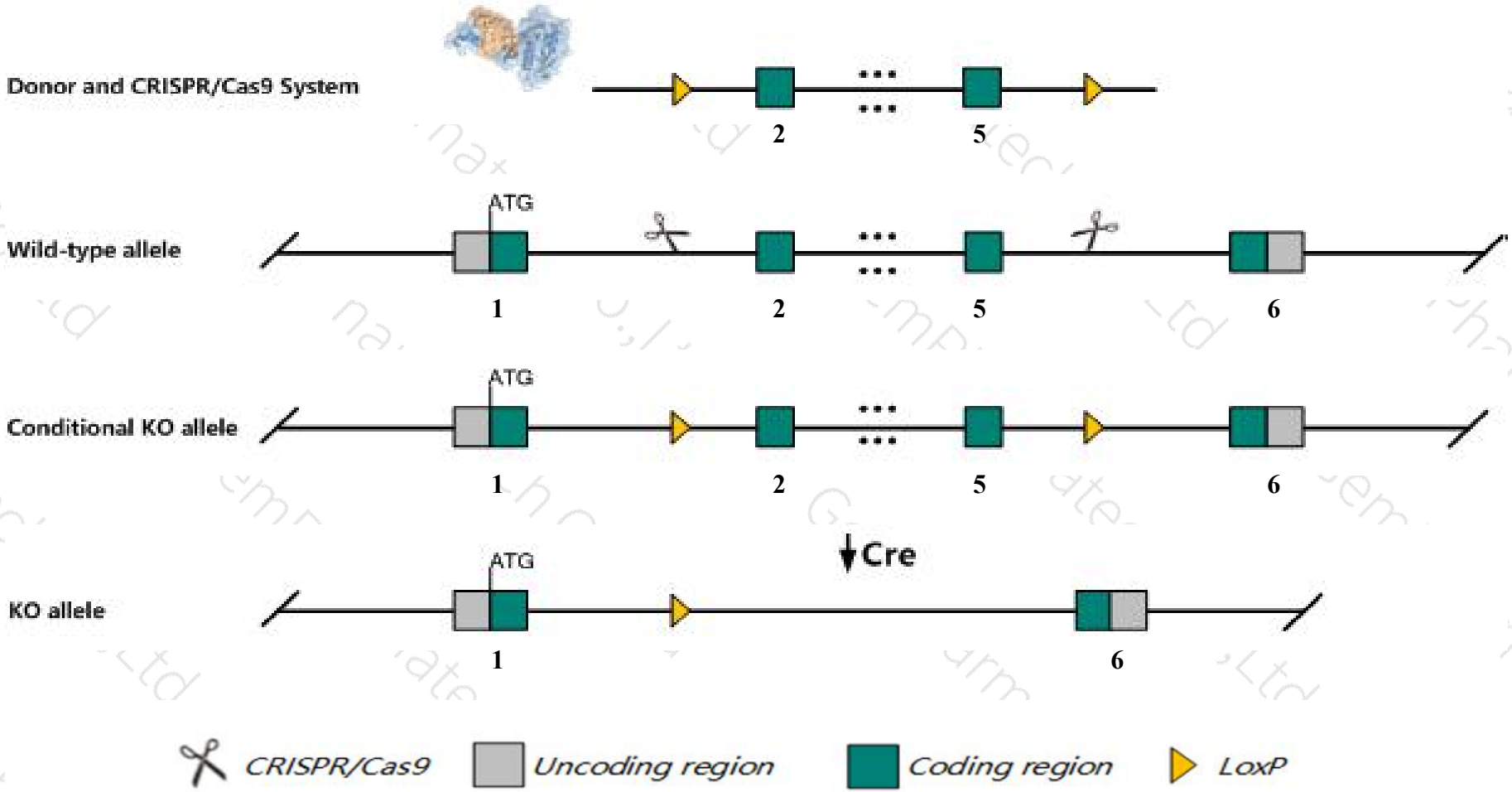
Project Overview

Project Name	***Myl1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Myl1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Myl1* gene has 8 transcripts. According to the structure of *Myl1* gene, exon2-exon5 of *Myl1-201*(ENSMUST00000027151.11) transcript is recommended as the knockout region.The region contains 424bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Myl1* 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,homozygotes for a targeted null mutation exhibit developmental delay, fail to form mesoderm, and die by embryonic day 8.5.

➢ The *Myl1* 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）

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Myl1 myosin, light polypeptide 1 [*Mus musculus* (house mouse)]

Gene ID: 17901, updated on 9-Jun-2020

Summary

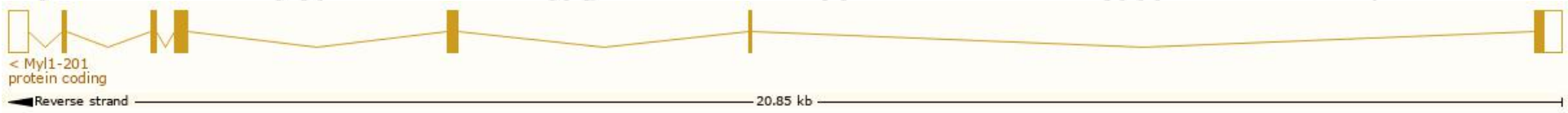
Official Symbol	Myl1 provided by MGI
Official Full Name	myosin, light polypeptide 1 provided by MGI
Primary source	MGI:MGI:97269
See related	Ensembl:ENSMUSG00000061816
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Mylf; MLC1f; MLC3f; AI325107
Summary	Myosin is a hexameric ATPase cellular motor protein. It is composed of two heavy chains, two non-phosphorylatable alkali light chains, and two phosphorylatable regulatory light chains. This gene encodes a myosin alkali light chain expressed in fast skeletal muscle. Multiple transcript variants encoding different isoforms have been identified for this gene. [provided by RefSeq, Jul 2008]
Expression	Biased expression in mammary gland adult (RPKM 118.5), limb E14.5 (RPKM 25.3) and 3 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

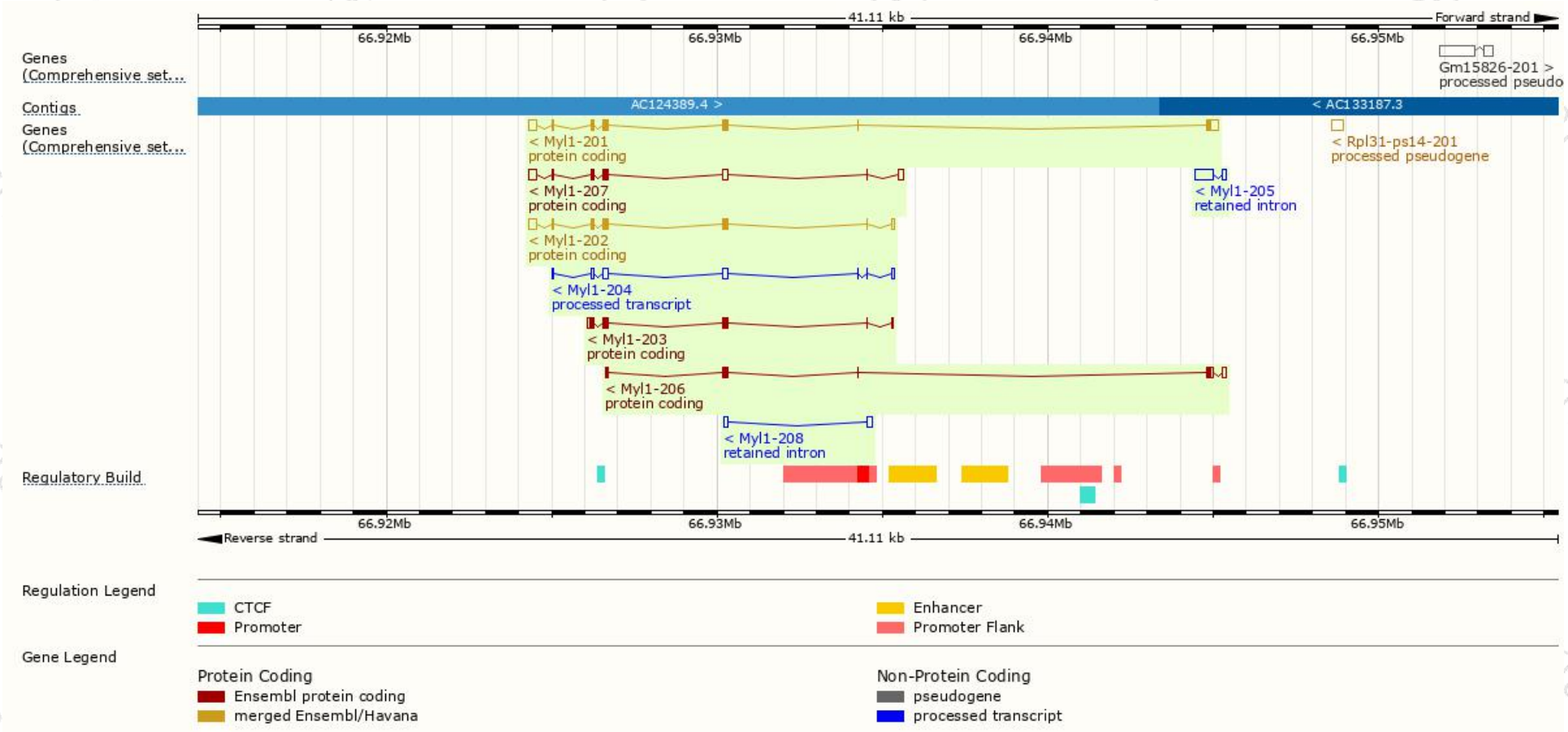
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Myl1-201	ENSMUST00000027151.11	1082	188aa	Protein coding	CCDS15024	P05977 Q545T7	TSL:1 GENCODE basic
Myl1-202	ENSMUST00000119429.7	803	150aa	Protein coding	CCDS48284	P05977 Q545G5	TSL:1 GENCODE basic APPRIS P1
Myl1-207	ENSMUST00000186202.6	874	92aa	Protein coding	-	A0A087WRZ7	TSL:5 GENCODE basic
Myl1-203	ENSMUST00000120415.7	621	161aa	Protein coding	-	E9PWG4	TSL:1 GENCODE basic
Myl1-206	ENSMUST00000160100.1	571	124aa	Protein coding	-	E0CZ30	CDS 3' incomplete TSL:3
Myl1-204	ENSMUST00000150542.1	567	No protein	Processed transcript	-	-	TSL:5
Myl1-205	ENSMUST00000151328.2	662	No protein	Retained intron	-	-	TSL:2
Myl1-208	ENSMUST00000186346.1	319	No protein	Retained intron	-	-	TSL:3

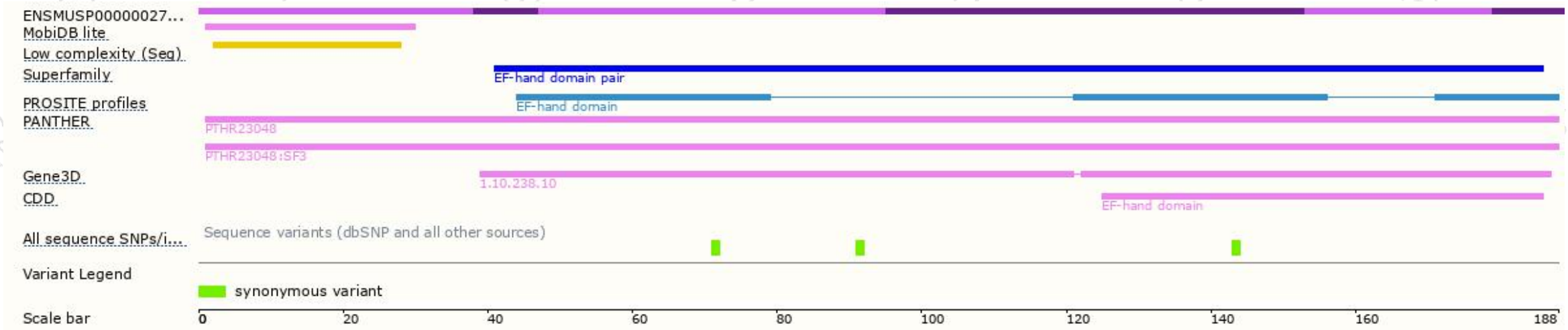
The strategy is based on the design of *Myl1-201* transcript,the transcription is shown below:



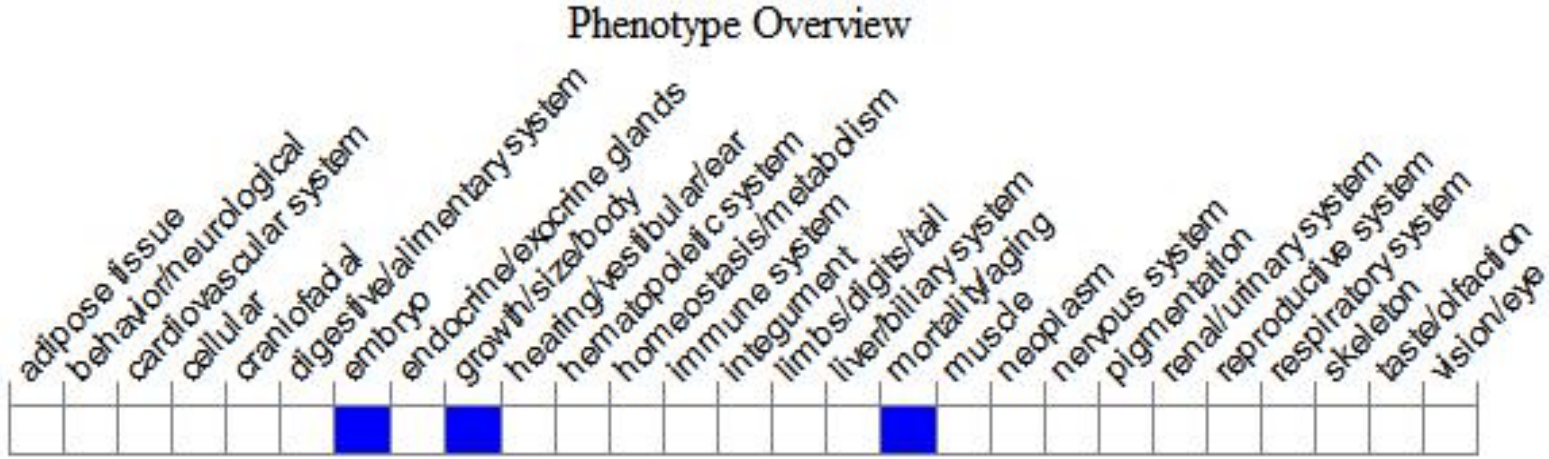
Genomic location distribution



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,homozygotes for a targeted null mutation exhibit developmental delay, fail to form mesoderm, and die by embryonic day 8.5.

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

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