Myot Cas9-CKO Strategy

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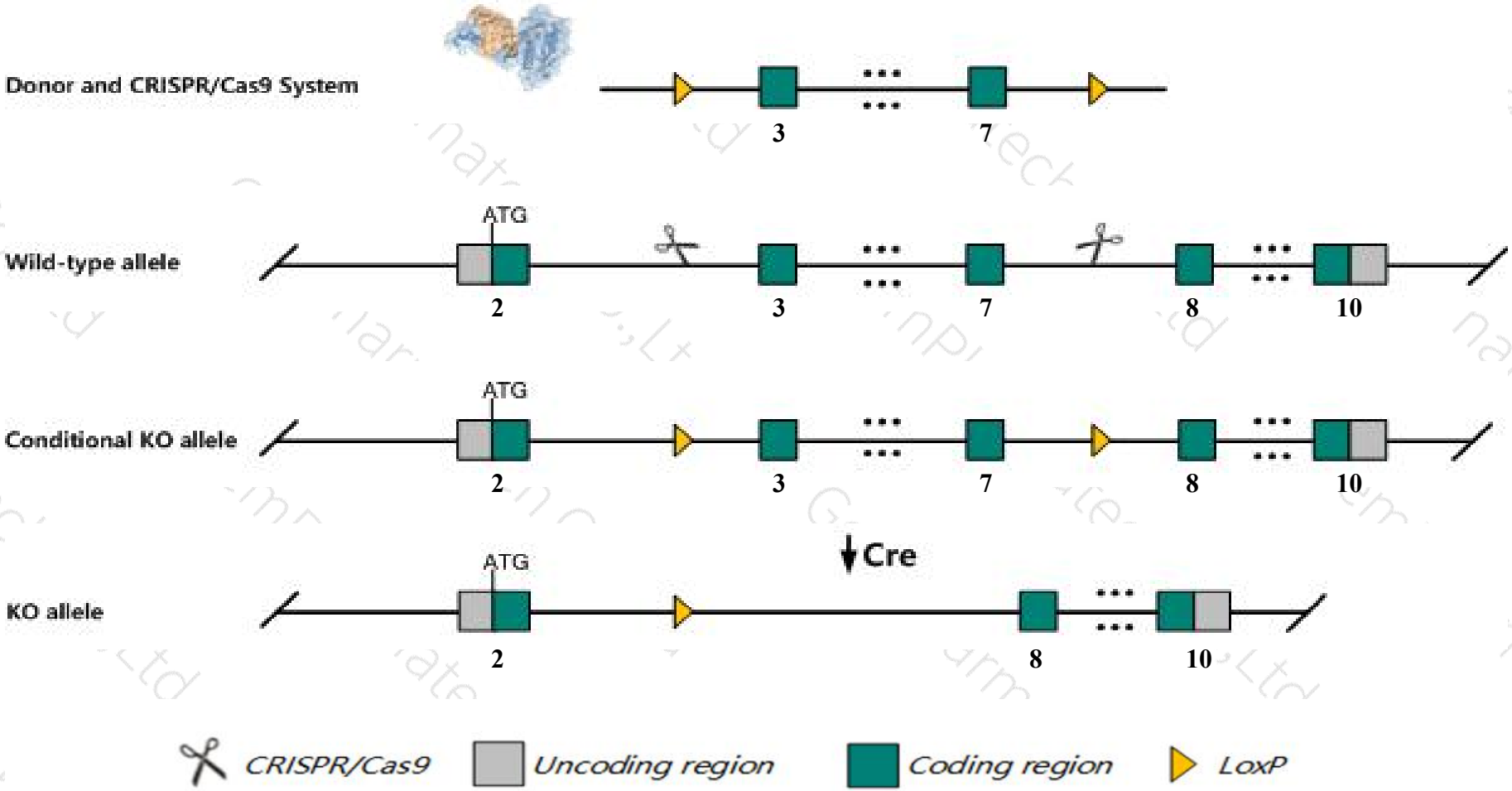
Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Myot* gene has 2 transcripts. According to the structure of *Myot* gene, exon3-exon7 of *Myot-201* (ENSMUST00000025349.11) transcript is recommended as the knockout region.The region contains 665bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Myot* 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, mice homozygous for a null allele are viable and fertile with normal skeletal and cardiac muscle morphology and function, growth rate, survival, and internal organ morphology.

- The *Myot* 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）

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Myot myotilin [*Mus musculus* (house mouse)]

Gene ID: 58916, updated on 12-Aug-2019

Summary

Official Symbol	Myot provided by MGI
Official Full Name	myotilin provided by MGI
Primary source	MGI:MGI:1889800
See related	Ensembl:ENSMUSG00000024471
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	Ttid; 5530402I04Rik
Expression	Biased expression in mammary gland adult (RPKM 26.3), heart adult (RPKM 5.5) and 4 other tissues See more
Orthologs	human all

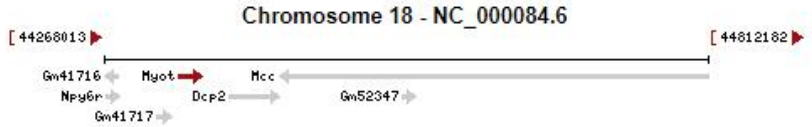
Genomic context

Location: 18; 18 B3

See Myot in Genome Data Viewer

Exon count: 10

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	18	NC_000084.6 (44334053..44355740)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	18	NC_000084.6 (44493728..44515376)

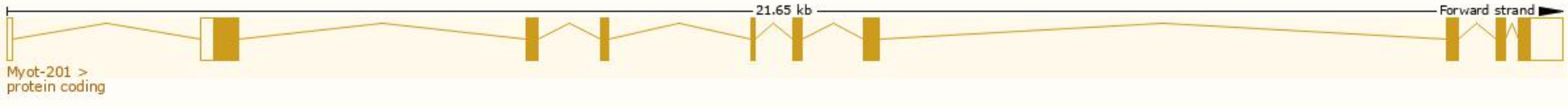


Transcript information（Ensembl）

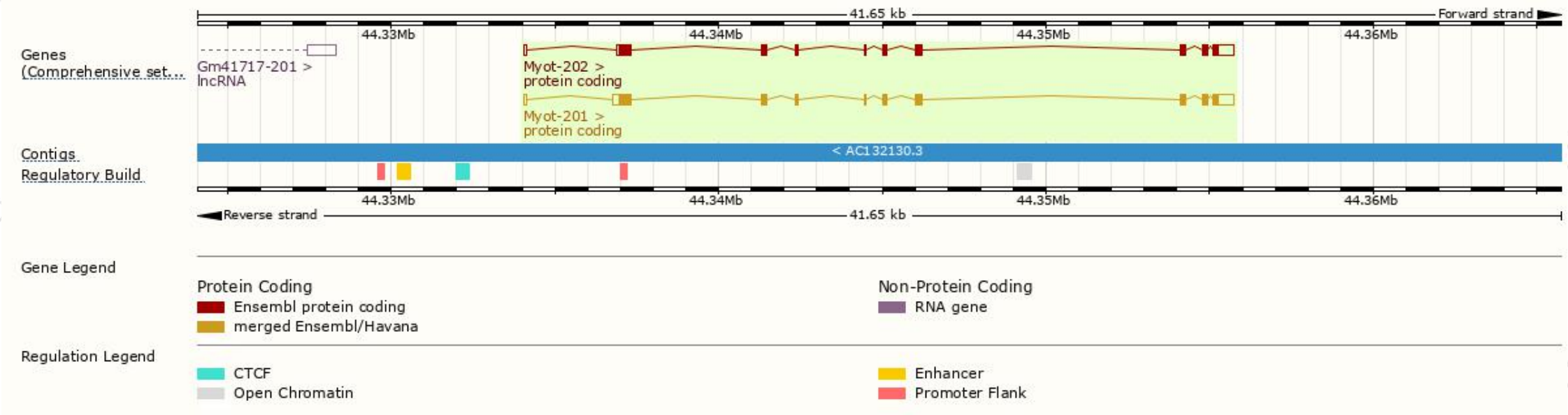
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Myot-201	ENSMUST00000025349.11	2209	496aa	Protein coding	CCDS29228	A0A509 Q9JIF9	TSL:1 GENCODE basic APPRIS P1
Myot-202	ENSMUST00000115498.1	2087	496aa	Protein coding	CCDS29228	A0A509 Q9JIF9	TSL:5 GENCODE basic APPRIS P1

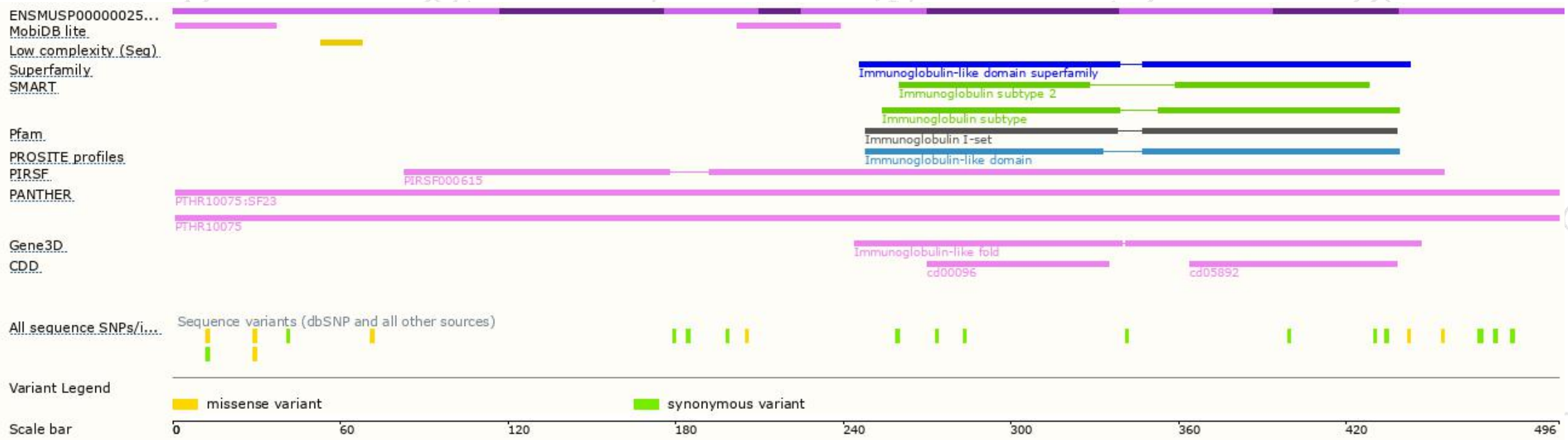
The strategy is based on the design of *Myot-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

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