Myl2 Cas9-KO *Strategy*

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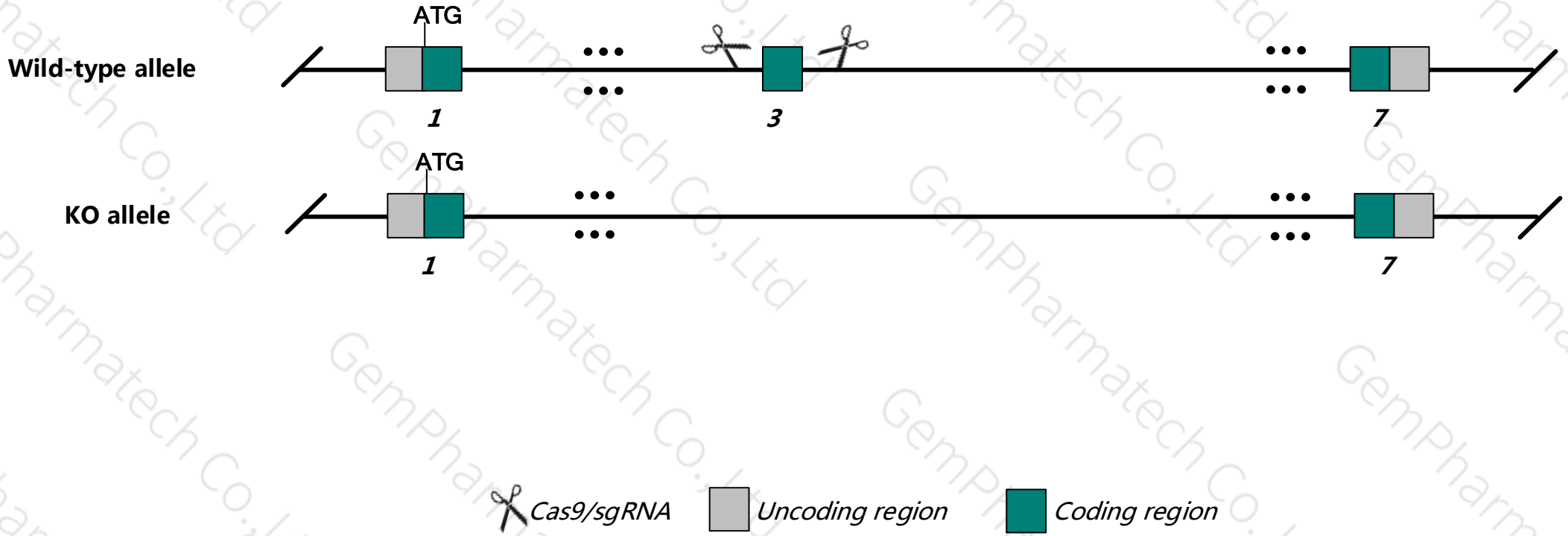
Design Date: 2019-7-29

Project Overview

Project Name	***Myl2***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Myl2* gene. The schematic diagram is as follows:



Technical routes

- The *Myl2* gene has 12 transcript.According to the structure of *Myl2* gene, exon3 of *Myl2*-201 (ENSMUST00000014080.12) transcript is recommended as the knockout region.The region contains 76bp coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Myl2* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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.

Notice

- According to the existing MGI data，Mice heterozygous for a knock-in allele exhibit embryonic growth retardation and die between E12.5 and E14.5 with abnormal heart development characterized by a single ventricle, complete absence of the interventricular groove and septum, and a thin myocardium compact layer.
- The *Myl2* gene is located on the Chr5. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

集萃药康 GemPharmatech

Myl2 myosin, light polypeptide 2, regulatory, cardiac, slow [*Mus musculus* (house mouse)]

Gene ID: 17906, updated on 25-Jun-2019

Summary

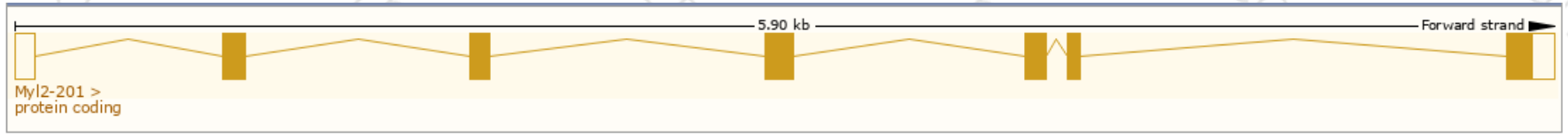
Official Symbol	Myl2 provided by MGI
Official Full Name	myosin, light polypeptide 2, regulatory, cardiac, slow provided by MGI
Primary source	MGI:MGI:97272
See related	Ensembl:ENSMUSG00000013936
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	MLC-2; Mlc2v; Mylpc; MLC-2v; MLC-2s/v
Expression	Restricted expression toward heart adult (RPKM 1539.2) See more
Orthologs	human all

Transcript information（Ensembl）

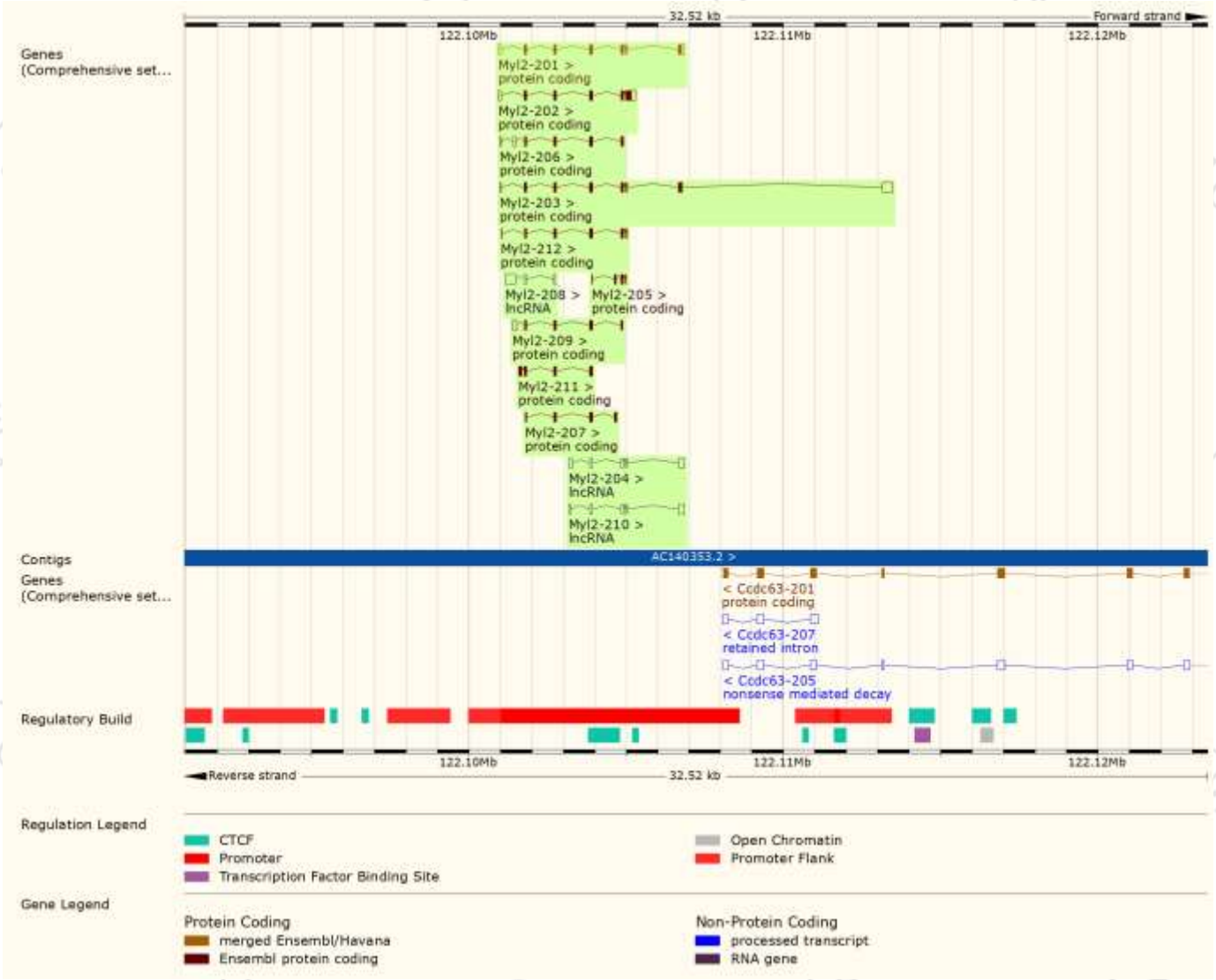
The gene has 12 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Myl2-203	ENSMUST00000111751.7	889	166aa	Protein coding	CCDS39252	P51667	TSL:3 GENCODE basic APPRIS P1
Myl2-201	ENSMUST00000014080.12	662	166aa	Protein coding	CCDS39252	P51667	TSL:1 GENCODE basic APPRIS P1
Myl2-202	ENSMUST00000111750.7	729	176aa	Protein coding	-	E9Q8Y0	TSL:2 GENCODE basic
Myl2-206	ENSMUST00000139213.7	476	98aa	Protein coding	-	D3YW14	CDS 3' incomplete TSL:5
Myl2-209	ENSMUST00000150535.7	473	93aa	Protein coding	-	D3Z0I3	CDS 3' incomplete TSL:5
Myl2-212	ENSMUST00000155612.7	433	115aa	Protein coding	-	D3YUI7	CDS 3' incomplete TSL:5
Myl2-211	ENSMUST00000153816.5	367	123aa	Protein coding	-	F6RBQ5	CDS 5' and 3' incomplete TSL:2
Myl2-207	ENSMUST00000146733.4	312	102aa	Protein coding	-	A0A0G2JE15	CDS 3' incomplete TSL:3
Myl2-205	ENSMUST00000126006.2	184	62aa	Protein coding	-	F6XCE3	CDS 5' and 3' incomplete TSL:5
Myl2-204	ENSMUST00000123913.7	528	No protein	lncRNA	-	-	TSL:3
Myl2-208	ENSMUST00000147178.4	469	No protein	lncRNA	-	-	TSL:5
Myl2-210	ENSMUST00000152744.1	467	No protein	lncRNA	-	-	TSL:3

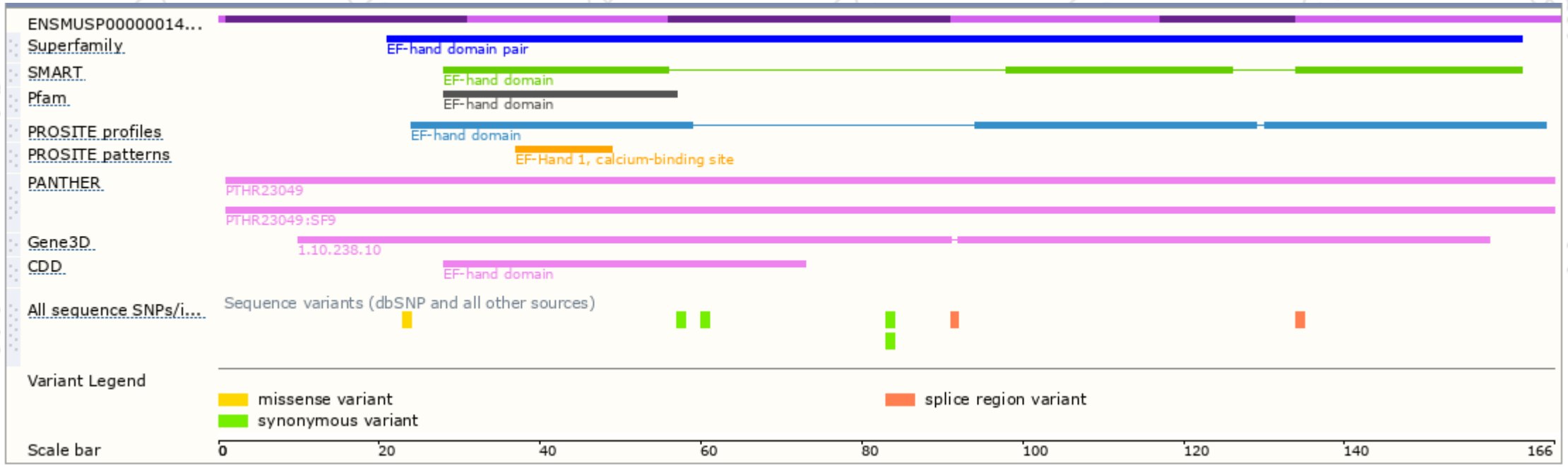
The strategy is based on the design of *Myl2*-201 transcript,The transcription is shown below



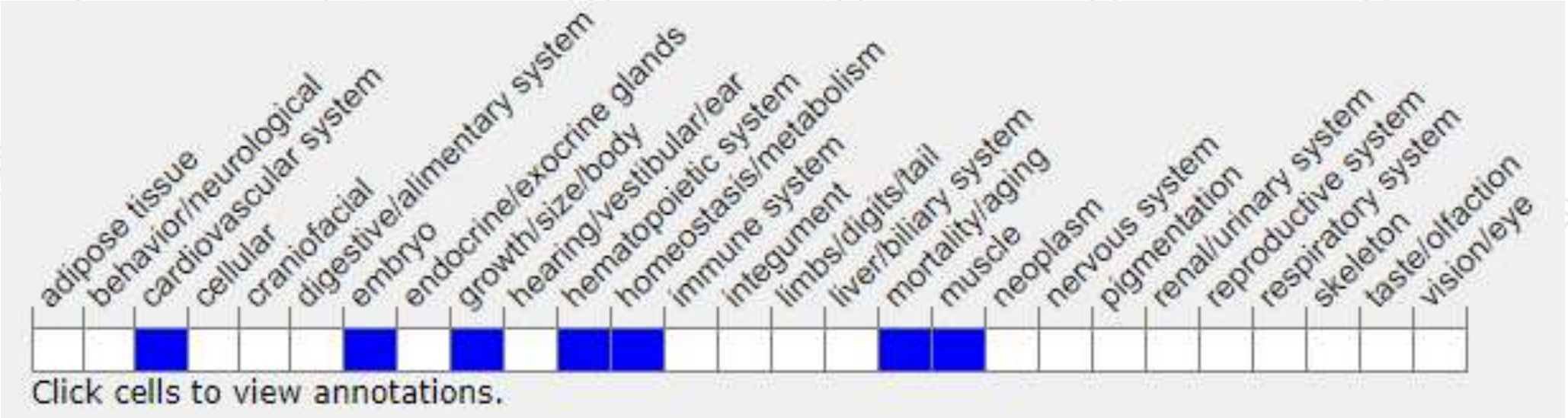
Genomic location distribution



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/) .

Mice heterozygous for a knock-in allele exhibit embryonic growth retardation and die between E12.5 and E14.5 with abnormal heart development characterized by a single ventricle, complete absence of the interventricular groove and septum, and a thin myocardium compact layer.

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

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