



Models to
Accelerate Innovation



CSS750-*H11-Myh6-CreER* Mouse Model Strategy

CRISPR-Cas9 Technology

Designer

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Reviewer

Ruirui Zhang

Date

2022-10-17

Project Overview



Project Name	CSS750-H11-Myh6-CreER
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Project Type	Cas9-KI(H11)
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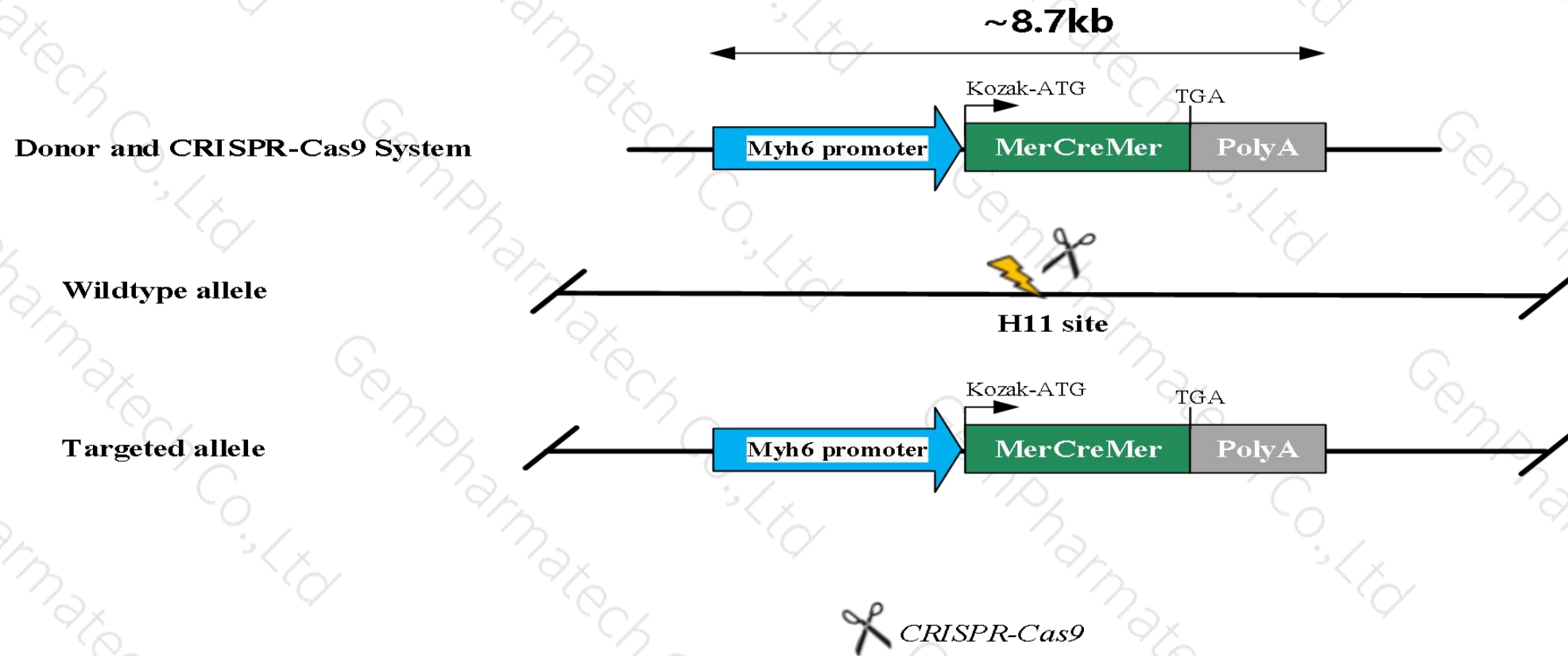
Background	D000750&CSS750
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Timeline	5-8 Months
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Strategy

This model will use CRISPR-Cas9 technology to edit. The schematic diagram is as follow:





Technical Description

- H11, located on mouse chromosome 11, is a safe site for foreign gene insertion. The foreign gene integrated into this site can be expressed stably and efficiently without destroying the function of endogenous gene^[1].
- In this project, the *Myh6-CreER-*loxyA** fragment will be inserted into H11 site of the mouse genome by CRISPR-Cas9 technology. Briefly, the donor vector and gRNA will be constructed in vitro. Cas9, donor vector and gRNA will be microinjected into fertilized eggs of D000750&CSS750 mice to obtain positive F0 generation mice. The F0 positive mice will be bred with D000750&CSS750 mice to obtain positive F1 mice. Pups from both F0 and F1 generations will be genotyped by PCR, followed by on-target sequencing analysis.

[1] Hippenmeyer, S., et al., Genetic mosaic dissection of *Lis1* and *Ndel1* in neuronal migration. *Neuron*, 2010. 68(4): 695-709.



Note

- The H11 localization is located on the Chr11. If the knockin 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 the currently available information in the existing databases. Due to the complexity of gene expression regulation, the effect of this strategy on gene expression cannot be completely predicted at the present technology level.



Sequence of MerCreMer (3012bp)

ATGGGAGATCCACGAAATGAAATGGGTGCTTCAGGAGACATGAGGGCTGCCAACCTTTGGCCAAGCCCTCTTGTGATTAAGCACACTAAGAAGAATAGCCCTGCCTTGTCCTTGACAGCTGACCAGATGGTCAGT
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AGCAGAGGGCTTCCCCAACACGATCTGA

<http://www.addgene.org/browse/sequence/239589/>

<http://www.addgene.org/124184/sequences/>



Summary of mouse *Myh6* promoter from JAX

B6.FVB(129)-A1 $cf^{Tg(Myh6-cre/Esr1^*)1Jmk/J}$

Strain #:005657

RRID:IMSR_JAX:005657 

Common Name: α MHC-MerCreMer

The alpha-MHC-MerCreMer (α MHC-MerCreMer) transgene has the mouse cardiac-specific alpha-myosin heavy chain promoter (α MHC or alpha-MHC; *Myh6*) directing expression of a tamoxifen-inducible Cre recombinase (MerCreMer) to juvenile and adult cardiac myocytes. These α MHC-MerCreMer transgenic mice allow the creation of bitransgenic mice for Cre-lox studies of temporally regulated deletion of *loxP*-flanked targeted genes in cardiac

<https://www.jax.org/strain/005657>

The Promoter of Mouse *Myh6*

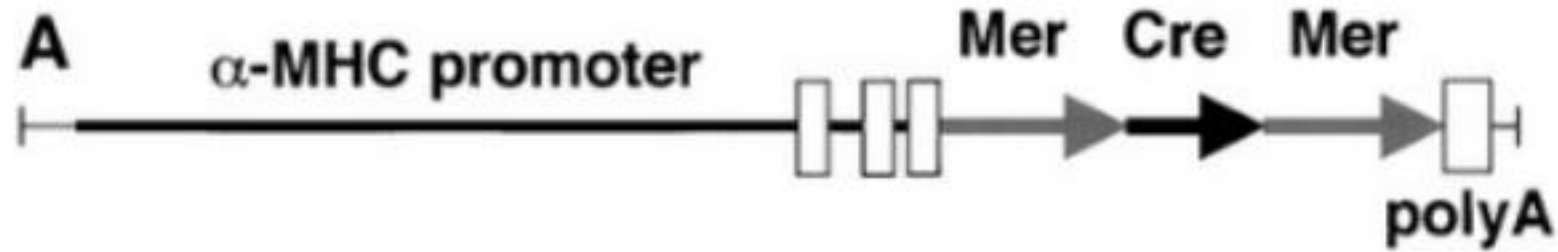
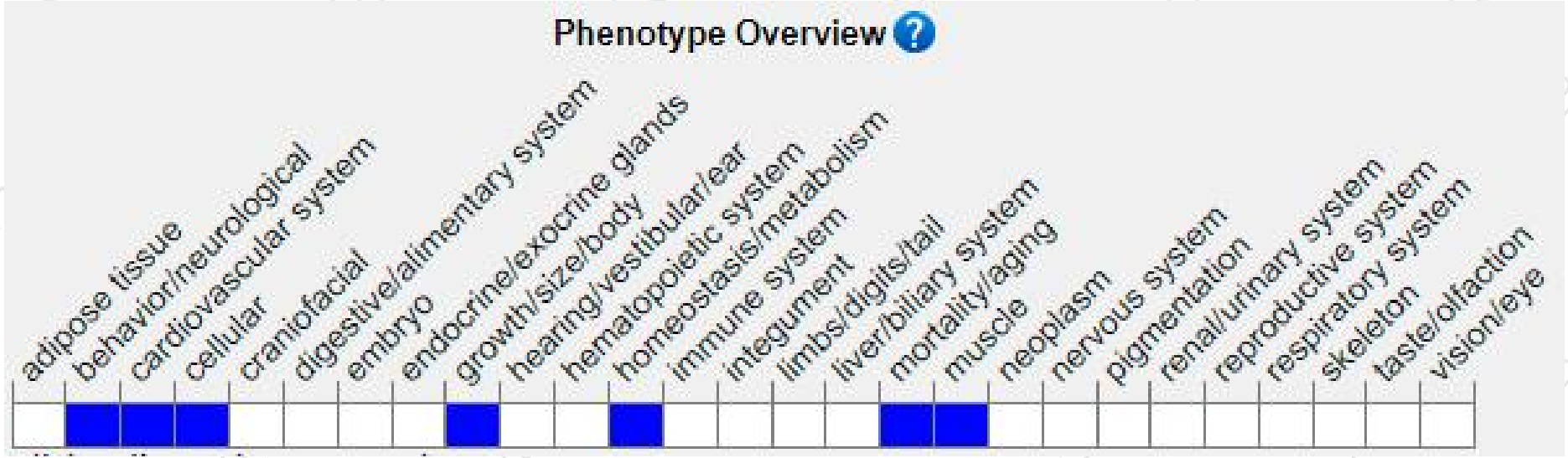


Figure 1. A, cDNA encoding the mutant estrogen receptor ligand-binding domain (Mer) flanking Cre recombinase (Cre) was subcloned downstream of the α -MHC 5.5-kb cardiac-specific promoter, which was used to generate transgenic mice (FVB strain). B, Western blotting of heart protein

Dawinder S. Sohal, et al., Temporally regulated and tissue-specific gene manipulations in the adult and embryonic heart using a tamoxifen-inducible Cre protein. *Circ Res.* 2001;89:20-25.

Phenotype Information (MGI)



Mice homozygous for a knock-out allele exhibit embryonic lethality associated with heart defects while heterozygotes show cardiac myofibrillar disarray, cardiac dysfunction and fibrosis. Mice heterozygous for different knock-in alleles may develop hypertrophic or dilated forms of cardiomyopathy.

<http://www.informatics.jax.org/marker/MGI:97255>



Basic Information of Mouse *Myh6* Gene

Gene name	Mouse <i>Myh6</i>
Gene ID (NCBI)	17888
Gene link (NCBI)	https://www.ncbi.nlm.nih.gov/gene/17888
Gene link (Ensembl)	http://asia.ensembl.org/Mus_musculus/Gene/Summary?g=ENSMUSG00000040752;r=14:55179378-55204384
Chromosome location	Chr 14



Mouse *Myh6* Gene Information (NCBI)



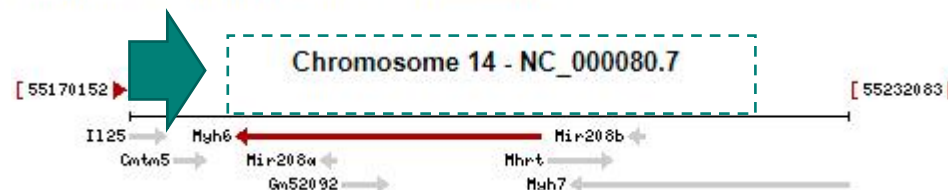
Myh6 myosin, heavy polypeptide 6, cardiac muscle, alpha [*Mus musculus* (house mouse)]

[Download Datasets](#)

Gene ID: 17888, updated on 26-Sep-2022

Summary

Official Symbol	Myh6 provided by MGI
Official Full Name	myosin, heavy polypeptide 6, cardiac muscle, alpha provided by MGI
Primary source	MGI:MGI:97255
See related	Ensembl:ENSMUSG00000040752 AllianceGenome:MGI:97255
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	Myhca; Myhc-a; alphaMHC; alpha-MHC; A830009F23Rik
Summary	Enables microfilament motor activity. Involved in cardiac muscle contraction. Acts upstream of or within several processes, including adult heart development; muscle cell development; and regulation of heart contraction. Located in Z disc and stress fiber. Part of myosin complex. Is expressed in several structures, including brown fat; embryo mesenchyme; great vessel of heart; heart; and skeletal musculature. Used to study dilated cardiomyopathy; dilated cardiomyopathy 1EE; and hypertrophic cardiomyopathy 14. Human ortholog(s) of this gene implicated in atrial heart septal defect (multiple); heart conduction disease (multiple); and intrinsic cardiomyopathy (multiple). Orthologous to human MYH6 (myosin heavy chain 6). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Restricted expression toward heart adult (RPKM 1317.1) See more
Orthologs	human all



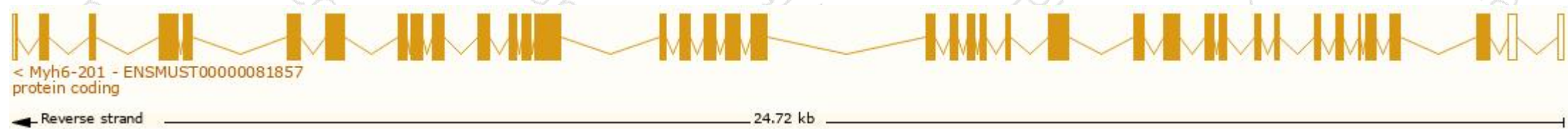
Mouse *Myh6* Transcript Information (Ensembl)



The gene has 7 transcripts. All transcripts are shown below:

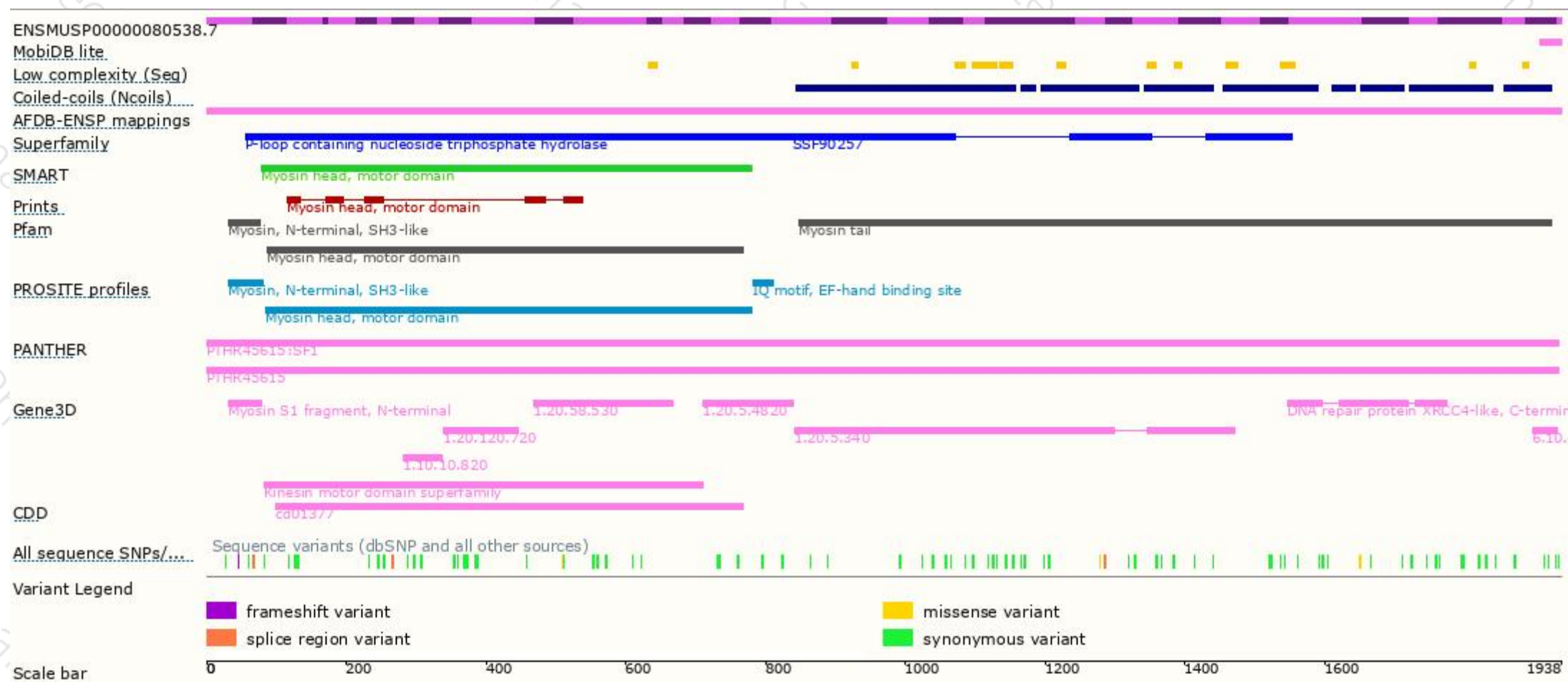
Show/hide columns (1 hidden)							Filter		
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags		
ENSMUST00000081857.14	Myh6-201	6113	1938aa	 Protein coding	CCDS36927	B2RQQ1 Q02566	Ensembl Canonical	GENCODE basic	APPRIS P1 TSL:1
ENSMUST00000226297.2	Myh6-205	6008	1938aa	 Protein coding	CCDS36927	B2RQQ1 Q02566	GENCODE basic APPRIS P1		
ENSMUST00000228731.2	Myh6-207	542	162aa	 Protein coding		A0A2I3BPY4	CDS 5' incomplete		
ENSMUST00000124930.8	Myh6-203	411	94aa	 Protein coding		Q1WNP4	TSL:1	CDS 3' incomplete	
ENSMUST00000111456.2	Myh6-202	376	63aa	 Protein coding		B8JJH3	TSL:3	CDS 3' incomplete	
ENSMUST00000227905.2	Myh6-206	718	No protein	 Retained intron		-	-		
ENSMUST00000131892.2	Myh6-204	511	No protein	 Retained intron		-	TSL:3		

The strategy is based on *Myh6*-201, which contains 39 exons, is 6113 bps long, and encodes 1938 amino acids.





Mouse *Myh6* Protein Information





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