H11-Myh6-iCre-ployA Cas9-KI Strategy

Designer:	**Yanhua Shen**
Reviewer:	**Huimin Su**
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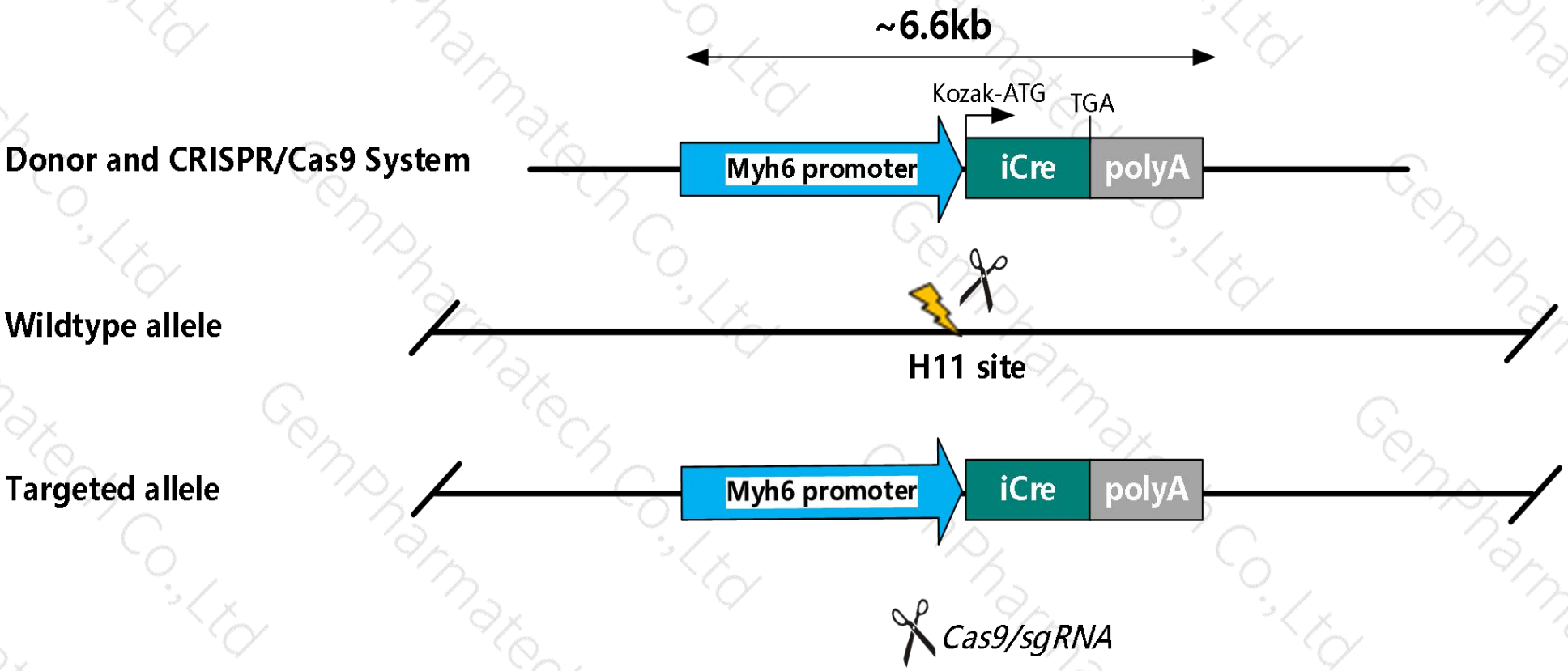
Project Overview

Project Name	***H11-Myh6-iCre-ployA***
Project type	**Cas9-KI**
Strain background	**C57BL/6J**

Knockin strategy

The *Myh6-iCre-ployA* fragment was inserted into H11 site of mice and the schematic diagram is as follows:



Summary of mouse *Myh6* promoter from JAX

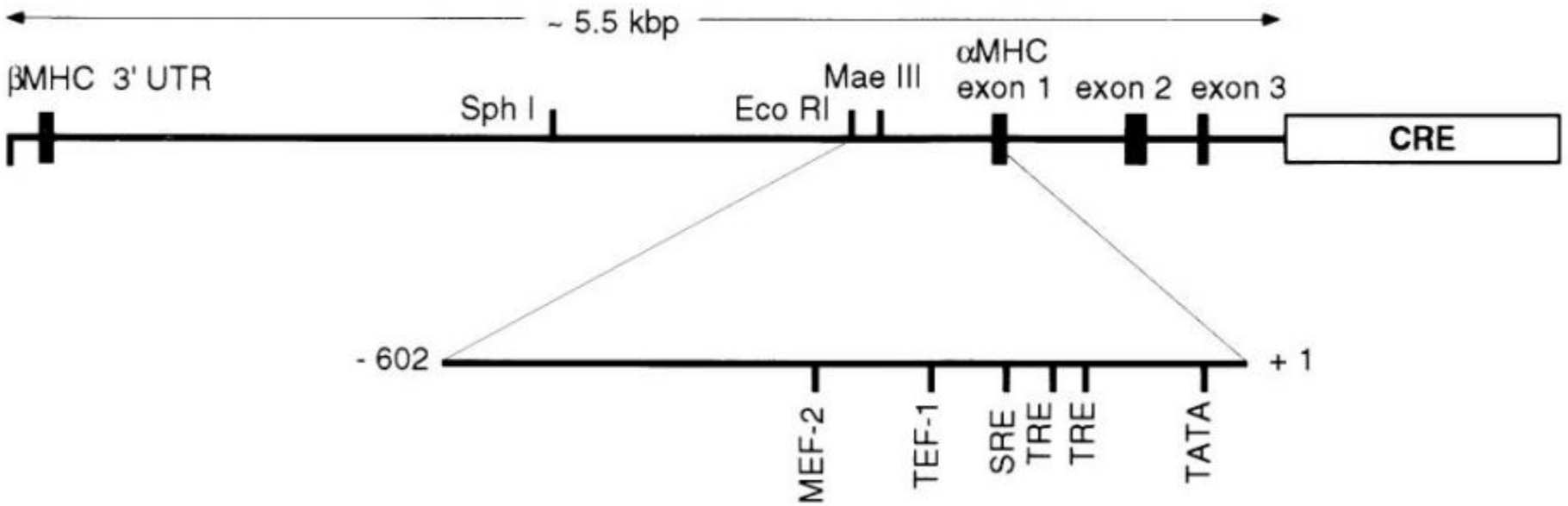
Tg(Myh6-cre)2182Mds

Allele Symbol: Tg(Myh6-cre)2182Mds MGI↗

Allele Name	transgene insertion 2182, Michael D Schneider
Allele Type	Transgenic (Recombinase-expressing)
Allele Synonym(s)	MCH-cre; MHC-Cre; MHCalphaCre; MHCcre; Tg(Myhca-cre)2182Mds; alpha-MHC-Cre^{+}; alphaMhc-Cre; alphaMyHC-Cre
Gene Symbol and Name	Tg(Myh6-cre)2182Mds MGI↗ , transgene insertion 2182, Michael D Schneider
Gene Synonym(s)	MHC-Cre; MHCalphaCre; MHCcre; Tg(Myhca-cre)2182Mds; Tg(Myhca-cre)2182Mds; alpha-MHC-Cre^{+}; alphaMhc Cre; alphaMyHC-Cre
Promoter	*Myh6*, myosin, heavy polypeptide 6, cardiac muscle, alpha, murine, murine
Molecular Note	The transgenic construct contained the Myh7 3' untranslated region, the Myhca promoter, Myhca noncoding exons 1 and 2, and the exon 3 splice acceptor site 180 bp upstream and driving the expression of the cre recombinase sequence. The Myhca promoter drives expression in cardiac tissue.

https://www.jax.org/strain/011038

The Promoter of Mouse *Myh6*



https://www.jax.org/strain/011038

Ramtin Agah. Targeted Expression of Cre Recombinase Provokes Cardiac-restricted, Site-specific Rearrangement in Adult Ventricular Muscle In Vivo. Gene Recombination in Postmitotic Cells. July 1997, 169–179.

cDNA (TTC = S). The cardiac-specific murine α-myosin-heavy chain (MyHC) promoter (α-5.5, provided by J. Robbins, University of Cincinnati, Cincinnati, OH) (21) was ligated as a SacI–HindIII fragment 180 bp upstream from the nls-Cre translational start site. The resulting αMyHC-Cre expression vector comprises the βMyHC 3' untranslated region, αMyHC promoter, αMyHC noncoding exons 1 and 2 (with the corresponding introns), and exon 3 splice acceptor sequence, 180 bp upstream from the nls-Cre ATG start site.

Technical routes

- 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.

- In this study, the *Myh6-iCre-ployA* gene fragment was inserted into H11 site of mice by CRISPR/Cas9 technology. The brief process is as follows: the donor vector and sgRNA were constructed in vitro, Cas9,donor and sgRNA were microinjected into the fertilized eggs of C57BL/6J mice, and F0 generation mice were obtained. The F0 positive mice were mated with C57BL/6J mice by PCR, sequencing, and southern blot, then the stable inheritance of F1 positive mice model was obtained.

Notice

- According to the existing JAX data, cre recombinase is expressed in cardiac tissue.
- Due to the insert region is large (~6.6 kb), the mouse offspring need a southern blot.
- H11 is located on Chr11. Please take the loci in consideration when breeding the Knock-in mice with other gene modified (e.g., iCre) strains, if the other gene is also on Chr11, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Gene information （NCBI)

集萃药康 GemPharmatech

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

Gene ID: 17888, updated on 12-Aug-2019

Summary

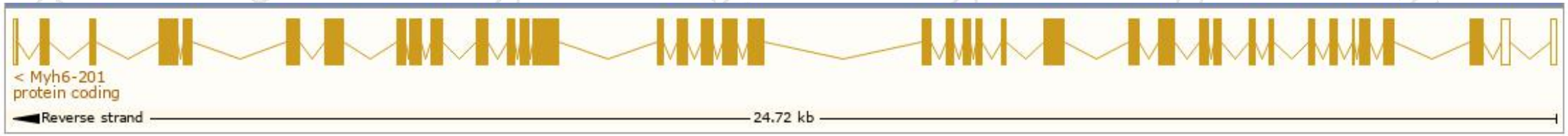
Official Symbol	Myh6 provided by MGI
Official Full Name	myosin, heavy polypeptide 6, cardiac muscle, alpha provided by MGI
Primary source	MGI:MGI:97255
See related	Ensembl:ENSMUSG00000040752
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Myhca; Myhc-a; AA517445; alphaMHC; alpha-MHC; A830009F23Rik
Expression	Restricted expression toward heart adult (RPKM 1317.1) See more
Orthologs	human all

Transcript information （Ensembl）

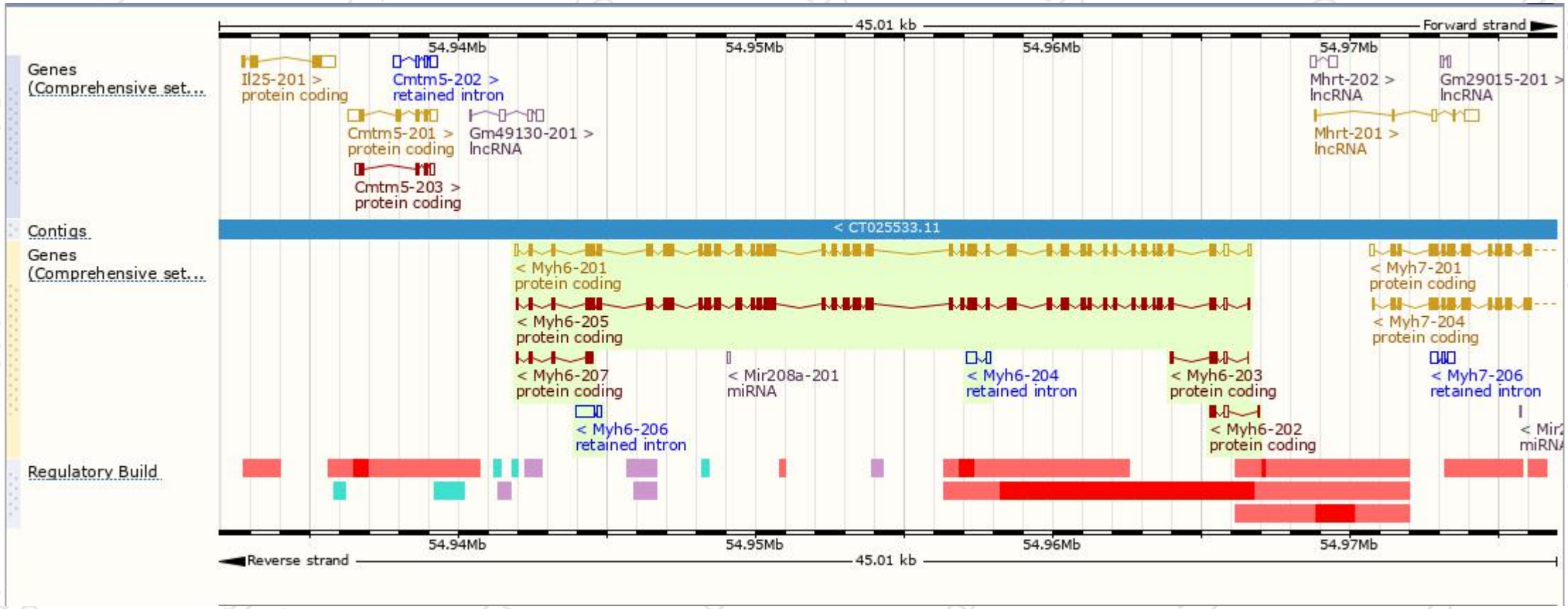
The gene has 7 transcripts, and the transcript is shown below：

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Myh6-201	ENSMUST00000081857.13	6113	1938aa	Protein coding	CCDS36927	B2RQQ1 Q02566	TSL:1 GENCODE basic APPRIS P1
Myh6-205	ENSMUST00000226297.1	6008	1938aa	Protein coding	CCDS36927	B2RQQ1 Q02566	GENCODE basic APPRIS P1
Myh6-207	ENSMUST00000228731.1	542	162aa	Protein coding	-	A0A2I3BPY4	CDS 5' incomplete
Myh6-203	ENSMUST00000124930.7	411	94aa	Protein coding	-	Q1WNP4	CDS 3' incomplete TSL:1
Myh6-202	ENSMUST00000111456.1	376	63aa	Protein coding	-	B8JJH3	CDS 3' incomplete TSL:3
Myh6-206	ENSMUST00000227905.1	718	No protein	Retained intron	-	-	-
Myh6-204	ENSMUST00000131892.1	511	No protein	Retained intron	-	-	TSL:3

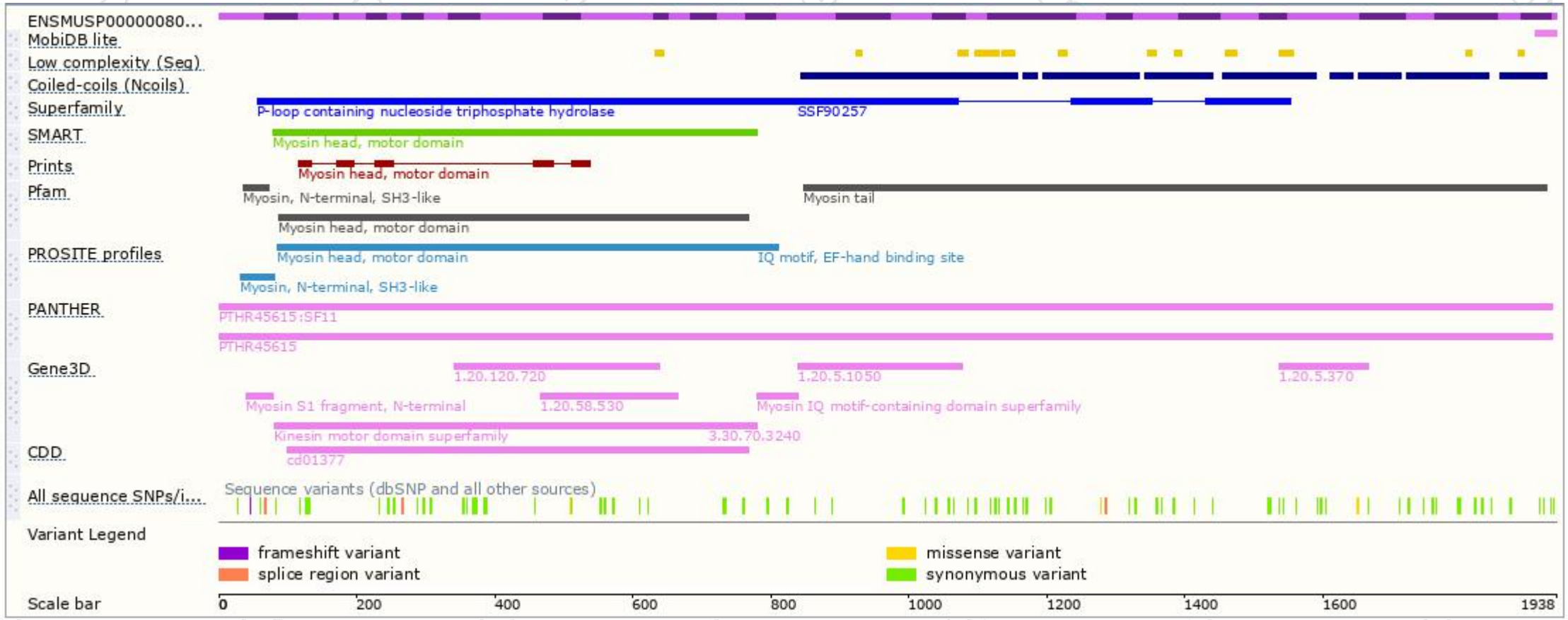
The strategy is based on the design of *Myh6-201* transcript,The transcription is shown below



Genomic location distribution



Protein domain



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

Tel: 025-5864 1534

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