

***Myf5-P2A-iCre* Cas9-KI Strategy**

Designer:

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Reviewer

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Design Date:

2019-8-7

Project Overview

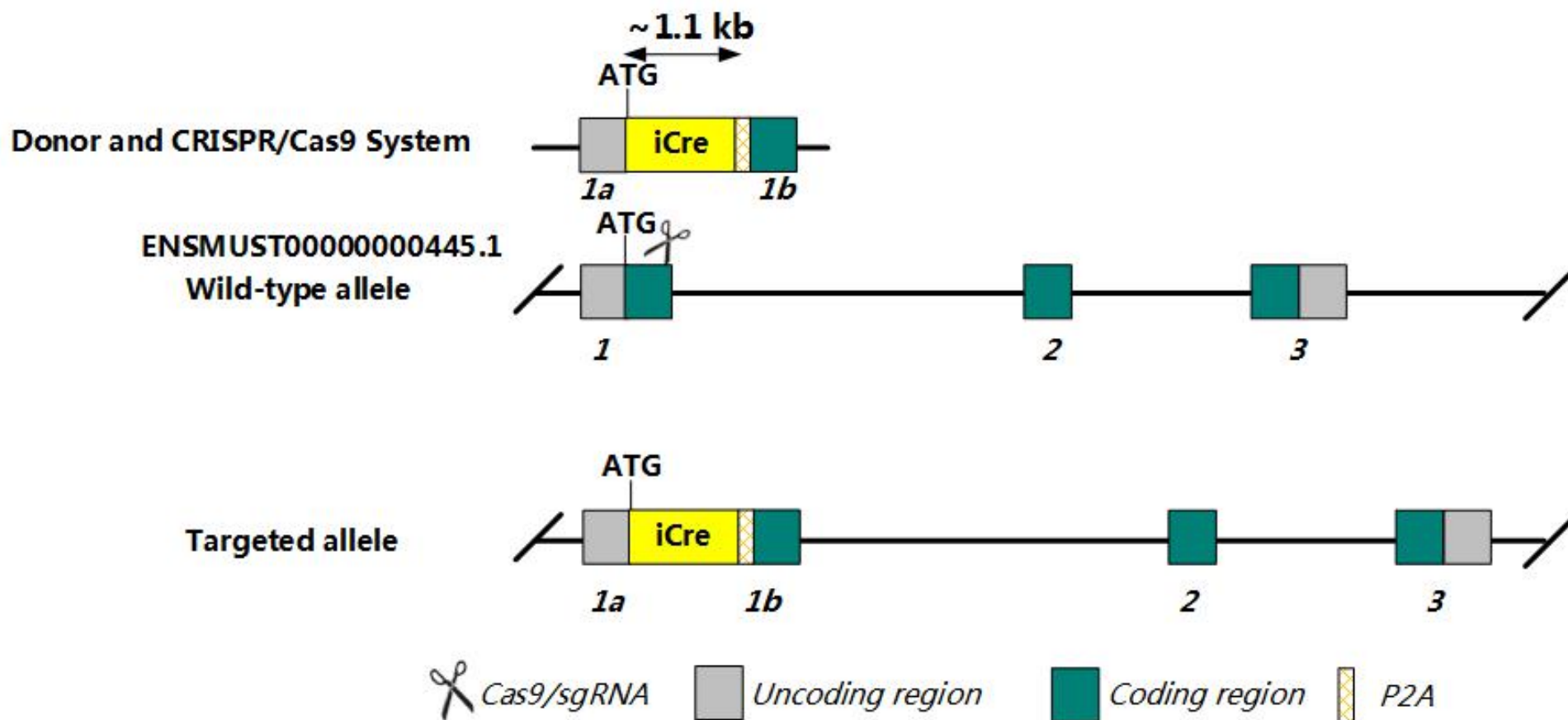
Project Name	<i>Myf5-P2A-iCre</i>
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Project type	Cas9-KI
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Strain background	C57BL/6J
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Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Myf5* gene. The schematic diagram is as follows:



- The *Myf5* gene has 1 transcript. According to the structure of *Myf5* gene, *Myf5-201*(ENSMUST00000000445.1) is selected for presentation of the recommended strategy.
- *Myf5-201* gene has 3 exons, with the ATG start codon in exon1 and TGA stop codon in exon3.
- We make *Myf5-iCre-P2A* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near start coding(ATG) of *Myf5* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in iCre-P2A before start coding(ATG) of *Myf5* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

- According to the existing JAX data, Cre recombinase occurs skeletal muscle and the dermis, and in several ectopic locations.
- The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide.
- Insertion of iCre may affect the regulation of the 5' end of the *Myf5* gene.
- There will be 1 to 2 amino acid synonymous mutation in exon1 of *Myf5* gene in this strategy.
- The *Myf5* gene is located on the Chr10. 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 genetic information in existing databases. Due to the complexity of gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)

Myf5 myogenic factor 5 [*Mus musculus* (house mouse)]

Gene ID: 17877, updated on 2-Jul-2019

Summary

- Official Symbol

Myf5 provided by [MGI](#)
- Official Full Name

myogenic factor 5 provided by [MGI](#)
- Primary source

[MGI:MGI:97252](#)
- See related

[Ensembl:ENSMUSG00000000435](#)
- 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

Myf-5; bHLHc2; B130010J22Rik
- Expression

Biased expression in limb E14.5 (RPKM 2.7), ovary adult (RPKM 0.6) and 1 other tissue [See more](#)
- Orthologs

[human](#) [all](#)

Genomic context

Location: 10 D1; 10 55.95 cM

See Myf5 in [Genome Data Viewer](#)

Exon count: 4

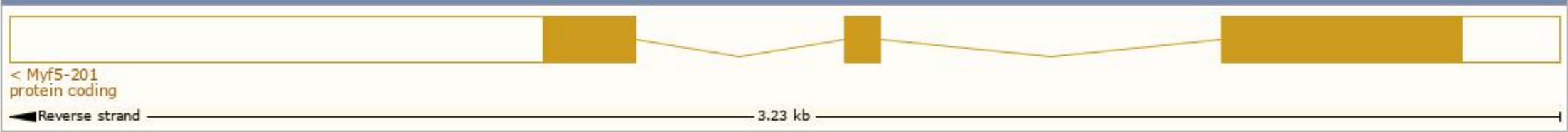
Annotation release	Status	Assembly	Chr	Location
106	current	GRCm38.p4 (GCF_000001635.24)	10	NC_000076.6 (107482908..107486927, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	10	NC_000076.5 (106919964..106923190, complement)

Transcript information (Ensembl)

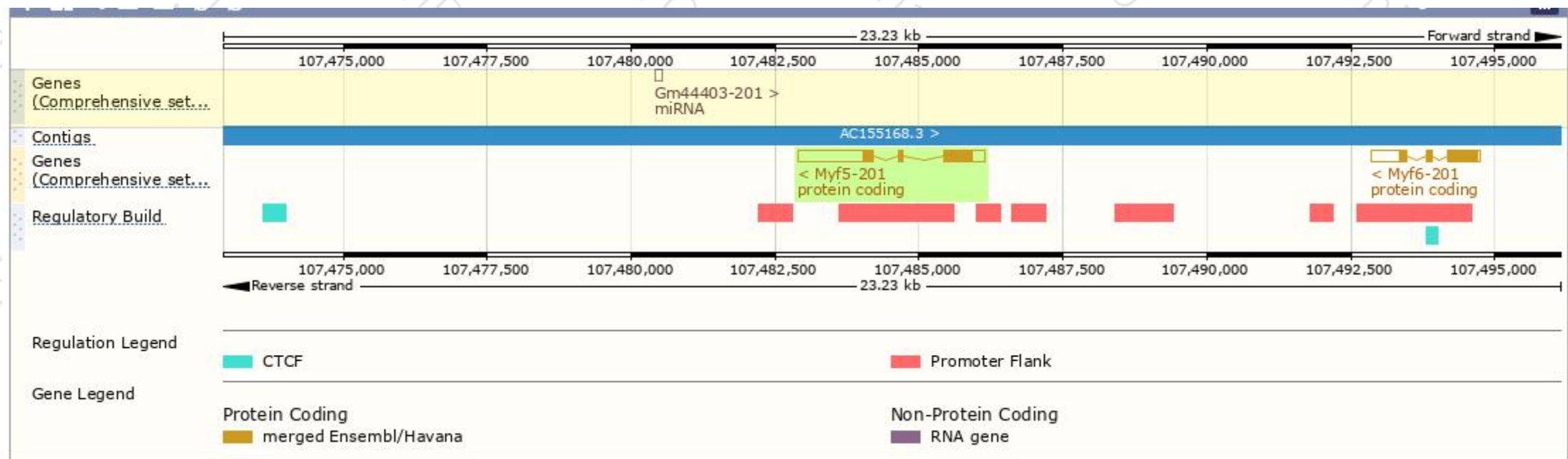
The gene has 1 transcript, and the transcript is shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Myf5-201	ENSMUST00000000445.1	2083	255aa	Protein coding	CCDS24161	A2RSK4 P24699	TSL:1 Gencode basic APPRIS P1

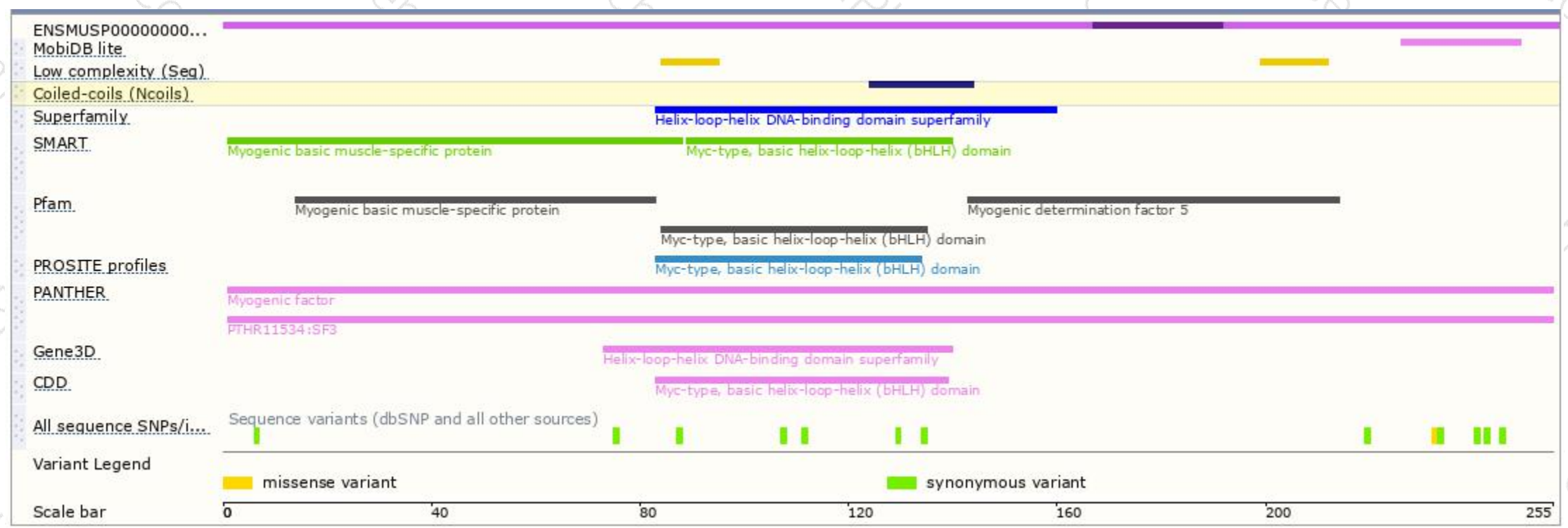
The strategy is based on the design of *Myf5-201* transcript, The transcription is shown below



Genomic location distribution

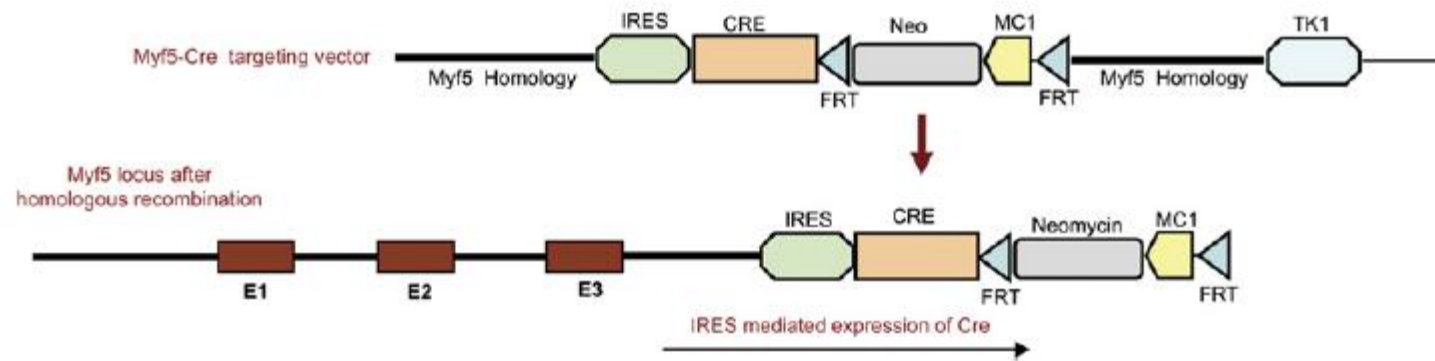


Protein domain



Targeted Progress (from MGI)

B Myf5- Cre mouse: Targeting strategy.



(B) The Myf5-targeting vector contains an encephalomyocarditis virus internal ribosomal entry site (IRES) fused to the Cre recombinase cDNA (Cre). This is followed by neomycin resistance gene (Neo) expressed by MC1 promoter and flanked by two FRT sequences to allow removal of MC1-Neo by breeding to a flippase-expressing mouse if desired. This entire construct is flanked by sequences homologous to region of the Myf5 3'UTR. A thymidine kinase 1 (TK1)-negative-selection cassette was placed after the Myf5 homology region. This strategy enables transcription of a bicistronic *Myf5-IRES-Cre* RNA leading to expression of Cre recombinase without interfering with *Myf5* expression.

Halдар M; Hancock JD; Coffin CM; Lessnick SL; Capecchi MR. A conditional mouse model of synovial sarcoma: insights into a myogenic origin. *Cancer Cell*.2007 11 (4) 375-88.


Targeted Progress (from JAX)

➡ Detailed Description

This strain expresses Cre recombinase from the endogenous *Myf5* locus. When crossed with a strain containing *loxP* site flanked sequence of interest, Cre-mediated recombination results in tissue-specific deletion of the target. **Recombination occurs skeletal muscle and the dermis, and in several ectopic locations.** Homozygotes for this allele have a perinatal lethal phenotype and die at birth. Homozygotes display abnormal rib development and some fusions of the cervical or thoracic vertebrae. This mutant mouse strain represents a model that may be useful in studies of skeletal development.

In an attempt to offer alleles on well-characterized or multiple genetic backgrounds, alleles are frequently moved to a genetic background different from that on which an allele was first characterized. This is the case for the strain above. It should be noted that the phenotype could vary from that originally described. We will modify the strain description if necessary as published results become available.

Allele Symbol: ***Myf5^{tm3(cre)Sor}*** 

Allele Name	targeted mutation 3, Philippe Soriano
Allele Type	Targeted (Recombinase-expressing)
Allele Synonym(s)	Mif5-Cre; Myf5 ^{Cre-SO} ; Myf5 ^{Cre} ; Myf5 ^{CreSOR} ; myf5-CreTG
Gene Symbol and Name	<i>Myf5</i>  , myogenic factor 5
Gene Synonym(s)	B130010J22Rik; B130010J22Rik; Myf-5; RIKEN cDNA B130010J22 gene; bHLHc2
Expressed Gene	<i>cre</i> , cre recombinase, bacteriophage P1
Site of Expression	skeletal muscle, dermis, and several ectopic locations
Strain of Origin	129S4/SvJaeSor
Chromosome	10
Molecular Note	A cre cDNA was inserted between a SacI site and a KpnI site in the 5'UTR of the Myf5 gene. An adjacent loxP-flanked neomycin cassette was removed by Cre-mediated recombination.
Mutations Made By	Dr. Philippe Soriano, Mount Sinai School of Medicine

<https://www.jax.org/strain/007893>

Targeted Progress (from JAX)

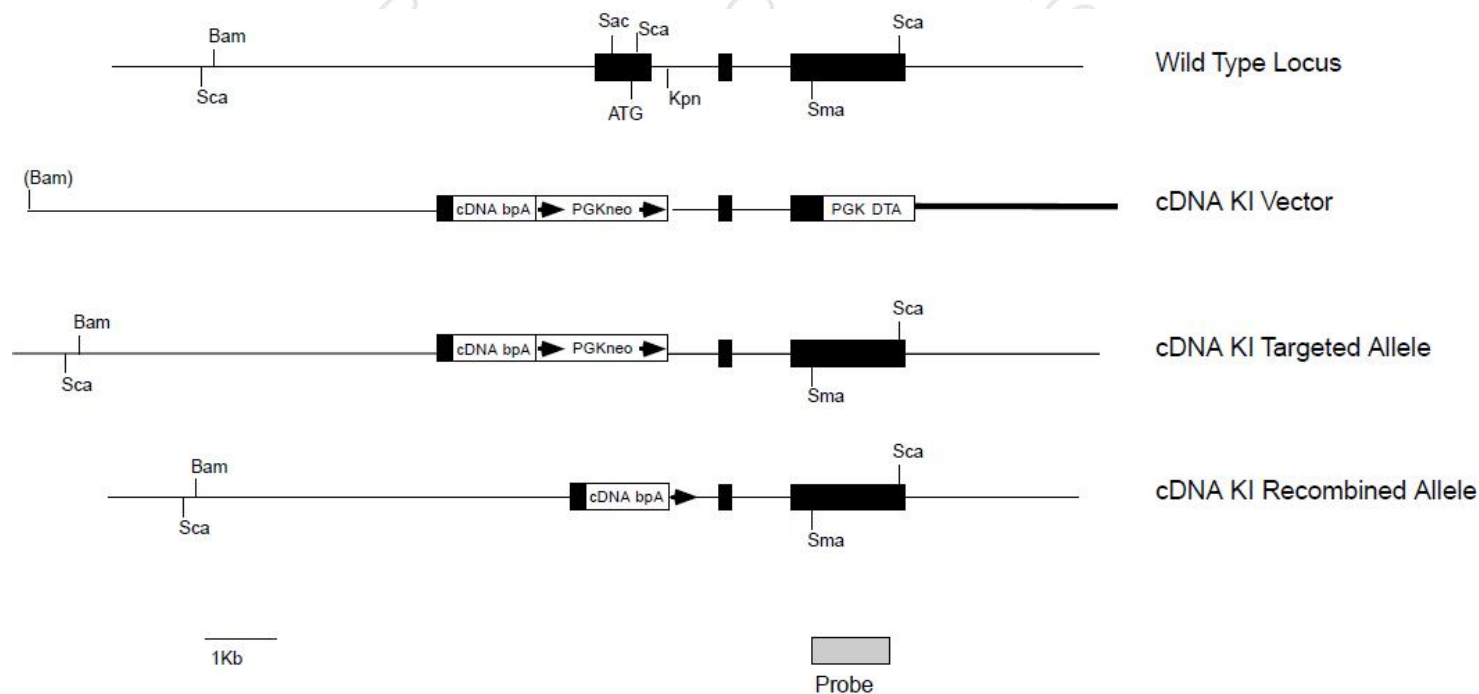


Fig. 5. Targeted insertions at the *Myf5* locus. *PDGFA*, *Cre*, or *MyoD* cDNAs (cDNA) were inserted between the *SacI* site in the 5'UTR and a *KpnI* site in the first intron of the *Myf5* gene. This deletion removes the basic helix-loop-helix DNA binding domain, a cysteine/histidine rich region and the activation domain, as well as all methionine encoding exons, and should be a null mutation in the gene. The cDNAs are followed by a bovine growth hormone polyadenylation sequence (bpA) and PGKneobpA selection cassette (PGKneo) flanked by loxP sites (arrowheads). A PGKDTA cassette is used for negative selection. Black boxes denote exons and the heavy line corresponds to the plasmid backbone. *Sca*, *ScaI*; *Bam*, *BamHI*; *Sac*, *SacI*; *Kpn*, *KpnI*; *Sma*, *SmaI*; ATG, initiator methionine of the *Myf5* gene. The probe used to characterize homologous recombination events by Southern blot is shown by a gray box.

Tallquist MD; Weismann KE; Hellstrom M; Soriano P. Early myotome specification regulates PDGFA expression and axial skeleton development. *Development*. 2000 127 (23) 5059-70

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