Genotyping *Myf5*$^{Cre/+}$

(*Myf5*$^{tm3(cre)Sor}$/J)

11-21-13
JDW

MGI #: 2135565

From: Talquist MD, Weisman KE, Hellstrom M, Soriano P. Early myotome specification regulates PDGFA expression and axial skeleton development. Development, 127, 5059-5070, 2000.

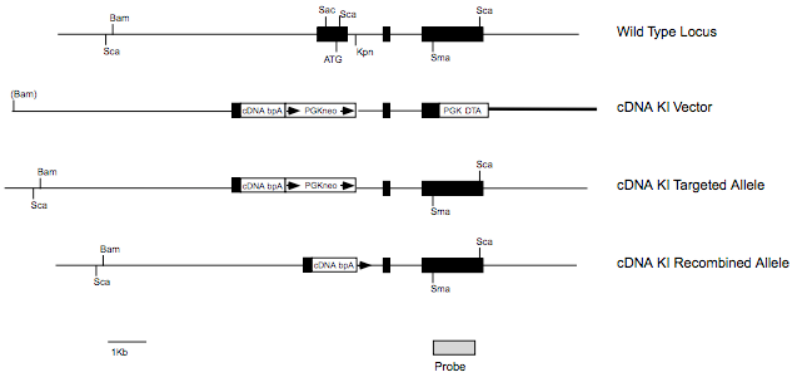

Fig. 5. Targeted insertions at the Myf5 locus. PDGFA, Cre, or MyoD cDNAs (cDNA) were inserted between the SacI site in the 5′UTR and a KpnI site in the first intron of the Myf5 gene. This deletion removes the basic helix-loop-helix DNA binding domain, a cysteine/histidine rich region and the activation 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.

A targeting vector containing nuclear localized *cre* coding sequence, *loxP* site flanked neomycin resistance cassette and a diphtheria toxin gene was inserted 90 bp upstream of the initiation codon to disrupt the targeted gene. The endogenous *Myf5* promoter drives expression of the Cre recombinase. The construct was electroporated into 129S4/SvJaeSor-derived AK7 embryonic stem (ES) cells. Correctly targeted ES cells were injected into recipient blastocysts. The resulting chimeric animals were crossed to Cre recombinase expressing strain to remove the neomycin selection cassette and then backcrossed to C57BL/6J mice for at least 5 generations.

Primers:

JDW 157 (Myf5 WT For / oIMR7659): 5’-CGT AGA CGC CTG AAG AAG GTC AAC CA
JDW 158 (Myf5 WT Rev / oIMR7660): 5’-CAC ATT AGA AAA CCT GCC AAC ACC
JDW 159 (Myf5 Cre REV / oIMR7919): 5’-ACG AAG TTA TTA GGT CCC TCG AC

Mut=400 bp
WT=603 bp

PCR Conditions

20 uM JDW 157	0.5 ul
20 uM JDW 158	0.5 ul
20 uM JDW 159	0.5 ul
Qiagen 10X Buffer	2.5 ul
ddH2O	19.3 ul
Qiagen Taq	0.2 ul
10 mM dNTPs	0.5 ul
genomic DNA	1.0 ul
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	25 ul

Reaction Parameters
94-4:00
94-0:30
59-0:30
72-0:45
Repeat 2-4, 34 cycles
72-6:00
16-forever