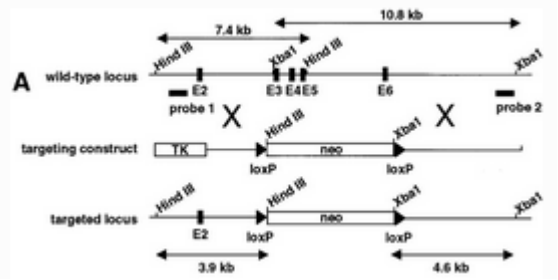
09.09.10 MS
08.18.15 MLS

Genotyping Protocol: **MMRRC 10773**

Assay Type: PCR- can distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Sigma's Extract-N-Amp Tissue PCR Kit (Cat#XNAT2R). Kit directions for fresh or frozen tails were performed with a few minor modifications as follows: use 50 µl of Extraction Solution and 12.5 µl of Tissue Preparation Solution and 50 µl of Neutralization Solution B.

Strain Characteristics: Exons 3-6 of the sarcoglycan, beta gene (*Sgcb*) were replaced with a neomycin cassette. Details can be found in Durbeej et al (2000) Molecular Cell 5 (1) 141-51.



Primer Information:

1) Name: BSU4	Sequence: 5' - AGC GAC ATC GGG ATG CAG TT - 3'
2) Name: BSL5	Sequence: 5' - CTC CAC ATC TCC TCC CAT GT - 3'
3) Name: neoU	Sequence: 5' - GCC TGA AGA ACG AGA TCA GC - 3'
4) Name: BSLF	Sequence: 5' - CAG GAC AGT GCT CAG CAA GA - 3'

Primer location: BSU4 and BSL5 are located in exons 4 and 5, respectively, of *Sgcb*. neoU is located in the inserted neomycin cassette, and BSLF is located in Exon 6 of *Sgcb*, after the stop codon.

Assay Name: Beta Sarcoglycan PCR

PCR Master Mix Components:
Run separate reaction for KO gene and WT gene:

Master Mix for WT gene:

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
BSU4	IDT	25µM	0.3
BSL5	IDT	25µM	0.3
sterile water			5.4

Master Mix for KO gene:

Component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma	2X	10
neoU	IDT	25µM	0.3
BSLF	IDT	25µM	0.3
sterile water			5.4

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PCR Setup:
WT Final Reaction: 16µl master mix & 4µl DNA template (10-20 ng/µl)
KO Final Reaction: 16µl master mix & 4µl DNA template (10-20 ng/µl)

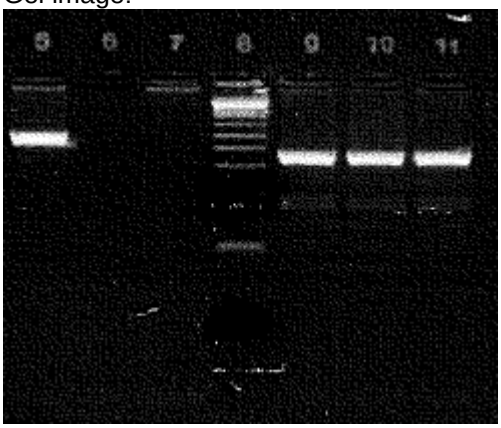
All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters (for both alleles):

1)	94°C	3 minutes
2)	94°C	30 seconds
3)	62°C WT & KO	30 seconds
4)	72°C	1 minute
5)	Repeat steps 2-4 34 times for a total of 35 cycles	
6)	72°C	10 minutes
7)	4°C	hold until refrigerate product

Product Analysis:
All products were analyzed on a 1% agarose gel with ethidium bromide staining
Wild type gene product: ~500bp
Knockout gene product: ~350 bp

Gel image:



Lane 5 displays a sample positive for the WT allele (500bp product). Lanes 6 and 7 display samples negative for the WT allele (no product).

Lanes 9, 10 and 11 displays samples positive for the mutant allele (350bp product).

Lane 8 displays 1Kb+ Ladder (Invitrogen Cat# 10787-018).