09.01.10 MS
02.07.17 MLS

Genotyping Protocol: **MMRRC 425**

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

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat# 69506). Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200µl of AE buffer once.

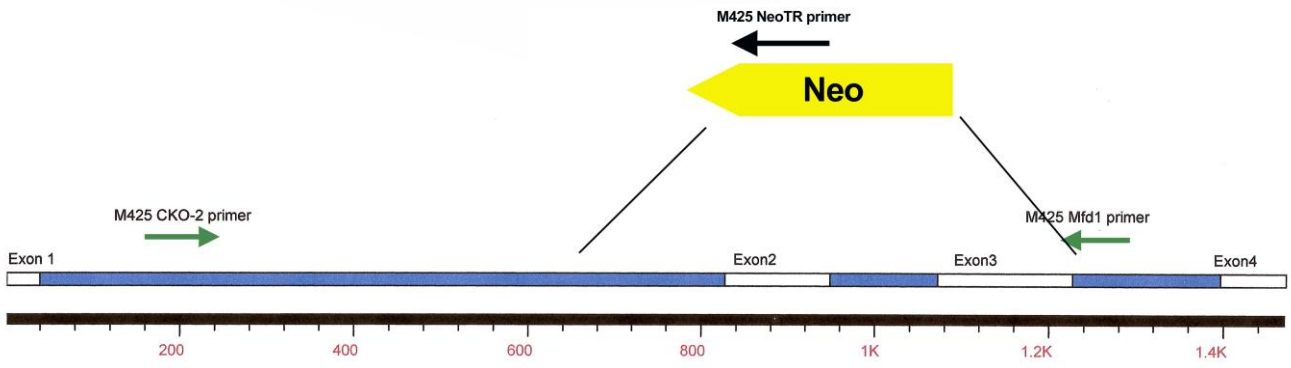
Strain Information: Exon 2, Exon 3, Intron 2, 571bp of Intron 1, and 65bp of Intron 3 of the sarcoglycan, alpha gene (*Sgca*) was replaced by a neomycin resistance gene in reverse transcriptional orientation. Details can be found in Duclos et al. (1998) J Cell Biol 142(6):1461-1471.

Primer Information:

1) Name: M425 CKO-2 Sequence: 5'-CAG GGC TGG GAG CTG GGT TCT G-3'
*common forward primer in intronic region between exon 1 & 2 of the *Sgca* protein (gene is on chromosome 11)

2) Name: M425 NeoTR Sequence: 5'-GCT ATC AGG ACA TAG CGT TGG CTA-3'
*reverse primer in the neomycin resistance gene

3) Name: M425 Mfd1 Sequence: 5'-CCC AGG GCC TTG ATG CCT-3'
* reverse primer in intronic region between exon 3 & 4 of the *Sgca* protein (gene is on chromosome 11)
* this area is deleted in mutant KO *Scga* allele



(exon & intron designations above are using the nomenclature scheme the authors indicated in their manuscript- please be aware there is a discrepancy between the publication and the transcript designations; manuscript starts exon numbering with ATG start site and disregards non-translated portion of transcript such that exon 1 in the manuscript is exon 2 in the ensemble database)

Assay Name: Alpha-Sarcoglycan KO PCR

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PCR Master Mix Components:

Master Mix for WT gene:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
M425 Mfd1	Sigma or IDT	25µM	0.3
M425 CKO-2	Sigma or IDT	25µM	0.3
FastStart *Taq*	Roche (Cat#12032953001)	5 U/µl	0.2
sterile water			13

Master Mix for KO gene:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTP	Promega	1.25mM	3.2
M425 CKO-2	Sigma or IDT	25µM	0.3
M425 NeoTR	Sigma or IDT	25µM	0.3
FastStart *Taq*	Roche	5 U/µl	0.2
sterile water			13

PCR Setup:

Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/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 WT and KO):
1) 95°C 5 minutes
2) 94°C 1 minute
3) 68°C 1 minute
4) 72°C 1 minute
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 10minutes
7) 4°C hold until refrigerate product

Product Analysis:
All products were analyzed on a 3% agarose gel with ethidium bromide staining
WT PCR assay = 1060 bp product
KO PCR assay = 600 bp product

Genotype results:
Wild type (WT) = 1060 bp product produced on WT PCR assay, no product with KO PCR assay
Homozygous mutant (HOM) = 600bp product produced on KO PCR assay, no product with WT PCR assay
Het= 1060bp product on WT PCR assay & 600bp product on KO PCR assay

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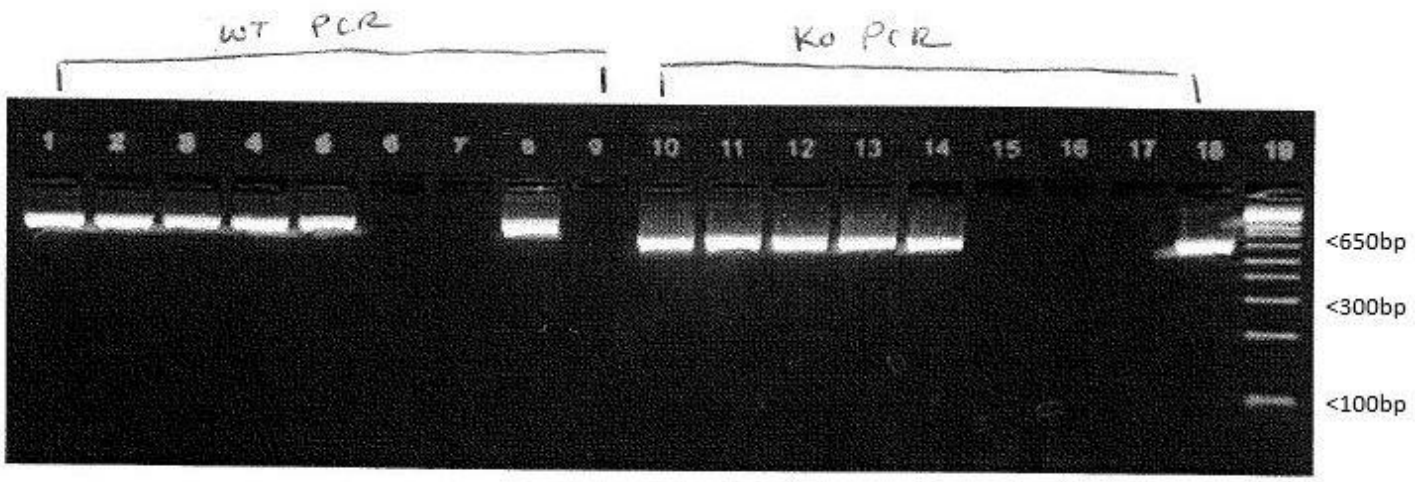
Example gel:



Lanes 1-9 are the WT PCR.
Lanes 1-5 display samples positive for the WT allele (1060bp product).
Lanes 6 and 7 are extraction and PCR blanks, respectively.
Lane 8 is a WT control (1060bp product) and Lane 9 is a homozygous control (no product).

Lanes 10-18 are the KO PCR.
Lanes 10-14 display samples positive for the KO allele (600bp product).
Lanes 15 and 16 are extraction and PCR blanks, respectively.
Lane 17 is a WT control (no product) and Lane 18 is a homozygous control (600bp product).

Lane 19 is 1kb+ size marker (Invitrogen Cat# 10787018)