

## Genotyping Protocol: **MMRRC 16841**

**Assay Type:** PCR - can distinguish heterozygous 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.

**Mutation Information:** A neo cassette was introduced into exon 3 of the *Myh9* locus to disrupt the gene and ablate the protein, nonmuscle myosin II-A.

**Primer Information:**

- 1) Name: M16841-228 Sequence: 5'-GGATGTTGTCCCGCCCATCA-3'
- 2) Name: M16841-351 Sequence: 5'-CCAAGGAGCGCTTTCATCCTAGGCTG-3'
- 3) Name: M16841-265 Sequence: 5'-CTAATTCCATCAGAAGCTGACTCT-3'

M16841-228 and M16841-351 bind to the *Myh9* gene. M16841-265 binds to the 3' UTR of bovine growth hormone sequence which is part of the targeting construct.

**Assay Name: 2AKO PCR**

**PCR Master Mix Components:**

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
M16841-228	Sigma	25µM	0.3
M16841-351	Sigma	25µM	0.3
M16841-265	Sigma	25µM	0.3
sterile water			5.1

**PCR Setup:**

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 Eppendorf Master Cycler or Applied Biosystems 2700 thermocycler.

**Cycle Parameters:**

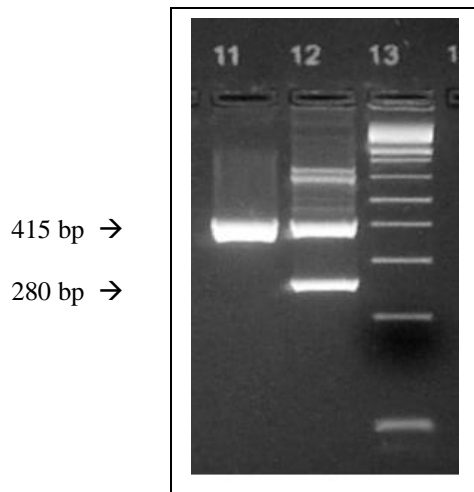
- 1) 94°C 2 minutes
- 2) 94°C 30 seconds
- 3) 60°C 30 seconds
- 4) 72°C 1 minute
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C 5 minutes
- 7) 4°C hold until refrigerate product

**Product Analysis:**

All products were analyzed on a 3% agarose gel with ethidium bromide staining; 45 minutes @100V.  
Expected sizes:

Wild type = 415 bp  
Heterozygous = 415 bp + 280 bp  
Homozygous mutant = 280 bp

**Example of Gel:**



Well 11 is a wild-type control. Well 12 is a heterozygous control. Well 13 is 1Kb Plus DNA ladder (Invitrogen Cat. # 10787-018). Note: larger non-specific amplicons may be present in the heterozygous samples and should be disregarded.