

09.15.10 MS
09.07.11 MS updated

Genotyping Protocol: **MMRRC 12035**

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 animal tissues were performed with a few minor modifications as follows: Use only 50 µl of Extraction Solution, 12.5 µl Tissue Preparation Solution and 50 µl of Neutralization Solution B.

Primer Information:

- 1) Name: M14242 411 Sequence: 5'-CGG AGG ATC AAC GAC GAG ATC G-3'
- 2) Name: M14241 412 Sequence: 5'-CGC GTA GTC GGG GAC GTC AAA AGC-3'

Assay Name: PPAR PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
PPAR 4	Sigma-Genosys	25µM	0.3
PPAR 5	Sigma-Genosys	25µM	0.3
nuclease free water			5.4

PCR Setup:

Final Reaction: 16µl master mix & 4µl 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:

- 1) 94°C 3 minutes
- 2) 94°C 1 minute
- 3) 56°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 the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

Alignment Marker: QX Alignment Marker 15bp/1000bp (Cat# 929521)

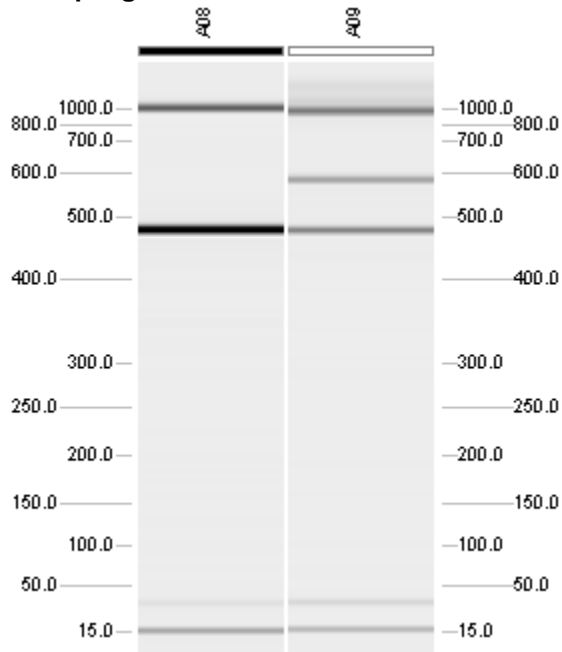
Size Marker: QX DNA Size Marker 50-800bp (Cat# 929556)

Method: AH320 Injection: 20s at 2kV
Separation: 320s at 6kV

Expected Products:

Hom = 572 bp
Het = 464 bp and 572 bp
WT = 464 bp

Example gel:



Lane A08 displays a WT sample (464bp band).
Lane A09 displays a heterozygous sample (464bp and 572bp bands).

Please note: the 15bp and 1000bp bands are reference markers specific to the QIAxcel method and do not represent expected products.