

Genotyping Protocol: MMRRC 425

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

DNA Extraction: DNA from tail snips was extracted using Sigma's RedExtract-N-Amp Tissue PCR Kit. Kit directions for animal tissues were performed with a few minor modifications as follows: Use only 50 μ l of Extraction Solution, 12.5 μ l Tissue Preparation Solution and 50 μ l of Neutralization Solution B.

Primer Information:

- | | |
|---------------------|---|
| 1) Name: M425 Mfd1 | Sequence: CCC AGG GCC TTG ATG CCT |
| 2) Name: M425 CKO-2 | Sequence: CAG GGC TGG GAG CTG GGT TCT G |
| 3) Name: M425 NeoTR | Sequence: GCT ATC AGG ACA TAG CGT TGG CTA |

PCR Master Mix Components:

component	manufacturer	concentration	μ l/rxn
REDExtract-N-Amp PCR Reaction Mix	Sigma	2X	10
M425 Mfd1	IDT	25 μ M	0.3
M425 CKO-2	IDT	25 μ M	0.3
M425 NeoTR	IDT	25 μ M	0.3
sterile water			5.1

PCR Setup:

Final Reaction: 16 μ l master mix & 4 μ l DNA template

All reactions were performed in 200 μ l 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 30 seconds
- 3) 69°C 30 seconds
- 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

Wildtype = 1000 bp

KO = 600 bp