

09.22.10 MS
04.18.13 MLS

Genotyping Protocol: **MMRRC 14241**

Assay Type: PCR – cannot 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.

Strain Description: This strain carries a transgene made up of the tetO promoter driving expression of the catalytic subunit of pertussis toxin (PTX).

Primer Information:

- 1) Name: M14241 GO 498 Sequence: 5'-GGA ACG TCC GGT CAG ATG GTC GA-3'
2) Name: M14241 GO 500 Sequence: 5'-CCA TAG AAG ACA CCG GGA CCG-3'

Primer Location:

M14241 GO 498: Catalytic subunit 1 of *Bordetella pertussis* toxin gene (PTX).
M14241 GO 500: tetO promoter

Assay Name: tetO-PTX PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
M14241 GO 498	Sigma	25µM	0.3
M14241 GO 500	Sigma	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 30 seconds
- 3) 66°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 the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

Alignment Marker: QX Alignment Marker 15bp/3Kb (Cat# 929522)

Size Marker: QX DNA Size Marker 100-3Kb (Cat# 929553)

Method: AM320

Injection: 10s at 5KV

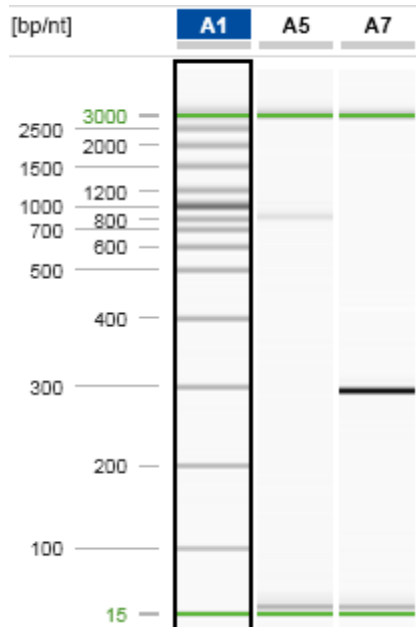
Separation: 320s at 6KV

Transgene positive = 291 bp

Transgene negative: no product

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Example gel:



Lane A1 displays a 15bp-3kb size marker.

Lane A5 displays a transgene negative sample (800bp non-specific band visible; the 291bp transgene product is not present).

Lane A7 displays a transgene positive sample (291 bp product).

Please note: the 15bp and 3kb bands are reference markers specific to the Qiaxcel method and do not represent expected products.