

09.01.10 MS
05.12.14 MLS

Genotyping Protocol: **MMRRC 424**

Strain Characteristics: Random integration of a 110-kb human apolipoprotein (a) YAC

Assay Type: PCR - cannot 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.

Primer Information:

1) Name: F apo (a) Sequence: 5'-AAC CCA CTC CAT CTG CAT GT-3'
2) Name: R apo (a) Sequence: 5'-TAG GAC ACC TGG CTG AGC TT-3'

Primer location: F apo (a) and R apo (a) are both located in the YAC apo (a) transgene.

Assay Name: YAC apo(a) Transgene PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
F apo (a)	IDT	25µM	0.3
R apo (a)	IDT	25µM	0.3
FastStart Taq	Roche (Cat# 12032953001)	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:

1) 94°C 5 minutes
2) 94°C 30 seconds
3) 68°C 30 seconds
4) 72°C 1 minute
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 7minutes
7) 4°C hold until refrigerate product

Product Analysis:

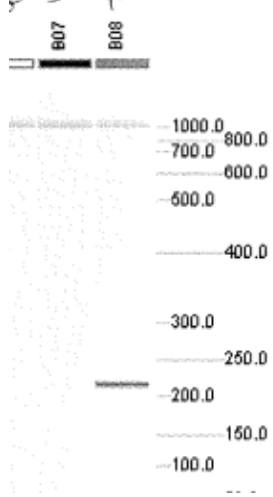
For analysis on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004):
Alignment Marker: QX Alignment Marker 15bp/1kb (Cat# 929521)
Size Marker: QX DNA Size Marker 50bp-800bp (Cat# 929561)
Method: AM320 Injection: 10s at 5kV
 Separation: 320s at 6kV

Expected products:

Transgene positive: 211bp
Transgene negative: no product

09.01.10 MS
05.12.14 MLS

Example gel:



Lane B07 displays a transgene negative sample (no product)
Lane B08 displays a transgene positive sample (211bp product)

Please note: the 1kb band is a reference marker specific to the QIAxcel method and does not represent an expected product.*