05.06.16 MS

Genotyping Protocol: MMRRC 34324

Assay Type: Can distinguish wild type, heterozygous and 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 200ul of AE buffer once.

Strain Description: Exon 1b of the mouse *Prkaca* gene is disrupted by the insertion of a loxp-flanked neomycin resistance cassette and a mutation of the translational initiation codon. Details can be found in Nolan et al (2004) Proc Natl Acad Sci USA 101(37):13483-8.

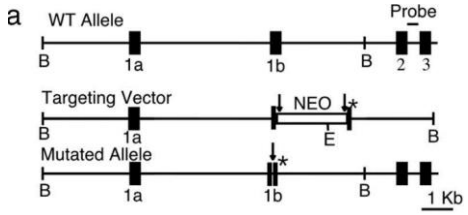


Image from Nolan et al (2004) Proc Natl Acad Sci U S A 101(37):13483-8.

Primer Information:

1) Name: M34324mut.F Sequence: 5'-TGT TCC CAC CCT ATC ACT CC-3'
2) Name: M34324mut.R Sequence: 5'-CGG TCT CGA CGA CGC GCC TCA-3'
3) Name: M34324wt.F Sequence: 5'-CGA GCC ACC GTA ATG CTA GT-3'
4) Name: M34324wt.R Sequence: 5'-TCA GGT TTT CTA GCC CAG GA-3'

Primer Location: M34324mut.F is located upstream of Exon 1b of *Prkaca*; M34324mut.R is located in the inserted neomycin cassette. M34324wt.F and M34324wt.R are located on either side of Exon 1b of *Prkaca*.

Assay Names: Prkaca PCR

MUT 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
M34324mut.F	Sigma	25µM	0.3
M34324mut.R	Sigma	25µM	0.3
FastStart *Taq*	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

PCR Setup:
Final Reaction: 19 µl master mix & 1 µl extracted DNA (10-20ng/µl).

All reactions were performed in 200 µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

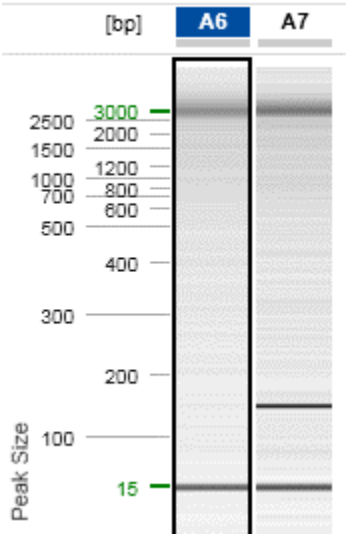
1) 95°C 3 minutes
2) 94°C 20 seconds
3) 61°C 25 seconds
4) 72°C 30 seconds
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 10 minutes
7) 4°C hold until refrigerate product

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

Expected product: 180bp

Example Gel:



Lane A6 displays a sample negative for the mutant allele (no product)
Lane A7 displays a sample positive for the mutant allele (180bp product)

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

WT 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
M34324wt.F	Sigma	25µM	0.3
M34324wt.R	Sigma	25µM	0.3
FastStart *Taq*	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

PCR Setup:
Final Reaction: 19 µl master mix & 1 µl extracted DNA (10-20ng/µl).

All reactions were performed in 200 µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

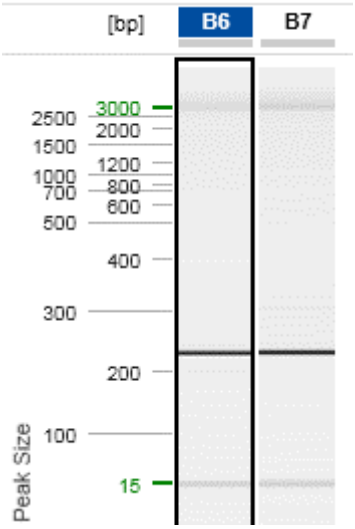
Cycle Parameters:
1) 95°C 3 minutes
2) 94°C 20 seconds
3) 61°C 25 seconds
4) 72°C 30 seconds
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 10 minutes
7) 4°C hold until refrigerate product

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

Expected product: 229bp

Example gel:



Lanes B6 and B7 display samples positive for the wild type allele (229bp product)

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