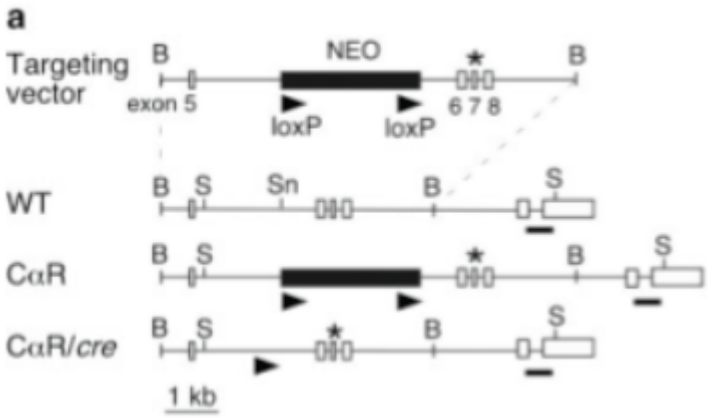
06.19.12 MS

Genotyping Protocol: **MMRRC 32825**

Assay Type: PCR followed by restriction digest- can 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.

Strain Description: This strain has a mutation in exon 7 of the protein kinase, cAMP dependent, catalytic, alpha gene (*Prkaca*) on Chromosome 8 that causes a tryptophan to arginine change at amino acid position 196. There is also a point mutation at the wobble position of Thr 195 which creates a *Mlu*I restriction site in the mutant allele. A neomycin cassette flanked by *loxP* sites and containing the SV40 promoter, the neomycin phosphotransferase gene, and SV40 polyadenylation sequences was inserted into a *SnaB*I restriction site between exons 5 and 6. Details can be found in Niswender et al. (2005) Genesis 43:109-119.



Primer Information:
1) Name: M32825.F Sequence: 5'-GGC TTG GAA CTC ATG ATG TAG-3'
2) Name: M32825.R Sequence: 5'-GAT GAG GAC TCC GAG AGC-3'

Primer location: M32825.F is located in intron 5 of the *Prkaca* gene. M32825.R is located in exon 8 of the *Prkaca* gene.

Assay name: Prkaca PCR/MluI

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M32825.F	Sigma	25µM	0.3
M32825.R	Sigma	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:

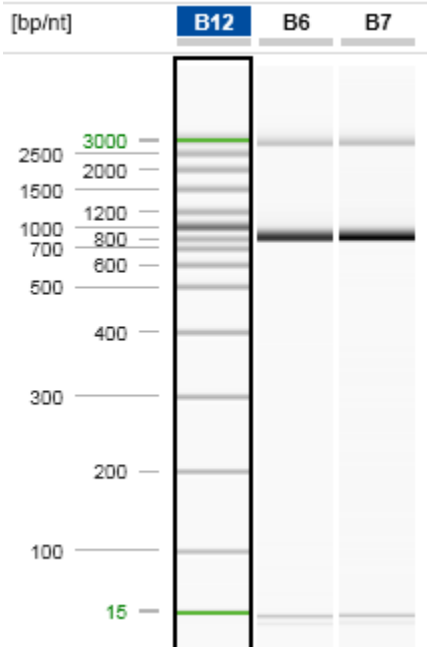
1)	95°C	3 minutes
2)	94°C	30 seconds
3)	64°C	30 seconds
4)	72°C	1 minute
5)	Repeat steps 2-4 34 times for a total of 35 cycles	
6)	72°C	10 minutes
7)	4°C	hold until refrigerate product

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 100bp-2.5kbbp (Cat# 929559)
Method: AM320 Injection: 10s at 5KV
Separation: 320s at 6KV

Expected product: 835bp

Example gel:



Lane B12 displays a 15bp-3kb size marker
Lanes B6 and B7 display the pre-digest 835bp products that result from the PCR.

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

***Mlu*I Restriction Digest: (NEB Catalog #R0198S)**

***Mlu*I Recognition Site:**
5′… A/CGCGT …3′
3′… TGCGC/A …5′

*Mlu*I restriction endonuclease sites (bold, italicized, underlined in WT sequence below)
Mutant Sequence, *Prkaca* gene:

TTTGTTTCATTTTTGACACAGGGTCTCACTTTGTAGTCTGGGCTGGCTTGGAACTCATGATGTAGCCCAGGCTAGCCTCGGACATATGCTAGTG
CTTCTGCCTCTGCTTCTCAGAGTGCTGTAGTGACAGATATGTCCTACTATGTCAGGCTCCCAAGTTTTTACAATAGTTTGGGTCTCTGTCAGGC
TGCCCTTGGAATCTTGGACTCATGCAGTCCCCCTGCCTCAGCCTCAGCCACATAGCGGGGACCAGTGGTACATGGCATCCCCAGCCTTTAGTCT
ACATTTGATAAGCTAGACACGGGGTGTCTTGCTCTGTGGGTACACAGACCCACATGCTGGACCACTTGGGGATGTGGTCATATTCACCCACACA
TCCCCTCCCGGCTTGTCCCACCAG**CGAGCCCCATGCCCGTTTCTACGCGGCGCAGATCGTCCTGACCTTTGAGTATCTGCACTCCCTGGACCTC**
ATCTACCGGGACCTGAAGCCCGAGAATCTTCTCATCGACCAGCAGGGCTATATTCAGGTGCCCGAGGCCGGGGGAGGGCACTCGAGGGCACATT
TGGAGCCTGCAGCCCTTCTCTCTACCAACTGCTCATTCTTGTGCCTACAG**GTGACAGACTTCGGTTTTGCCAAGCGTGTGAAAGGCCGTA/CGC**

GTACCTTGTGTGGGACCCCTGAGTACTTGGCCCCCGAGATTATCCTGAGCAAAGTAGGCACCTCAACCAGCCTGCCCCACCCCTGAGGCCTACT
CTACCTCACTAGCCCGCCCCACCCCTGAGGAATCACCTCCCTCTTCACCTTGCCTCATCGAGTGGCCCCCCCATCTTGCTCTAGGGCTACAACA
AGGCTGTGGACTGGTGGGCTCTCGGAGTCCTCATCTACGAGATGGCTGCTGGTTACCCACCCTTCTTCGCTGACCAGCCTATCCAGATCTATGA
GAAAATCGTCTCTGGGAAGGTGAGGCCAGGATACGGATTTCAGCTCTGGAAGGAATCAAAACAGCCTATCACATGTCCTCA

Key:
M32825.F
M32825.R
Exon 6
Exon 7
Exon 8
MluI cut site (A/CGCGT***)***

Restriction Digest:
10 µl PCR product from PCR above
0.4 µl *Mlu*I (NEB – 10U/µl)
2 µl 10X Buffer 4 (NEB)
7.6µl water
20 µl reaction

Incubate at 37°C overnight.

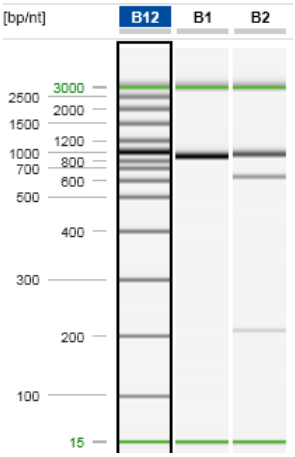
Product Analysis:
All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004):

Alignment Marker: QX Alignment Marker 15bp/3kb (Cat# 929522)
Size Marker: QX DNA Size Marker 100bp-2.5kb (Cat# 929559)
Method: AM320 Injection: 20s at 8kV
Separation: 320s at 6kV

Important! Dilute restriction enzyme samples with equal parts DNA Dilution Buffer prior to loading on the Qiaxcel*

Predicted Products:
Wild type (WT): 1 band (835bp)
Heterozygous (Het): 3 bands (835bp, 609bp, 226bp)
Homozygous mutant (Hom): 2 bands (609bp, 226bp)

Example Gel:



Lane B12 displays a 15bp-3kb size marker
Lane B1 displays a wild-type sample (835bp product)
Lane B2 displays a heterozygous sample (835bp, 609bp and 226bp products)

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