

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	199
MMRRC Strain Name	B6;129X1- <i>Prkaca</i> ^{tm1Gsm} /Mmnc
Gene Name(s)	Protein kinase, cAMP dependent, catalytic, alpha (Prkaca)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	1/30/12

MMRRC# 199 PCR Reaction

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10 uM Primer F	1
10 uM Primer R	1
Taq	0.5
DNA	2

2,2 Primer Reaction (WT-F and WT-R) and (MutF and MutR)

Thermal Cycler:

94°C for 30 sec

60°C for 30 sec

72°C for 60 sec

30x

Taq: **Apex and Chromataq 5X Buffer**

Bands: MUTANT: 270bp

WT: 250bp

Primer sequences 5' to 3':

PrkacaWT-F(199) : CTGACCTTTGAGTATCTGCAC

PrkacaWT-R(199): GTCCACACAAGGTCCAAGTA

PrkacaMut-F(199) : AGACTACTGCTCTATCACTGA

PrkacaMut-R(199) : GTGGTTTGTCCAAACTCATCAATGT

Run on 2.0% agarose gel in TAE.