

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	200
MMRRC Strain Name	B6.129X1- <i>Prkacb</i> ^{tm2Gsm} /Mmnc
Gene Name(s)	Protein kinase, cAMP dependent, catalytic, beta (<i>Prkacb</i>)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	4/28/11

MMRRC# 200 PCR Reaction

	<u>1X</u>
DNA	2.0
5X Buffer	5.0
10mM dNTPs	0.5
10uM Primer (Each)	1.0
Taq	0.5
ddH ₂ O	11.0
25mM MgCl ₂	2.0

Note: Add 1ul of each 10uM primer to mix.

Thermal Cycler:

94°C for 45 sec

60°C for 60 sec

72°C for 60 sec

30x

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

Bands: MUTANT: ~375bp

WT: ~430bp

Primer sequences 5' to 3':

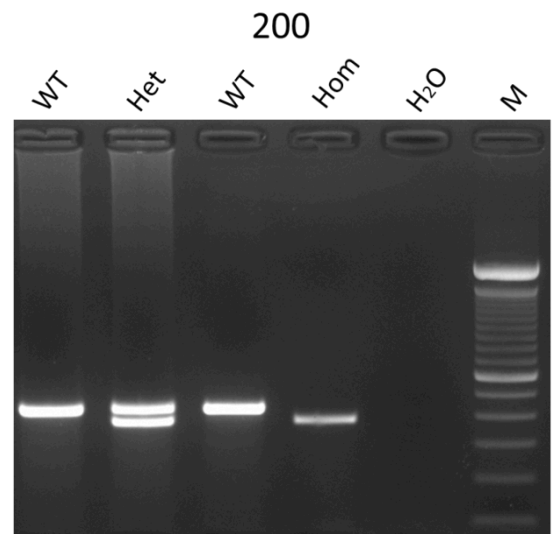
CBWT-F: GCT CCA GCT TCC ATT AAC CCC AC

CBWT-R: CAC TTC CAA TTG CAG AAA AGC ATA CAG

N752S: GAT GGA TTG CAC GCA GGT TCT

N1131AS : AGG TAG CCG GAT CAA GCG TAT

Run on 2.0% agarose gel in TAE.



M: 100 bp DNA ladder (Invitrogen)