

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	34291
MMRRC Strain Name	C57BL/6J- <i>Prkcb</i> ^{m1Btlr} /Mmnc
Gene Name(s)	protein kinase C, beta / mutation 1 (<i>Prkcb</i> ^{m1Btlr})
Breeding Protocol(s)	Random Intra-Strain Mating
Protocol Date	7/29/13

MMRRC #34291 PCR Reaction

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 59°C for 30 sec
 Step 4: 72°C for 30 sec
 Step 5: 35x from step 2 to step 4
 Step 6: 72°C for 7 min

Taq: Apex and Chromataq 5X Buffer

	1X
ddH ₂ O	13
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10 μM Primer Forward	1
10 μM Primer Reverse	1
Apex Taq	0.5
DNA	2

Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

E- For (34291): AAGTATGGGGTGCTGACTTTGGTGT

E- Rev (34291): CATCTCAAAGCGAGCAGAATACAGTTGT

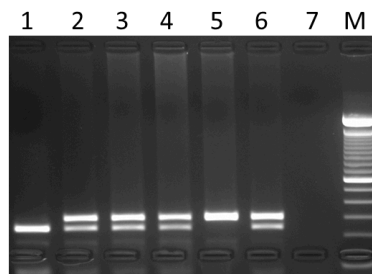
Bands expected: WT and MUTANT: 293 bp

Note: This genotyping requires *DraIII* restriction digestion.

Master mix for each sample

9.6 μl H₂O
 4.0 μl NEB Buffer 3, 10X
 0.4 μl BSA (100 μg/ml)
 1.0 μl *DraIII*

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Lane 1: Hom; Lane 2-4, 6: Het; Lane 5: WT; Lane 7: H₂O;
 M: 100 bp DNA ladder (Invitrogen)

Add 15ul Mix to each 25ul PCR product: incubate at 37 °C for overnight.

Expect Bands expected after *DraIII* digestion: MUTANT: 236bp

WT: 293bp

Run on a 2% agarose gel.