

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	30045
MMRRC Strain Name	FVB.BKa- <i>Ptprc^b</i> /Mmnc
Gene Name(s)	Ptprcb - protein tyrosine phosphatase, receptor type, C
Breeding Protocol(s)	Intra-Strain Mating
Protocol Date	1/6/14

MMRRC# 30045 PCR Reaction

	<u>1X</u>
DNA	2.0
5X Buffer	5.0
10mM dNTPs	0.5
20 uM Primer F/R	1.0
Taq	0.5
ddH ₂ O	13.5
25mM MgCl ₂	2.0

Reaction requires *BspE1* restriction digest

10ul H₂O
 4ul NEB Buffer 3, 10X
 1ul ***BspE1***

Add 15ul Mix to each 25ul PCR product:

Incubate at 37 °C for 12 hours
 Heat inactive 80 °C for 20min

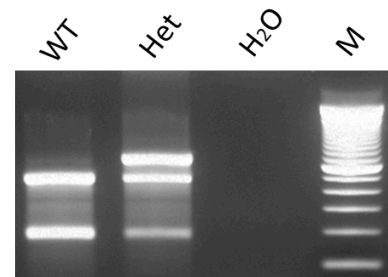
Thermal Cycler:

Step 1: 94°C for 45 sec
 Step 2: 55°C for 45 sec
 Step 3: 72°C for 60 sec
 36x

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

Bands: MUTANT: 472bp and 184bp
 WT: 656bp

30045



M: 100 bp DNA ladder (Invitrogen)

Primer sequences 5' to 3': Primers are 10uM with respect to each primer (20uM total).

30045For: 5'-CTCACAGGCACATGAACGAT

30045Rev: 5'-CGCTTCAAGCATGTCTTCTG

Run on 2.0% agarose gel in TAE.