

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	14109
MMRRC Strain Name	B6.129S6- <i>Pparg</i> ^{tm1.2Mgn} /Mmnc
Gene Name(s)	Peroxisome proliferator activated receptor gamma (<i>Pparg</i>)
Breeding Protocol(s)	Backcross to C57BL/6J (homo lethal)
Protocol Date	8/27/10

MMRRC# 14109 PCR Reaction

NOTE: Run two, 2 primer reactions using the following primer pairs: (PPAR4 & PPAR1) and (PPAR4 & PPAR5). Combine in gel.

Thermal Cycler:

Step 1: 94°C for 6 min
 Step 2: 94°C for 1 min
 Step 3: 60°C for 30 sec
 Step 4: 72°C for 30 sec
 Steps 2-4: 40 cycles
 Step 5: 72°C for 7 min
 Step 6: 4°C hold

	<u>1X</u>
DNA	1.0
10X Chromataq Buffer	5.0
10mM dNTPs	1
20 uM Primer F/R	1.5
Taq	1.0
ddH ₂ O	6.0
25mM MgCl ₂	2.0

Taq: **Apex**

Bands: MUTANT: 551bp
 WT: 464bp

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

PPAR4: GCT CCT GAG TGC TAA TAT TAA AG

PPAR1: GAT AAG ACA GCA CAA CAA TGT TC

PPAR5: CCA TGG ACT AAT GCT GTA ATA TTA

Run on 1.0% agarose gel in TAE. (Run gel approximately half way)