

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	34625
MMRRC Strain Name	B6.129P2- <i>Ptges3</i> ^{Gt(RST271)Byg} /Mmnc
Gene Name(s)	prostaglandin E synthase 3 (cytosolic) (Ptges3)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	2/28/13

MMRRC # 34625 PCR Reaction

2,2 Primer Reaction: (WTF + WTR) for wild-type and (PLAPF + PLAPR) for the mutant reaction

Thermal Cycler:

Step 1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 60°C for 30 sec

Step 4: 72°C for 30 sec

Step 2 to 4 Cycles: 35

Step 5: 72°C for 7 min

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

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

Bands: MUTANT: 391bp WT: 369bp

Primer sequences 5' to 3':

WTF: TCCCCCTTGAACCTCTACTCTTTC

WTR: AGTGTCCACGGAAGCCAGAAG

PLAPF: AGGGGTGCCAGGACATCGCTAC

PLAPR: TCCACGAAGAGGAAGAAGCCGC

Run on 2% agarose gel in TAE.