

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11638
MMRRC Strain Name	B6.129P2- <i>Ptger1</i> ^{tm1Dgen} /Mmnc
Gene Name(s)	prostaglandin E receptor 1 (subtype EP1) (<i>Ptger1</i>)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	3/17/14

MMRRC #11638 PCR Reaction

	<u>1X</u>
ddH ₂ O	12
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10 mM Primer 1	1
10 mM Primer 2	1
10 mM Primer 3	1
Taq	0.5
DNA	2

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 45 sec
 Step 3: 61°C for 45 sec
 Step 4: 72°C for 60 sec
 Step 2 to 4 Cycles: 35
 Step 5: 72°C for 7 min

Taq: Apex Taq and Chromataq 5X Buffer

Bands: MUTANT: 558bp
 WT: 272bp

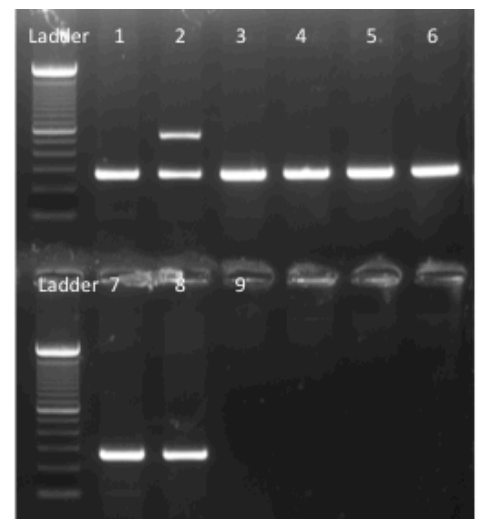
Primer sequences 5' to 3':

NIH1175-GS2: ACCATGCAGCCACCCAGGAAATGAC

NIH1175-GS3: CCAACAGGCGATAATGGCACATCAC

NIH1175-Neo: GGGGATCGATCCGTCCTGTAAGTCT

Run on 2.0% agarose gel in TAE.



<u>Lanes</u>	<u>Genotype</u>
1	Wild-type
2	Heterozygous
3	Wild-type
4	Wild-type
5	Wild-type
6	Wild-type
7	Wild-type
8	Wild-type Control
9	Negative Control