

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	12830
MMRRC Strain Name	B6.129S6-S1pr2 ^{tm1Rlp} /Mmnc
Gene Name(s)	Sphingosine-1-phosphate receptor 2 (S1pr2)
Breeding Protocol(s)	Sib-mating
Protocol Date	12/16/13

MMRRC# 12830 PCR Rxn

	<u>1X</u>
DNA	2.0
5X Buffer	5.0
10mM dNTPs	0.5
10 uM Primer F/R	1/1
Taq	0.5
ddH ₂ O	13.0
25mM MgCl ₂	2.0

NOTE: Run 2 reactions for each sample (WT: pr1 + pr2) and (Mut: pr1 + pr3).

Thermal Cycler:

95°C for 5 min
 95°C for 30 sec
 55°C for 60 sec
 72° C for 60 sec
 35x
 72°C for 7 min

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

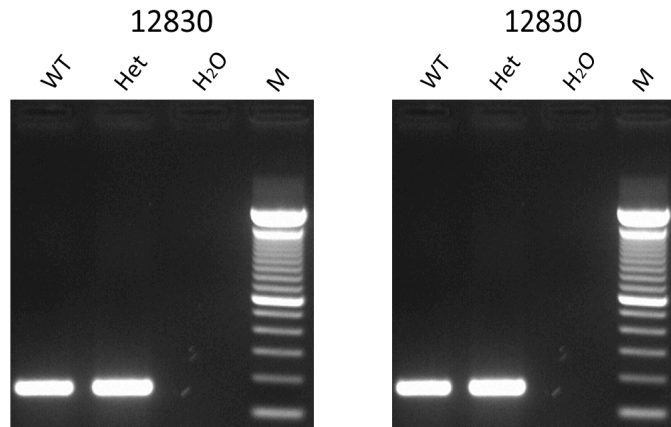
Bands: MUTANT: 220bp
 WT: 170bp

Primers are 10uM with respect to each primer.

Primer sequences 5' to 3':

S1P2 pr 1 FOR 5'- GCA GTG ACA AAA GCT GCC GAA TGC TGA TG
 S1P2 pr 2 REV 5'- AGA TGG TGA CCA CGC AGA GCA CGT AGT G
 S1P2 pr 3 REV 5'-TGA CCG CTT CCT CGT GCT TTA CGG TAT CG

Run on 2.0% agarose gel in TAE.



Primers: S1P2 pr1 + S1P2pr2

M: 100 bp DNA ladder (Invitrogen)

Primers: S1P2 pr1 + S1P2pr3

M: 100 bp DNA ladder (Invitrogen)