

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	12038
MMRRC Strain Name	B6.129S6- <i>S1pr3</i> ^{tm1Rlp} /Mmnc
Gene Name(s)	Sphingosine-1-phosphate receptor 3 (S1pr3)
Breeding Protocol(s)	Sib-mating
Protocol Date	12/2/14

MMRRC #12038 PCR Reaction

Thermal Cycler:

94°C for 5 min
 94°C for 30 sec
 55°C for 30 sec
 72°C for 30 sec
 35x
 72°C for 7 min

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

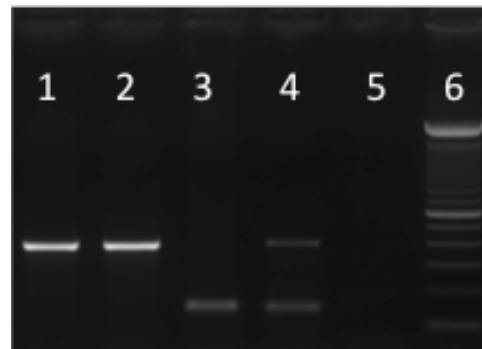
Bands: MUTANT: ~380bp
 WT: 137bp

Primer sequences 5' to 3':

P4 (12038): TCAGTATCTTCACCGCCATT
 P5 (12038): AATCACTACGGTCCGCAGAA
 P6 (12038): GTGCAATCCATCTTGTTCAAT

Run on 2.0% agarose gel in TAE.

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM Primer 4	1
10uM Primer 5	1
10uM Primer 6	1
Taq	0.5
DNA	2



1. Homozygous Sample
2. Homozygous Sample
3. Wild-Type Control
4. Heterozygous Control
5. Negative Control
6. 100bp Marker