

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	35
<b>MMRRC Strain Name</b>	B6;129S- <i>S1pr1</i> <sup>tm1Rlp</sup> /Mmnc
<b>Gene Name(s)</b>	Endothelial differentiation sphingolipid G-protein coupled receptor 1 ( <i>S1pr1</i> )
<b>Breeding Protocol(s)</b>	Intra-strain mating (homozygous lethal) Backcross to C57BL/6J
<b>Protocol Date</b>	10/21/13

### MMRRC# 35 PCR Reaction

	<u>1X</u>
DNA	1.0
5X Buffer	5.0
10mM dNTPs	1.0
30 uM Primer F/R	1.5
Taq	1.0
ddH <sub>2</sub> O	13.5
25mM MgCl <sub>2</sub>	2.0

**Thermal Cycler:**  
 94°C for 60 sec  
 55°C for 60 sec  
 72°C for 120 sec  
 45x

Taq: **Apex and Chromataq 10X Buffer**

**Bands:** MUTANT: 250bp  
 WT: 400bp

Primers are 10uM with respect to each primer (20uM total).

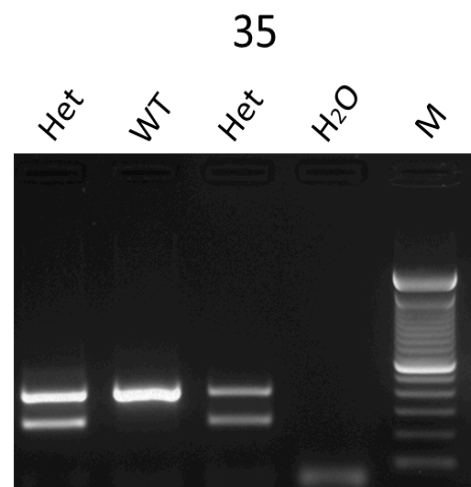
**Primer sequences 5' to 3':**

**neo290:** TCGCCTTCTTGACGAGTTCTTCTGAG

**5288R:** TGCTGCGGCTAAATTCCATG

**4900F:** CCATCCTCTACTGCAGGATCT

Run on 2.0% agarose gel in TAE.



M: 100 bp DNA ladder (Invitrogen)