

Genotyping protocol

EMMA ID: EM:10854

Gene symbol: S1pr2

Strain designation: C57BL/6N-S1pr2^{stdf}/WtsiOulu

Primers:

<u>Primer name</u>	<u>Primer sequence (5'- 3')</u>
S1pr2_F	TGGGTGTTTTTCATCATCTGC
S1pr2_R	GTCCCGTCACTCCCTTCC

Products:

<u>Allele</u>	<u>Primer 1</u>	<u>Primer 2</u>	<u>Product size (bp)</u>
Wild-type	S1pr2_F	S1pr2_R	228
Mutant*	S1pr2_F	S1pr2_R	228

*mutation is a GC transition in the S1pr2 gene, resulted in a threonine to arginine change (T289R). After amplification the product is sequenced.

Reaction mix:

<u>Component</u>	<u>Volume (µl)</u>
Genomic DNA (~50-100 ng)	1.0
Primer 1 (10 µM)	1.0
Primer 2 (10 µM)	1.0
10x Buffer (15 mM MgCl ₂)	3.0
MgCl ₂ (25 mM)	1.0
dNTPs (25 mM)	0.25
DNA polymerase (5 U/µl)	0.1
H ₂ O	22.65
Total	30.0

PCR program:

<u>Time</u>	<u>Temperature (+ °C)</u>	<u>Cycles</u>
4 min	94	
1 min	94	30
1 min	52	
1 min	72	
10 min	72	
∞	4	