

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	12831
MMRRC Strain Name	B6.129P2- <i>Sphk2</i> ^{Gt(pGT1dTMPfs)1Kly} /Mmnc
Gene Name(s)	Sphingosine kinase 2 (Sphk2)
Breeding Protocol(s)	Sib-mating
Protocol Date	12/16/13

MMRRC# 12831 PCR Reaction

**2,2 Primer Reactions : WT (FOR 1 + REV 1) and
MUT (FOR 1 + REV 2)**

Thermal Cycler:

95°C for 5 min
 95°C for 30 sec
 57°C for 60 sec
 72°C for 3 min
 Steps 2-4, 36x
 72°C for 10 min

	1X
DNA	2.0
5X Buffer	5.0
10mM dNTPs	0.5
20 uM Primer 1 & 2	1/1
Taq	0.5
ddH ₂ O	13.0
25mM MgCl ₂	2.0

Taq: Apex and Chromataq 5X Buffer

Bands: MUTANT: 924bp
 WT: 629bp

Primer sequences 5' to 3':

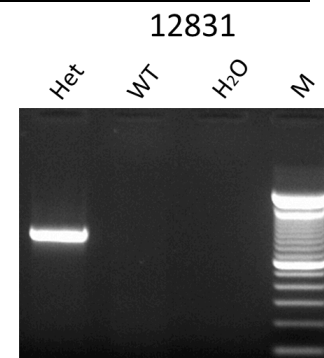
Primers are 10uM with respect to each primer (20uM total).

Sphk2 FOR 5'- TTT CCT GGG ATG TTA ATC TCA TTC TTC

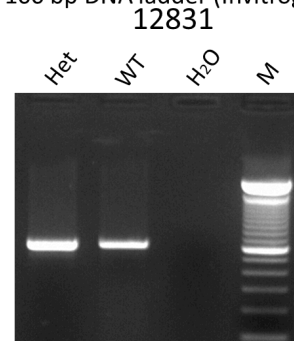
Sphk2 REV1 5'- CAG CCC CTC CGG AGT CTG CAC TAC

Sphk2 REV2 5'- CTT CTT CTT TGG TTT TCG GGA CCT GGG AC

Run on 2.0% agarose gel in TAE.



Primers: Sphk2 FOR + Sphk2 REV2
 M: 100 bp DNA ladder (Invitrogen)



Primers: Sphk2 FOR + Sphk2 REV1
 M: 100 bp DNA ladder (Invitrogen)