MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	31829
MMRRC Strain Name	B6.129S1-Rgs9^{tm1Citb}/Mmnc
Gene Name(s)	regulator of G-protein signaling 9 (Rgs9)
Breeding Protocol(s)	Intra-Strain Mating (-/+ x -/+) Backcross to C57BL/6J
Protocol Date	12/9/13

MMRRC# 31829 PCR Reaction

NOTE: Run 2 reactions for each sample (RGS9F +R; and RGS9neo-3 + RGS9neo-T).

Thermal Cycler:
94°C for 30 sec
62°C for 40 sec
72°C for 60 sec
35x

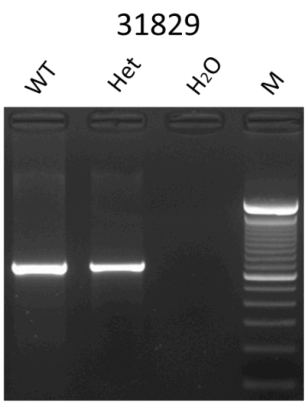
Taq: **Apex and Chromataq 5X Buffer**

Bands: MUTANT: 300bp
WT: 691bp

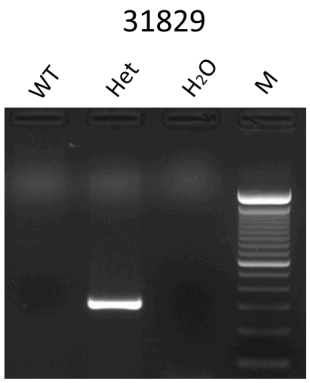
	1X
DNA	2.0
5X Buffer	5.0
10mM dNTPs	0.5
20 uM Primer F/R	1.0
Taq	1.0
ddH_2O	13.5
25mM MgCl2	2.0

Primer sequences 5' to 3': Primers are 10uM with respect to each primer (20uM total).
RGS9R: 5'-CACTGAGGCATGACCCCCAC-3'
RGS9F: 5'-CCTGCCTCTCCTGCCTTTCG-3'
Neo3: 5'-CGGCGAGGATCTCGTCGTGACCCA-3'
NeoT: 5'-GAACAAACTACCCAACACCCGTGCGT-3'

Run on 2.0% agarose gel in TAE.



Primer: RGS9R + RGS9RF
M: 100 bp DNA ladder (Invitrogen)



Primer: Neo-3 + NeoT
M: 100 bp DNA ladder (Invitrogen)

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