

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	30618
MMRRC Strain Name	129S-Gt(ROSA)26Sor ^{tm1(Tfrc*)Nco} /Mmnc
Gene Name(s)	Gene trap ROSA 26 [Gt(ROSA)26]
Breeding Protocol(s)	Sib-mating (Homozygote x Homozygote)
Protocol Date	11/25/13

MMRRC# 30618 PCR Reaction

NOTE: Run two, 2-primer reactions: (64a & 65) and (64a & 102). Run separately.

Thermal Cycler:

94°C for 2min
 94°C for 30 sec
 55°C for 45 sec
 72°C for 60 sec (10 times)
 94°C for 30 sec
 55°C for 45 sec
 72°C for 45 sec + 5 sec 30 times (5 sec extension/cycle)
 72°C for 7 min
 4°C Forever

	<u>WT/MT</u>
DNA	1.0/2.0
5X Buffer	5.0
10mM dNTPs	0.5
20 uM Primer F/R	1.0
Taq	1.0
ddH ₂ O	14.0/13.5
25mM MgCl ₂	1.5/2.0

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

Bands: MUTANT: 614bp
 WT: 259bp

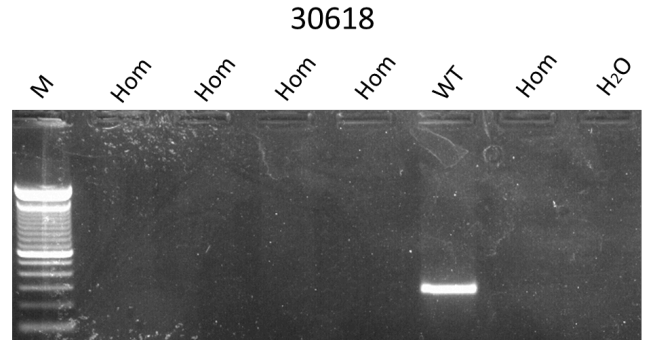
Primer sequences 5' to 3': Primers are 10uM with respect to each primer (20uM total).

PS-64A: GAGGGGAGTGTGCAATA

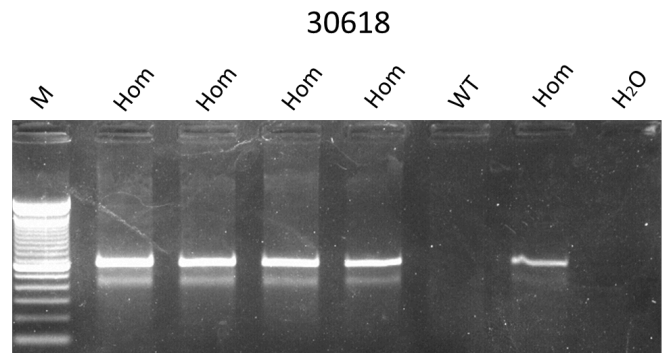
PS-65: CTTCCCACAAAACCGAA

PS-102: TCTGACACTAGCCTTC

Run on 2.0% agarose gel in TAE.



Primer: RG2WTF & RG2WTR; M: 100 bp DNA ladder (Invitrogen)



Primer: RG2WTF & RG2MTR; M: 100 bp DNA ladder (Invitrogen)