

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11652
MMRRC Strain Name	B6;129S5- <i>Rgs10</i> ^{Gt(IRESBetageo)421Lex} /Mmnc
Gene Name(s)	regulator of G-protein signalling 10 (Rgs10)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	8/12/13

MMRRC #11652 PCR Reaction

	<u>1X</u>
ddH ₂ O	12
5X Buffer	4
25mM MgCl ₂	2
10mM dNTPs	0.5
10 uM Primer 1297-5'	1
10 uM Primer LTR-rev	1
10 uM Primer 1297-3'	1
Taq	0.5
DNA	2

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 15 sec
 Step 3: 65°C for 30 sec; Decrease 1°C /cycle
 Step 4: 72°C for 40 sec
 Step 5: go to step 2, 10 cycles
 Step 6: 94°C for 15 sec
 Step 7: 55°C for 30 sec
 Step 8: 72°C for 40 sec
 Step 9: go to step 6 to 8, 30 cycles
 Step 10: 72°C for 7 min

Taq: Apex and Chromataq 5X Buffer

Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

Primer 1297-5': CCTTCCTGAGCACTGGACAAGTATG

Primer LTR-rev: ATAAACCCTCTTGCAGTTGCATC

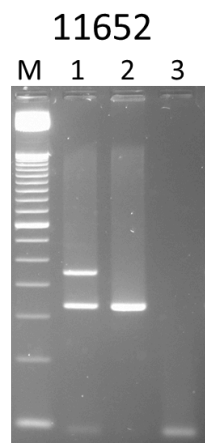
Primer 1297-3': AGGTGCTATGAAGCCTGGTTTGC

Bands:

WT: (Primers 1297-5' and Primer 1297-3'): 165 bp

MUTANT: (Primers 1297-5' and Primer LTR-rev): 223 bp

Run on 2 % agarose gel in TAE.



Lane 1: Het; Lane 2: WT; Lane 3: H₂O;
 M: 50 bp DNA ladder (Invitrogen)