

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	10601
MMRRC Strain Name	129S6/SvEvTac-Tg(Alb1-Ren)1Unc/Mmnc
Gene Name(s)	Transgene insertion 1, University of North Carolina (Tg(Alb1-Ren)1Unc)
Breeding Protocol(s)	Random Intra-Strain Mating
Protocol Date	7/29/13

MMRRC #10601 PCR Reaction

	<u>1X</u>
ddH ₂ O	12
5X Buffer	5.0
25 mM MgCl ₂	2
10 mM dNTPs	0.5
10 μM Primer P1	1
10 μM Primer P2	1
10 μM Primer P3	1
Taq	0.5
DNA	2

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 56°C for 30 sec
 Step 4: 72°C for 45 sec
 Step 5: Step 2 to 4; Cycles: 35
 Step 6: 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.

P1 (10601): TGGGATTCTAACCCCTGAGGACC

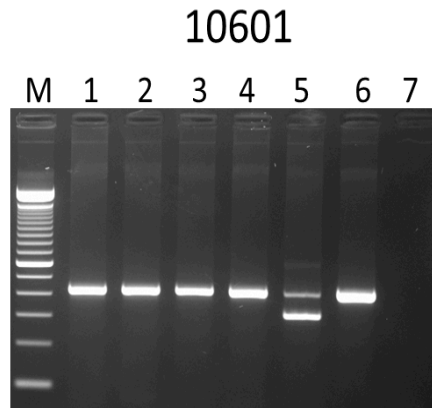
P2 (10601): CACAGATTGTAAGTCAAATCTGTCTG

P3 (10601): GTTCTTCTGAGGGGATCGGC

Bands expected: WT: primers P1 + P2, 425 bp

Mut: primers P1 + P3, 330 bp

Run on 2% agarose gel in TAE.



Lane 1-4, 6: WT; Lane 5: Het; Lane 7: H₂O; M: 100 bp DNA ladder (Invitrogen)