

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	295
MMRRC Strain Name	STOCK Tg(Ascl1-EGFP)AU176Gsat/Mmnc
Gene Name(s)	Achaete-scute complex homolog 1 (Ascl1)
Breeding Protocol(s)	Outcross to Swiss Webster (Taconic)
Protocol Date	1/21/14

MMRRC #295 PCR Reaction

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25 mM MgCl ₂	2
10 mM dNTPs	0.5
10 μM Primer F	1
10 μM Primer R	1
Taq	0.5
DNA	2

Thermal Cycler:

Step 1: 94⁰C for 5 min
 Step 2: 94⁰C for 30 sec
 Step 3: 60⁰C for 30 sec
 Step 4: 72⁰C for 45 sec
 Step 5: Step 2 to 4; Cycles: 35
 Step 6: 72⁰C for 7 min

Taq: Apex and Chromataq 5X Buffer

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

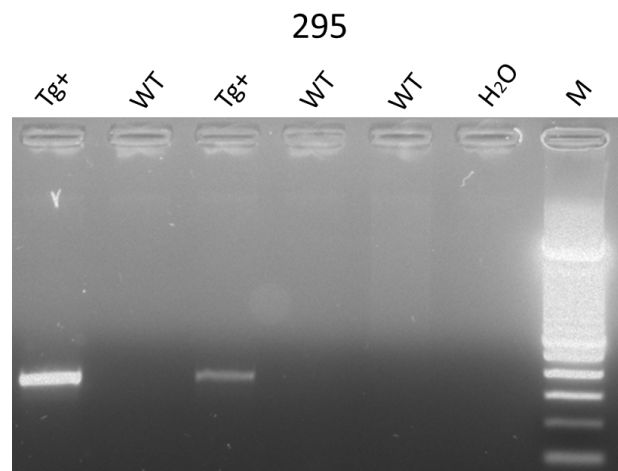
TM3a-RC (295): CGTTTCTCCCCTCTCCTTTTCTTCGT

TM-R (295): TAGCGGCTGAAGCACTGCACG

Bands expected: Tg: ~ 370 bp

WT: no band

Run on 1% agarose gel in TAE.



M: 100 bp DNA ladder (Invitrogen)