

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11534
MMRRC Strain Name	B6;129P2- <i>Tgfa</i> ^{tm1Unc} /Mmnc
Gene Name(s)	Transforming growth factor alpha (Tgfa)
Breeding Protocol(s)	Intra-strain Mating
Protocol Date	6/4/12

MMRRC# 11534 PCR Reaction

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

2,2 Primer Reaction (m13-14 + E4-2) for wild-type and (m13-14 + Neo-up) for mutant

Thermal Cycler:

94°C for 5 min
94°C for 30 sec
60°C for 30 sec
72°C for 60 sec
36x
72°C for 7 min

Taq: **Denville Taq (5 U/ul) and Chromataq 5X Buffer**

Bands: MUTANT: ~450bp
WT: ~200bp

Primer sequences 5' to 3':

Tgfa m13-14 (11535): GGTAAGGAGTCCTCGGTCAAGGGT

Tgfa E4-2 (11535): GATCAGCACACAGGTGATAATGAGGAC

Tgfa Neo-up (11535): TCTGTTGTGCCAGTCATAGCCGA

Run on 2.0% agarose gel in TAE.