

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11535
MMRRC Strain Name	B6;129- <i>Egf</i> ^{tm1Dle} <i>Tgfa</i> ^{tm1Unc} /Mmnc
Gene Name(s)	Epidermal growth factor (Egf) Transforming growth factor alpha (Tgfa)
Breeding Protocol(s)	Random Intra-Strain Mating
Protocol Date	9/13/10

MMRRC# 11535 PCR Reactions

	<u>1X</u>
DNA	1.0
5X Buffer	5.0
10mM dNTPs	1.0
30 uM Primer F/R	1.5
Taq	0.5
ddH ₂ O	13.5
25mM MgCl ₂	2.0

NOTE: There are two 2-primer reactions and one 3-primer reaction. The conditions are the same for both *Egf*(two 2-primer rxns) and *Tgfa* (all 3).

For *Tgfa*: ml3-l4 & E4-2 & Neo-Up.

For *Egf*: MI19-3 & MI19-Up & MI19-3 & PGK-2

Thermal Cycler:

94°C for 5 min

94°C for 45 sec

64°C for 45 sec

72°C for 60 sec

36 cycles

72°C for 10 min

4°C forever

Taq: **DENVILLE** and Chromataq 5X Buffer

Bands: **Egf:** MUTANT: ~600bp
WT: ~350bp
Tgf: MUTANT: ~450bp
WT: ~200bp

Primer sequences 5' to 3': Primers are 10uM with respect to each primer (30uM total).

Tgfa mI3-14 (11535): GGTAAGGAGTTCTCGGTCAAGGGT

Tgfa E4-2 (11535): GATCAGCACACAGGTGATAATGAGGAC

Tgfa Neo-up (11535): TCTGTTGTGCCCAGTCATAGCCGA

Egf MI19-3 (11535): ATA AGA TGC TGA TGA TGC TGA TGC

Egf MI19-Up (11535): AGC TGT CCT CTA TAG ACC TGG CTG

Egf PGK-2 (11535): CTG CAC GAG ACT AGT GAG ACG TGC

Run on 2.0% agarose gel in TAE.