

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	118
MMRRC Strain Name	C.129S2(B6)- <i>Tgfb1</i> ^{tm1Doe} /Mmnc
Gene Name(s)	Transforming growth factor, beta 1 (Tgfb1)
Breeding Protocol(s)	Backcross to Balb/cJ
Protocol Date	9/3/13

MMRRC #118 PCR Reaction

	<u>1X</u>
ddH ₂ O	12
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10 μM WT-F (118)	1
10 μM WT-R (118)	1
10 μM Mut (118)	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.

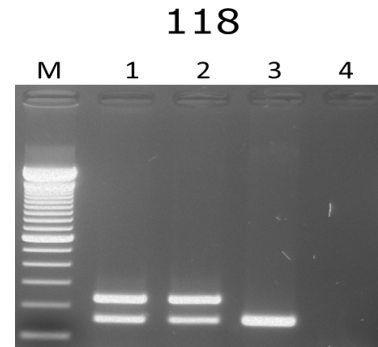
WT-F (118): TCC ACA GAG AAG AAC TGC TG

Mut (118): CAG GAC ATA GCG TTG GCT AC

WT-R (118): TTG CTG TAC TGT GTG TCC AG

Bands expected: WT 152bp
 Mut ~250

Run on 2% agarose gel in TAE.



Lane 1, 2: Het; Lane 3: WT; Lane 4: H₂O
 M: 100 bp DNA ladder Invitrogen