

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	42
MMRRC Strain Name	FVB/N-Tg(Dspp-TGFB1)3Kul/Mmnc
Gene Name(s)	Dentin sialophosphoprotein (Dspp) Transforming growth factor beta-1 (TGFB1)
Breeding Protocol(s)	Sib-mating or Backcross to FVB/N
Protocol Date	9/7/13

MMRRC #42 PCR Reaction

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

Thermal Cycler:

Step 1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 60°C for 30 sec

Step 4: 72°C for 30 sec

Step 5: 35x from step 2 to step 4

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.

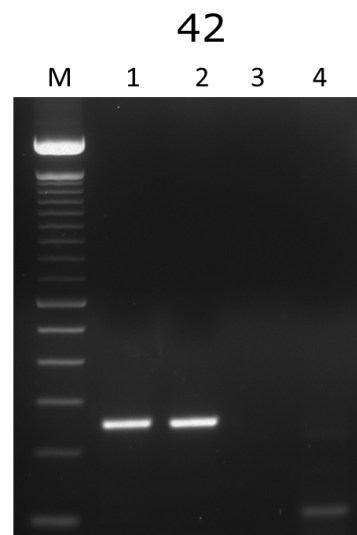
TgfB1-F 2: TGCCGGAACCTGTATTGCTCTCTC

TgfB1-R 2: TGTCACAGGAACAGTGGGCACTGA

Bands expected: Tg+: 249 bp

WT: No band

Run on 1% agarose gel in TAE.



Lane 1, 2: Tg+; Lane 3: WT; Lane 4: H₂O
M: 100 bp DNA ladder (Invitrogen)