

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	225
MMRRC Strain Name	STOCK Tg(Col2a1-lacZ)1Tlu/Mmnc
Gene Name(s)	Collagen, type II, alpha 1 (Col2a1) LacZ (E. Coli) - Beta galactosidase, Transgene insertion 1 (Tg(Col2a1-lacZ)1Tlu)
Breeding Protocol(s)	Backcross to B6D2
Protocol Date	9/30/13

MMRRC #225 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: 58⁰C for 30 sec
 Step 4: 72⁰C for 50 sec
 Step 5: Step 2 to 4; Cycles: 35
 Step 6: 72⁰C for 7 min

Taq: Apex and chromataq Buffer

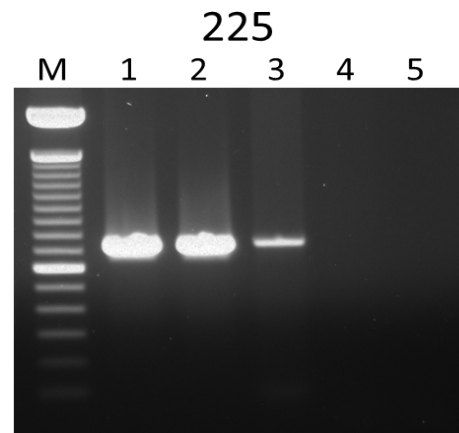
Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

b-gal fw: GACGTCTCGTTGCTGCATAA

b-gal rv: TCACACTCGGGTGATTACGA

Bands expected: MUTANT 730bp
WT no band

Run on 1% agarose gel in TAE.



Lane 1, 2, 3: Mut; Lane 4: WT; Lane 5: H₂O;
M: 100 bp DNA ladder (Invitrogen)