

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	10479
MMRRC Strain Name	FVB.Cg-Tg(KRT5-CCND1)1Sprd/Mmnc
Gene Name(s)	Keratin 36 (Krt36), cyclin D1 (Ccnd1)
Breeding Protocol(s)	Sib-mating
Protocol Date	12/16/13

MMRRC #10479 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: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 55°C for 30 sec
 Step 4: 72°C for 45 sec
 Step 5: 35 x from step 2 to step 4
 Step 6: 72°C for 7 min

Taq: Apex and Chromataq 5X Buffer

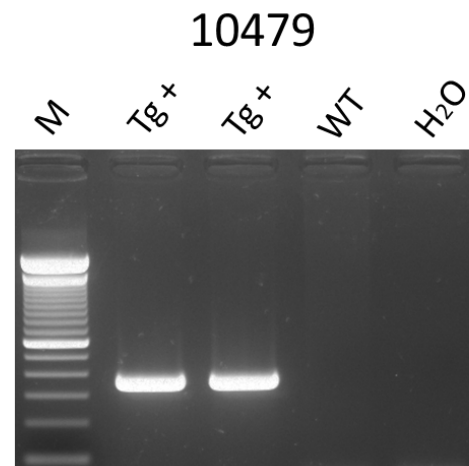
Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

K5D1b (10479): CAATAAGAATATTTCCACGCCA

R (10479): TTCAGGGTGTGTTTAGAATGG

Bands expected: Tg⁺, 350 bp

Run on 1% agarose gel in TAE.



M: 100 bp DNA ladder (Invitrogen)