

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	10623
MMRRC Strain Name	129S4/SvJae- <i>Cd80^{tm1Shr}</i> /Mmnc
Gene Name(s)	CD80 antigen (CD80)
Breeding Protocol(s)	Intra-Strain Mating
Protocol Date	1/27/14

MMRRC #10623 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 45 sec
 Step 5: 33 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.

TM-WT-F (10623): ACTCTCCTCATGAAGATGAGTCTGAAGACC

TM-WT-R (10623): GTAGAGGTCTCCACCTTGATCAATCAGG

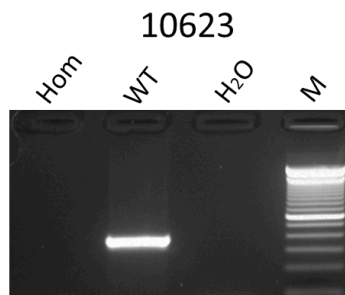
TM-Mut-F (10623): CGATGCCTGCTTGCCGAATATCAT

Bands expected:

WT: TM-WT-F (10623) & TM-WT-R (10623), 399 bp

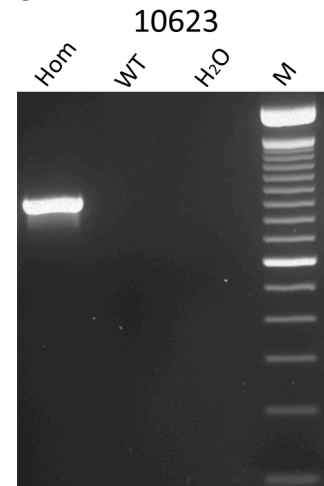
MUTANT; TM-WT-R (10623) & TM-Mut-F (10623), ~900 bp

Run on 2% agarose gel in TAE.



Primers: TM-WT-F (10623) & TM-WT-R (10623)

M: 100 bp DNA ladder (Invitrogen)



Primers: TM-WT-R (10623) & TM-Mut-F (10623)

M: 100 bp DNA ladder (Invitrogen)