

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	10625
MMRRC Strain Name	129S4/SvJae- <i>Cd80^{tm1Shr} Cd86^{tm2Shr}</i> /Mmnc
Gene Name(s)	CD80 antigen (CD80) CD86 antigen (CD86)
Breeding Protocol(s)	Intra-Strain Mating
Protocol Date	1/27/14

MMRRC #10625 PCR Reactions

There are four 2-primer reactions.

	<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

Taq: Apex and Chromataq 5X Buffer

CD80 PCR Reaction

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: 30x from step 2 to step 4

Step 6: 72°C for 7 min

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

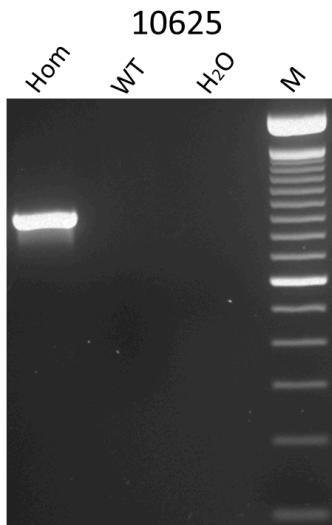
TM-WT-F (10623): ACTCTCCTCATGAAGATGAGTCTGAAGACC

TM-WT-R (10623): GTAGAGGTCTCCACCTTGATCAATCAGG

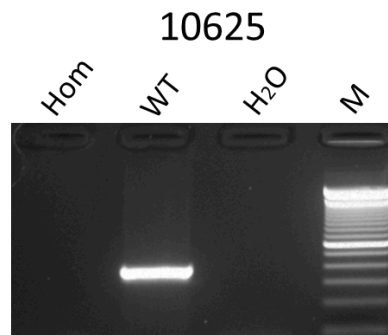
TM-Mut-F (10623): CGATGCCTGCTTGCCGAATATCAT

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

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



Primers: TM-WT-R(10623) & TM-Mut-F(10623)
M: 100 bp DNA ladder (Invitrogen)



Primers: TM-WT-F (10623) & TM-WT-R(10623)
M: 100 bp DNA ladder (Invitrogen)

CD86 PCR Reaction

Thermal Cycler:

Step 1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 62°C for 30 sec

Step 4: 72°C for 45 sec

Step 5: 30x from step 2 to step 4

Step 6: 72°C for 7 min

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

B7-2-Com-F (10624): AGATCCTAGCACCCACCTCTCCAGTACT

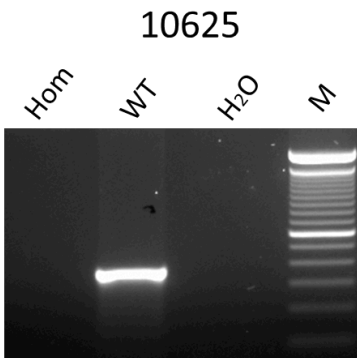
B7-2-WT-R (10624): CCCATGTCCTTGATCTGAACATTGTG

B7-1/2 Mut-R (10625): GTCGGAGACGCTGTCGAACTTTTC

Bands expected: WT: B7-2-Com-F (10624) + B7-2-WT-R (10624), 379 bp

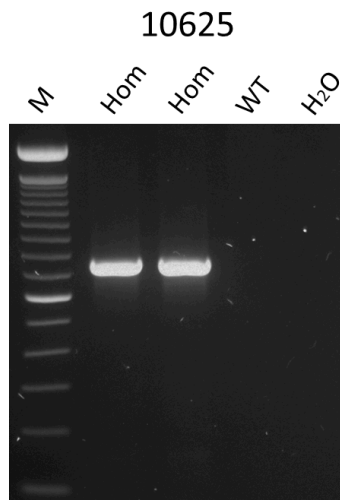
MUTANT: B7-2-Com-F (10624) + B7-1/2 Mut-R (10625), ~720 bp

Run on 2% agarose gel in TAE.



Primers: B7-2-Com-F (10624) + B7-2-WT-R (10624)

M: 100 bp DNA ladder (Invitrogen)



Primers: B7-2-Com-F (10624) + B7-1/2 Mut-R (10625)

M: 100 bp DNA ladder (Invitrogen)