

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	428
MMRRC Strain Name	B10.CB(Cg)-H2-Dma ^{tm1Liz} /Mmnc
Gene Name(s)	Histocompatibility 2, class II, locus Dma (H2-Dma)
Breeding Protocol(s)	Sib-mating
Protocol Date	12/6/13

MMRRC #428 PCR Reaction

	1X
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: 59°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

Taq: Apex and Chromataq 5X Buffer

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

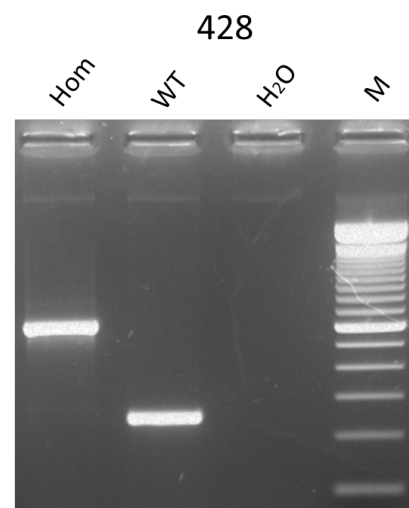
WT-F (427): CACATTCCGGCACACTCTATTCTG

Com-R (427): TCTGGACACTGGGATTT GACCTTC

Mut-F (427): CTTGCCCCAATAGCAGC CAGTCC

Bands expected: WT-F (427) + Com-R (427); WT 247 bp
 Mut-F (427) + Com-R (427); Mut ~600 bp

Run on 2% agarose gel in TAE.



M: 100 bp DNA ladder (Invitrogen)