

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	427
<b>MMRRC Strain Name</b>	BALB/cJ-H2-DMA <sup>tm1Liz</sup> /Mmnc
<b>Gene Name(s)</b>	Histocompatibility 2, class II, locus Dma (H2-Dma)
<b>Breeding Protocol(s)</b>	Sib-mating
<b>Protocol Date</b>	10/28/13

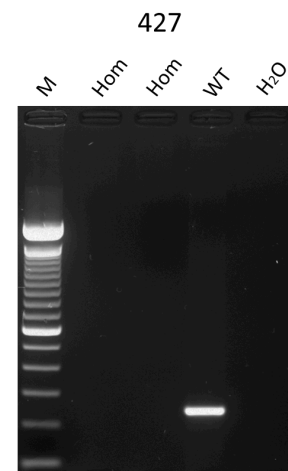
### MMRRC #427 PCR Reaction

	<b>1X</b>
ddH <sub>2</sub> O	13
5X Buffer	5.0
25 mM MgCl <sub>2</sub>	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**



Primers: WT-F(427) + Com-R(427)  
 M: 100 bp DNA ladder (Invitrogen)

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

WT-F (427): CACATTCCGGCACACTCTATTCTG

Com-R (427): TCTGGACACTGGGATTT GACCTTC

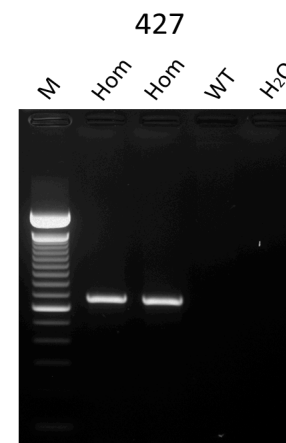
Mut-F (427): CTTGCCCCAATAGCAGC CAGTCC

#### Bands expected:

WT-F (427) + Com-R (427); WT 247 bp

Mut-F (427) + Com-R (427); Mut 691 bp

Run on 2% agarose gel in TAE.



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