

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	197
<b>MMRRC Strain Name</b>	B6;129- <i>Nfatc2</i> <sup>tm1Rao</sup> /Mmnc
<b>Gene Name(s)</b>	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2 ( <i>Nfatc2</i> )
<b>Breeding Protocol(s)</b>	Sib-Mate Backcross to C57BL/6J
<b>Protocol Date</b>	11/11/13

### MMRRC #197 PCR Reaction

	<b><u>1X</u></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: 61°C for 30 sec  
 Step 4: 72°C for 1 min  
 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.

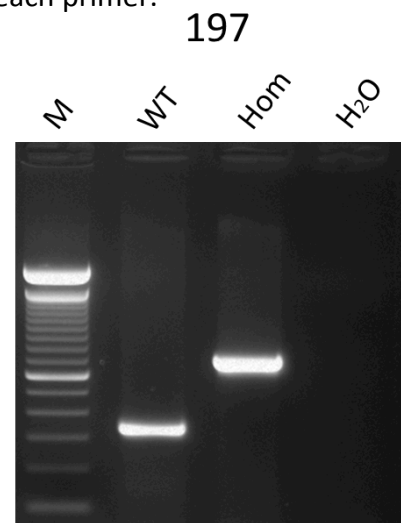
Com F (197): CAGCTTCATCCCCCATGAGCAGCT

WT R (197): TGGGGCTTGGGTTGGACCTCAA

Mut R (197): GGACATAGCGTTGGCTACCCGTGA

**Bands expected:** WT, Com-F + WT R, 340 bp  
 Mut, Com-F + Mut R, ~700 bp

Run on 2 % agarose gel in TAE.



M: 100 bp DNA ladder (Invitrogen)