

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	9968
<b>MMRRC Strain Name</b>	STOCK Del(11Irf1-D11Mit23)1Rub/Mmnc
<b>Gene Name(s)</b>	Interferon regulatory factor 1 (Irf1), Deletion, Chr 11 (Del(11Irf1-Csf2)1Rub, Colony Stimulating factor 2 (Csf2)
<b>Breeding Protocol(s)</b>	Sib-mating
<b>Protocol Date</b>	11/18/13

### MMRRC #9968 PCR Reaction

	<u>1X</u>
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: 60°C for 30 sec  
 Step 4: 72°C for 45 sec  
 Step 5: 35x 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.

P1 (9968): AAATTTAGCTGGCATCCGG

P2 (9968): TTGCCAGCCCACAAAAGA

P3 (9968): CATAGCCTGAAGAACGAGATC

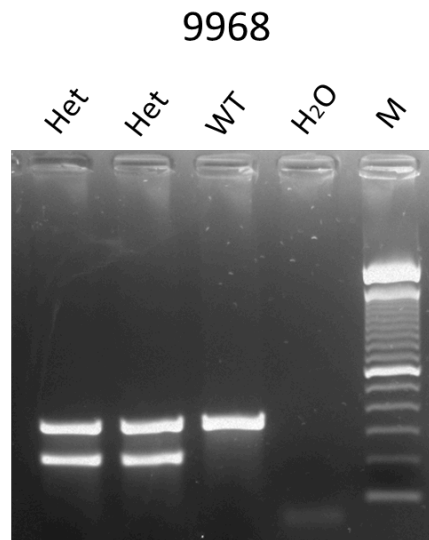
P4 (9968): GTAAAACGACGGCCAGT

**Bands expected:**

WT (P1 + P2): 343 bp

MUTANT (P3 + P4): 240 bp

Run on 2% agarose gel in TAE.



M: 100 bp DNA ladder (Invitrogen)