

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	10318
<b>MMRRC Strain Name</b>	B6.129S6- <i>Nfia</i> <sup>tm1Rmg</sup> /Mmnc
<b>Gene Name(s)</b>	Nuclear factor I/A (Nfia)
<b>Breeding Protocol(s)</b>	Backcross to C57BL/6NTac
<b>Protocol Date</b>	11/25/13

### MMRRC #10318 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

**Note:** Set up one 6-primer 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: 33x 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.

I2B9 (10318): TGCTGTGTTCTGGTCAGTCAAG

I2CC (10318): CAAAGCAAATCTCCATGCTCGG

neo57 (10318): GGAGAGGCTATTCGGCTATGAC

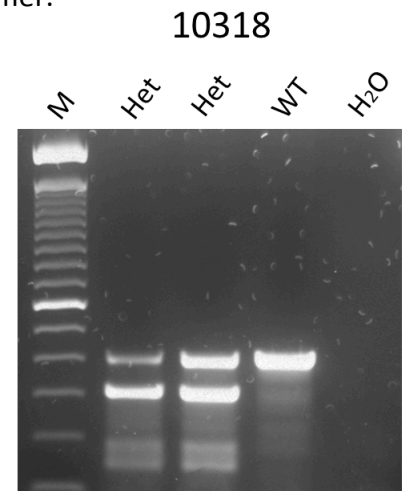
neo371R (10318): CGCATTGCATCAGCCATGATGG

sry1 (10318): AACAACTGGGCTTTGCACATTG

sry2 (10318): GTTTATCAGGGTTTCTCTAGC

**Bands expected:** WT I2B9 (10318) + I2CC (10318): 405 bp  
 Mutant neo57 (10318) + neo371R (10318), 315 bp  
 sry1 + sry2, 166 bp and 146 bp

Run on 2% agarose gel in TAE.



M: 100 bp DNA ladder (Invitrogen)