

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	16128
MMRRC Strain Name	B6.129- <i>Nasp</i> ^{tm1Mgor} /Mmnc
Gene Name(s)	Nuclear autoantigenic sperm protein (Nasp)
Breeding Protocol(s)	Backcross to C57BL/6J (homo lethal)
Protocol Date	8/27/10

MMRRC# 16128 PCR Reaction

NOTE: Run two, 2 primer reactions using the following primer pairs: (WT F/R) and (MUTANT F/R). Run separately.

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 45 sec
 Step 3: 55°C(WT) or 59°C(MUT) for 45 sec
 Step 4: 72°C for 1 min
 Step 5: Repeat steps 2-4, 34 cycles
 Step 6: 72°C for 10 min
 Step 7: 4°C hold

	<u>1X</u>
DNA	1.0
5X Chromataq Buffer	5.0
10mM dNTPs	0.5
20 uM Primer F/R	1.0
Taq	1.0
ddH ₂ O	14.5
25mM MgCl ₂	2.0

Taq: **Apex and Chromataq buffer**

Bands: MUTANT: ~300bp
 WT: 554bp

Primer sequences 5' to 3': Primers are 10uM with respect to each primer (20uM total).

WT F: GAA GCA AGG GAA GAG TTG AGA G

WT R: TTC ACT CTC AGA GGT AGC

MUTANT F: GTT TCC ACC CAA TGT CGA GCA AAC

MUTANT R: TTC AGT GAC AAC GTC GAG CAC

Run on 2.0% agarose gel in TAE.