

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	11169
<b>MMRRC Strain Name</b>	B6;SJL- <i>Nos2</i> <sup>+</sup> /Mmnc
<b>Gene Name(s)</b>	Nitric oxide synthase 2, inducible, macrophage/wild type ( <i>Nos2</i> <sup>+</sup> )
<b>Breeding Protocol(s)</b>	Sib-mating (Wild-type control)
<b>Protocol Date</b>	3/17/14

### MMRRC #11170 PCR Reactions

**Note: This strain (*iNOS*<sup>+/+</sup>; *hAPP*<sup>+/-</sup>; *hPSEN1*<sup>+/-</sup>) requires to genotype *iNOS*, *hAPP* and *hPSEN1*. The reagent mix, thermal cyclers and Taq are the same for the 3-allele genotyping.**

	<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: 62°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**

### iNOS Reaction

**Primer sequences 5' to 3'**: Primers are 10  $\mu$ M with respect to each primer.

NOS-A (11169-71): ATC AGC CTT TCT CTG TCT CC

NOS-B (11169-71): GGC TTT CTG TCT GTT CTC TC

TM-NOSF (11169): ATCAGCCTTTCTCTGTCTCCTGAATCCC

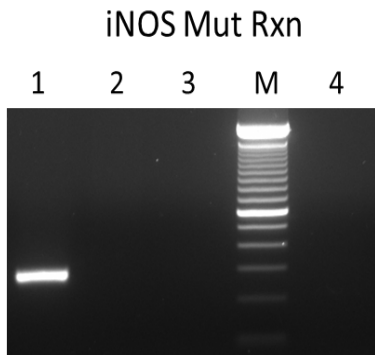
TM-NOSR-C (11169): GCCTGAAGAACGAGATCAGCAGCCT

**Bands expected:** WT: NOS-A + NOS-B: 413 bp

iNOS<sup>-/-</sup>: no band

iNOS mutant: TM-NOSF (11169) TM-NOSR-C (11169): 268 bp

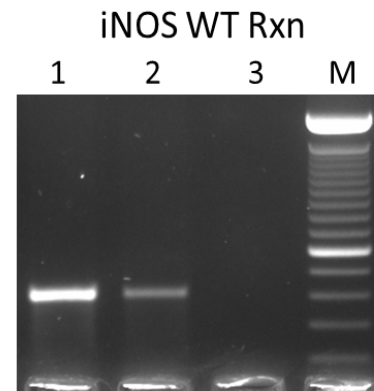
Run on 1% agarose gel in TAE.



Primers: TM-NOSF (11169) + TM-NOSR-C (11169)

Lane 1: Sperm CS#121414; Lane 2: B6; Lane 3: H2O

Lane 4: Sperm CS# 121222; M: 100 bp DNA ladder (Invitrogen)



Primers: NOS-A (11169-71) + NOS-B (11169-71)

Lane 1: Sperm CS# 121222; Lane 2: B6; Lane 3: H2O

M: 100 bp DNA ladder (Invitrogen)

**Note: The following PCR reaction was used for monitoring DNA quality (DNA quality control).**

**Primer sequences 5' to 3':** Primers are 10  $\mu$ M with respect to each primer

1502 F (11169-71): GTG GAT AAC CCC TCC CCC AGC CTA GAC CA

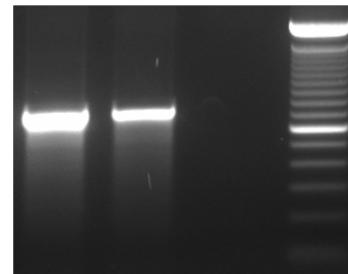
1501 R (11169-71): AAG CGG CCA AAG CCT GGA GGG TGG AAC A

**Bands expected:** 759 bp

Run on 1% agarose gel in TAE.

DNA quality control Rxn

1      2      3      M



Primers: 1502 F (11169-71) + 1501 R (11169-71)  
 Lane 1: Sperm CS# 121222; Lane 2: B6; Lane 3: H<sub>2</sub>O  
 M: 100 bp DNA ladder (Invitrogen)

### hAPP Reaction

**Primer sequences 5' to 3':** Primers are 10  $\mu$ M with respect to each primer

1502 F (11169-71): GTG GAT AAC CCC TCC CCC AGC CTA GAC CA

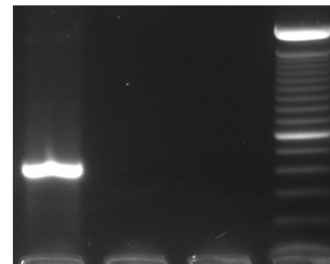
1503 R (11169-71): CTG ACC ACT CGA CCA GGT TCT GGG T

**Bands expected:** WT: no band  
 Tg+: ~400 bp

Run on 1% agarose gel in TAE.

hAPP Rxn

1      2      3      M



Primers: 1502 F (11169-71) + 1503 R (11169-71)  
 Lane 1: Sperm CS# 121222; Lane 2: B6; Lane 3: H<sub>2</sub>O  
 M: 100 bp DNA ladder (Invitrogen)

**hPS Reaction**

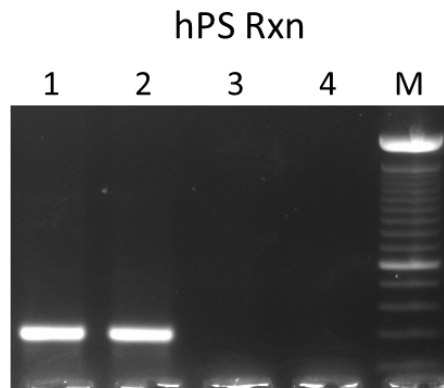
**Primer sequences 5' to 3'**: Primers are 10  $\mu$ M with respect to each primer.

TM-PS1-F (11169): GTG AAG GAA CCT TACTTCTGTGGTGTGAC

TM-PS1-R (11169): GTC CTT GGG GTC TTC TAC CTTTCTCTTCT

**Bands expected:** WT: No band  
Tg+: 300 bp

Run on 2% agarose gel in TAE.



Primers: TM-PS1-F (11169) + TM-PS1-R (11169)  
Lane 1: Human DNA; Lane 2: Sperm CS# 121222; Lane 3: B6  
Lane 4: H<sub>2</sub>O; M: 100 bp DNA ladder (Invitrogen)