

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11170
MMRRC Strain Name	B6;SJL- <i>Nos2</i> ⁺ Tg(APP _{SWE})2576Kha Tg(PSEN1)1Zhe/Mmnc
Gene Name(s)	Nitric oxide synthase 2, inducible, macrophage/wild type (<i>Nos2</i> ⁺) amyloid beta (A4) precursor protein (App Human) Presenilin 1 (PSEN1 Human)
Breeding Protocol(s)	Sib-mating mice with wild type iNOS alleles and the APP and PS1 transgenes, each of which was inherited from only one parent so as to avoid overdose.
Protocol Date	3/17/14

MMRRC #11170 PCR Reactions

Note: This strain (*iNOS*^{+/+}; *hAPP*^{+/⁰}; *hPSEN1*^{+/⁰}) requires to genotype iNOS, hAPP and hPSEN1. The reagent mix, thermal cyclers and Taq are the same for the 3-allele genotyping.

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25 mM MgCl ₂	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 μ 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

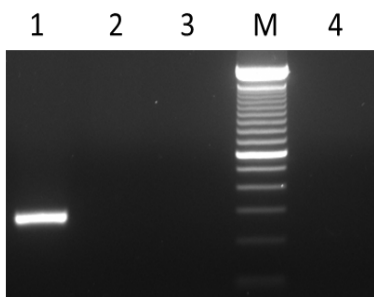
Bands expected: WT: NOS-A + NOS-B: 413 bp

iNOS^{-/-}: no band

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

Run on 1% agarose gel in TAE.

iNOS Mut Rxn

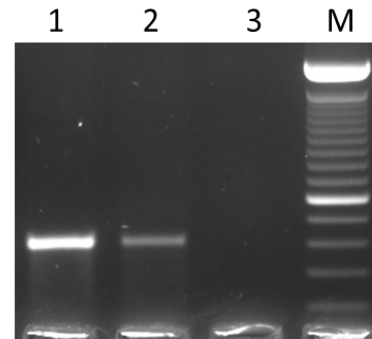


Primers: TM-NOSF (11169) + TM-NOSR-C (11169)

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

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

iNOS WT Rxn



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 μ M with respect to each primer

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

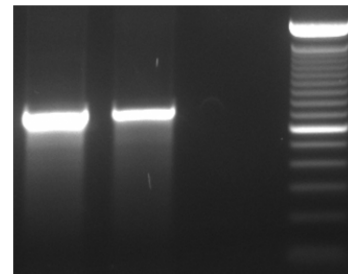
1501 R (11169-71): AAG CGG CCA AAG CCT GGA GGG TGG AAC A

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: H2O
 M: 100 bp DNA ladder (Invitrogen)

hAPP Reaction

Primer sequences 5' to 3': Primers are 10 μ M with respect to each primer

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

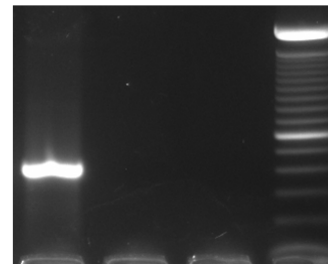
1503 R (11169-71): CTG ACC ACT CGA CCA GGT TCT GGG T

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: H2O
 M: 100 bp DNA ladder (Invitrogen)

hPS Reaction

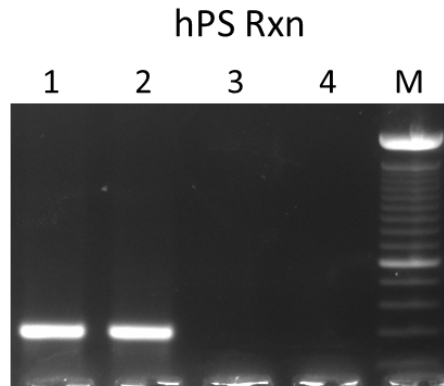
Primer sequences 5' to 3': Primers are 10 μ M with respect to each primer.

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: H₂O; M: 100 bp DNA ladder (Invitrogen)