MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11171
MMRRC Strain Name	B6;SJL-*Nos2*^{tm1Mrl} Tg(APPSWE)2576Kha Tg(PSEN1)1Zhe/Mmnc
Gene Name(s)	Nitric oxide synthase 2, inducible, macrophage/wild type (Nos2^{+}) 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.

	1X
ddH_2O	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**

The Mutant Mouse Resource and Research Center – University of North Carolina
CB# 7519, Chapel Hill, NC 27599
Phone: (919)966-4570 Fax: (919)843-1818
www.med.unc.edu/mmrrc www.mmrrc.org

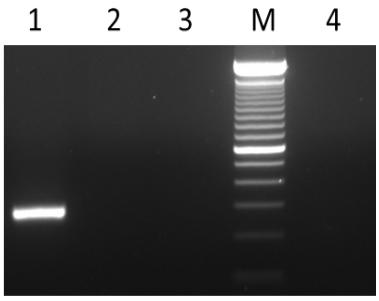
MMRRC at UNC
Mutant Mouse Resource and Research Center supported by NIH

iNOS Reaction

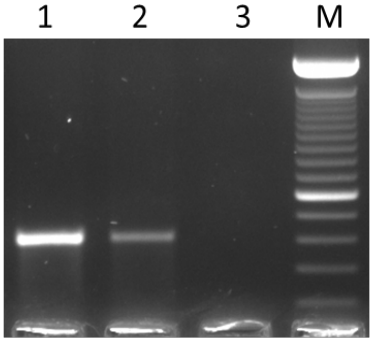
Primer sequences 5' to 3': Primers are 10 µM with respect to each primer.
NOS-A (11169-71): ATC AGC CTT TCT CTG TCT CC
NOS-B (11169-71): GGC TTT CTG TCT GTT CTC TC
TM-NOSF (11169): ATCAGCCTTTCTCTGTCTCCTGAATCCC
TM-NOSR-C (11169): GCCTGAAGAACGAGATCAGCAGCCT

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.



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)

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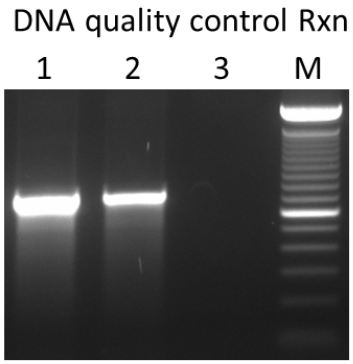
Note: The following PCR reaction was used for monitoring DNA quality (DNA quality control).

Primer sequences 5' to 3': Primers are 10 µM 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.



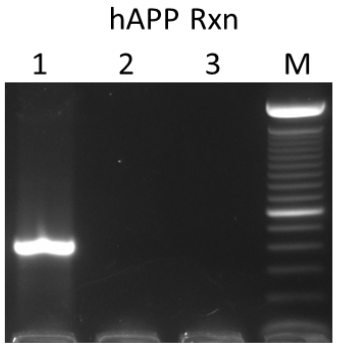
Primers: 1502 F (11169-71) + 1501 R (11169-71)
Lane 1: Sperm CS# 121222; Lane 2: B6; Lane 3: H_2O
M: 100 bp DNA ladder (Invitrogen)

hAPP Reaction

Primer sequences 5' to 3': Primers are 10 µM 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.



Primers: 1502 F (11169-71) + 1503 R (11169-71)
Lane 1: Sperm CS# 121222; Lane 2: B6; Lane 3: H_2O
M: 100 bp DNA ladder (Invitrogen)

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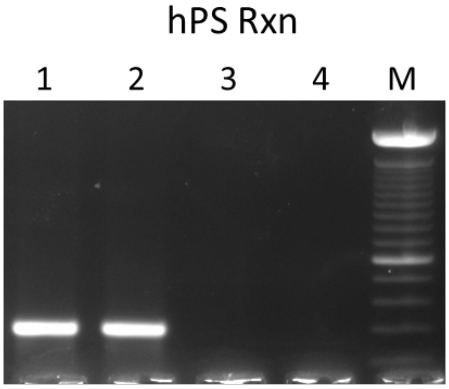
hPS Reaction

Primer sequences 5' to 3': Primers are 10 µM 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_2O; M: 100 bp DNA ladder (Invitrogen)

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