

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	30159
MMRRC Strain Name	STOCK Tg(Nelf-EGFP)FY2Gsat/Mmnc
Gene Name(s)	NMDA receptor synaptonuclear signaling and neuronal migration factor (Nsmf)
Breeding Protocol(s)	Backcross to SW/Tac
Protocol Date	9/1/11

MMRRC# 30159 PCR Reaction

Thermal Cycler:

Step 1: 94°C for 45 sec
 Step 2: 60°C for 45 sec
 Step 3: 72°C for 60 sec
 30x

Taq: **Apex and 10X Qiagen Buffer**

Bands:

MUTANT: Actin ~ 1kb (control), EGFP ~300bp
 WT: No Band

	1X
DNA	1.0
Qiagen 10X Buffer	2.0
Qiagen 25 mM MgCl ₂	0.5
Qiagen 10mM dNTPs	0.4
50 uM EGFP F/R	0.4
50 uM Actin F/R	0.8
Taq	0.2
ddH ₂ O	10.7
Qiagen Q solution	4.0

Primer sequences 5' to 3':

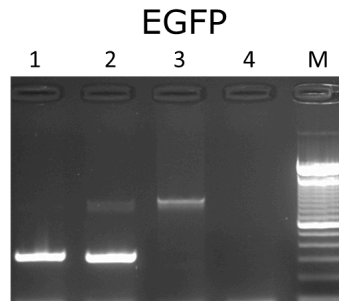
EGFP forward primer: CCT ACG GCG TGC AGT GCT TCA GC

EGFP reverse primer: CGG CGA GCT GCA CGC TGC GTC CTC

Actin PCR control/forward primer: GAT GAC GAT ATC GCT GCG CTG GTC G

Actin PCR control/reverse primer: GCC TGT GGT ACG ACC AGA GGC ATA CAG

Run on 2.0% agarose gel in TAE.



Lane 1, 2: EGFP+; Lane 3: WT; Lane 4: H₂O
 M: 100 bp DNA ladder Invitrogen