

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	232
MMRRC Strain Name	STOCK Tg(Chrm4-EGFP)Y86Gsat/Mmnc
Gene Name(s)	Cholinergic receptor, muscarinic 4 (Chrm4), Enhanced Green Fluorescent Protein (EGFP-Jelly Fish), transgene insertion Y86
Breeding Protocol(s)	Backcross to Swiss Webster (Taconic)
Protocol Date	12/7/11

MMRRC# 232 PCR Reaction

	<u>1X</u>
DNA	2.0
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	<u>10.7</u>
Qiagen Q solution	4.0

Thermal Cycler:

94°C for 45 sec

60°C for 45 sec

72°C for 60 sec

30x

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

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

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.