

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	234
MMRRC Strain Name	STOCK Tg(Pax6-EGFP)BV100Gsat/Mmnc
Gene Name(s)	Paired box gene 6 (Pax6)
Breeding Protocol(s)	Outcross to Swiss Webster (Taconic)
Protocol Date	11/18/13

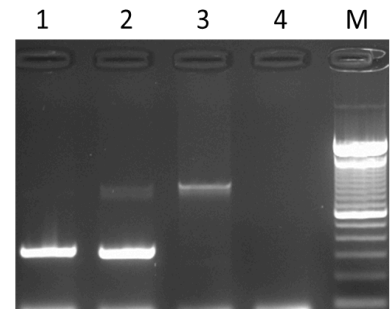
MMRRC# 234 PCR Reaction

	<u>1X</u>
DNA	1.0
Qiagen 10X Buffer	2.0
Qiagen 25 mM MgCl ₂	0.5
Qiagen 10mM dNTPs	0.4
100 uM EGFP F/R	0.4
100 uM Actin F?R	0.8
Taq	0.2
ddH ₂ O	10.7
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 Qiagen 10X Buffer** **EGFP**



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

Primers are 50uM with respect to each primer (100uM total).

Primer sequences 5' to 3':

EGFP Forward: 5'- CCT ACG GCG TGC AGT GCT TCA GC

EGFP Reverse: 5'- 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

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

Run on 2.0% agarose gel in TAE.