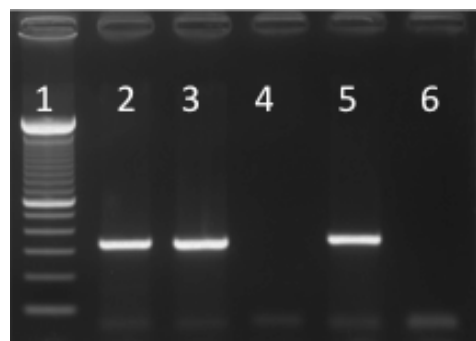


MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	293
MMRRC Strain Name	STOCK Tg(Fezf2-EGFP)CO61Gsat/Mmnc
Gene Name(s)	Fez family zinc finger 2 (Fezf2)
Breeding Protocol(s)	Outcross to Swiss Webster (Taconic)
Protocol Date	3/3/15

MMRRC# 293 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



1. 100bp Marker
2. Mutant Sample
3. Mutant Sample
4. Wild-type Control
5. Positive Control
6. Negative Control

Thermal Cycler:

94°C for 45 sec
 60 for 45 sec
 72°C for 60 sec
 30x

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

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

Primer sequences 5' to 3': Primers are 50uM with respect to each primer (100uM total).

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

Run on 2.0% agarose gel in TAE.