

MMRRC UNC – Genotyping Protocol

| | |
|-----------------------------|---------------------------------|
| MMRRC Strain ID | 12033 |
| MMRRC Strain Name | B6.Cg-Tg(Fos/EGFP)4-1Brth/Mmnc |
| Gene Name(s) | FBJ osetosarcoma oncogene (Fos) |
| Breeding Protocol(s) | Backcross to C57BL/6J |
| Protocol Date | 10/28/13 |

MMRRC# 12033 PCR Reaction

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

| | |
|--------------------------------|------------------|
| | <u>1X</u> |
| 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 | <u>10.7</u> |
| Qiagen Q solution | 4.0 |

Primer sequences 5' to 3':

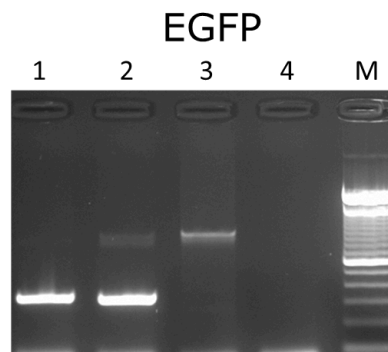
EGFP forward primer: CCT ACG GCG TGC AGT GCT TCA GC

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