

**GENOTYPING BY PCR PROTOCOL**  
**MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS**  
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 530-754-MMRRC

NAME OF PCR: STOCK Tg(EII3-EGFP)OS49Gsat/Mmcd MMRRC # 034341-UCD

**Protocol:**

| Reagent/ Constituent                                                                                                              | Volume (μL) |
|-----------------------------------------------------------------------------------------------------------------------------------|-------------|
| Water                                                                                                                             | 11.275      |
| 10x Buffer                                                                                                                        | 2.5         |
| MgCl <sub>2</sub> (stock concentration is 25mM)                                                                                   | 1.7         |
| Betaine (stock concentration is 5M)                                                                                               | 6.5         |
| dNTPs (stock concentration is 10mM)                                                                                               | 0.5         |
| DMSO                                                                                                                              | 0.325       |
| Primer 1 (stock concentration is 20μM)                                                                                            | 0.5         |
| Primer 2 (stock concentration is 20μM)                                                                                            | 0.5         |
| Taq Polymerase (5Units/μL)                                                                                                        | 0.2         |
| DNA extracted with <input type="checkbox"/> NaOH <input checked="" type="checkbox"/> Proteinase K <input type="checkbox"/> Other: | 1.0         |
| TOTAL VOLUME OF REACTION:                                                                                                         | 25μL        |

**Comments on protocol:**

- Use Touch-Down cycling protocol-first 10 cycles anneal at 65° C decreasing in temperature by 1.0° C; next 30 cycles anneal at 55° C.
- Betaine/DMSO is standardized due to high GC content in promoter regions and protocol may be tested without. Also, may adjust MgCl<sub>2</sub> to increase reaction or decrease non specific amplifications.

**Strategy:**

| Steps                                                     | Temp (°C)             | Time (m:ss) | # of Cycles |
|-----------------------------------------------------------|-----------------------|-------------|-------------|
| 1. Initiation/Melting HOT START? <input type="checkbox"/> | 94                    | 5:00        | 1           |
| 2. Denaturation                                           | 94                    | 0:15        | } 40x       |
| 3. Annealing } steps 2-3-4 will cycle in sequence         | 65 to 55 (↓1°C/cycle) | 0:30        |             |
| 4. Elongation                                             | 72                    | 0:40        |             |
| 5. Amplification                                          | 72                    | 5:00        | 1           |
| 6. Finish                                                 | 4                     | Hold        | n/a         |

**Primers:**

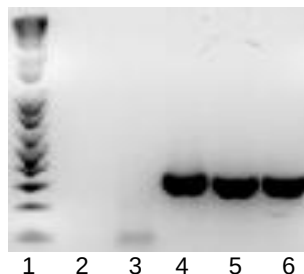
| Name              | Nucleotide Sequence (5' - 3') |
|-------------------|-------------------------------|
| 1: EII3 (34341) F | CACAGCAGAGGTGGCCCTCAC         |
| 2: GS eGFP R3     | GGTCGGGGTAGCGGCTGAA           |

**Electrophoresis Protocol:**

% Agarose: 1.5 V: 90

Estimated Running Time (min): 90

| Primer Combination                 | Band   | Genotype   |
|------------------------------------|--------|------------|
| 1 and 2                            | 325 bp | transgenic |
| <b>Tg copy # ~ 8 copies/genome</b> |        |            |



Lanes  
 1: 1kb+ ladder (Invitrogen, Cat. #10787-026)  
 2: ntc  
 3: wild-type & eGFP  
 4-6: EII3 +