

**GENOTYPING BY PCR PROTOCOL**  
**MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS**  
 2795 2nd Street, Suite 400, Davis, CA 95618  
[mmrrc@ucdavis.edu](mailto:mmrrc@ucdavis.edu)  
 530-754-MMRRC

NAME OF PCR: STOCK Tg(6030405A18Rik-EGFP)JP93Gsat/Mmcd MMRRC # 030515-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. 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                                              | 65 to 55 (↓1°C/cycle) | 0:30        |             |
| 4. Elongation                                             |                       | 0:40        |             |
| 5. Amplification                                          | 72                    | 5:00        | 1           |
| 6. Finish                                                 | 4                     | Hold        | n/a         |

**Primers:**

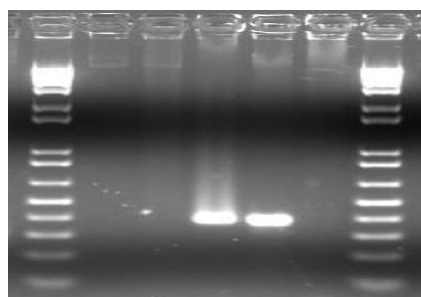
| Name                        | Nucleotide Sequence (5' - 3') |
|-----------------------------|-------------------------------|
| 1. 6030405A18Rik (30515) F1 | CTGTCATGATCGAGCAATGTCAGTT     |
| 2. GFP R2                   | TAGCGGCTGAAGCACTGCA           |

**Electrophoresis Protocol:**

Agarose: 1.5% V: 90

Estimated Running Time: 90 min.

| Primer Combination | Band   | Genotype   |
|--------------------|--------|------------|
| 1 and 2            | 400 bp | transgenic |



Lanes  
 1 & 7. 1 kb+ ladder  
 (Invitrogen, Cat. #10787-026)  
 2. Non-template control  
 3. Wild-type Control  
 4 & 5. 6030405A18Rik tg/+  
 6. Other GENSAT line

1 2 3 4 5 6 7