

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

NAME OF PCR: STOCK Tg(Chrna4-EGFP)ME19Gsat/Mmcd MMRRC # 032061-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          |
| <b>TOTAL VOLUME OF REACTION:</b>                                                                                                  | <b>25.00</b> |

**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:**

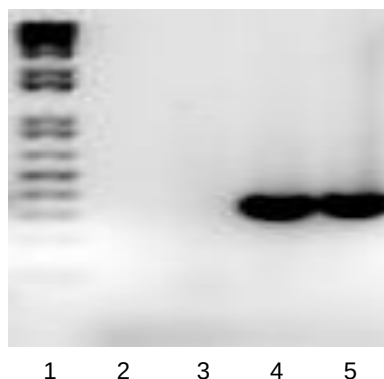
| Name                | Nucleotide Sequence (5' - 3') |
|---------------------|-------------------------------|
| 1. Chrna4 (32061) F | CGACACGGGGCATGAAGTTG          |
| 2. GS eGFP R3       | GGTCGGGGTAGCGGCTGAA           |

**Electrophoresis Protocol:**

Agarose: 1.5% V: 90

Estimated Running Time: 90 min.

| Primer Combination                  | Band   | Genotype   |
|-------------------------------------|--------|------------|
| 1 and 2                             | 350 bp | transgenic |
| <b>Tg copy # ~ 12 copies/genome</b> |        |            |



Lanes

1. 1 kb+ ladder (Invitrogen, Cat. #10787-026)
2. Non-Template Control
3. Wild-type & eGFP
- 4 & 5. Chrna4 tg/+