

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

NAME OF PCR: STOCK Tg(Ngf-EGFP)KI30Gsat/Mmcd MMRRC # 034399-UCD

Protocol:

| Reagent/ Constituent | Volume (µL) |
|---|-------------|
| Water | 11.275 |
| 10x Buffer | 2.5 |
| MgCl ₂ (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 MgCl₂ 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:

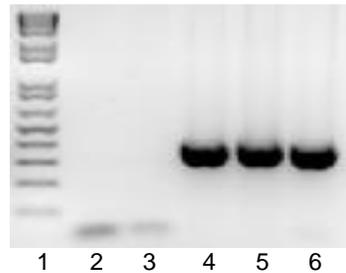
| Name | Nucleotide Sequence (5' - 3') |
|------------------|-------------------------------|
| 1: Ngf (34399) F | GCTCATCAAATGCACTGTCAAACCTTC |
| 2: GS eGFP R3 | GGTCGGGGTAGCGGCTGAA |

Electrophoresis

Agarose: 1.5% V: 90

Estimated Running Time: 90 min.

| Primer Combination | Band | Genotype |
|-------------------------------------|--------|------------|
| 1 and 2 | 350 bp | transgenic |
| Tg copy # ~ 32 copies/genome | | |



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