

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: STOCK Tg(Prkcd-glc-1/CFP,-cre)EH124Gsat/Mmucd MMRRC # 011559-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 | 65 to 55 (↓1°C/cycle) | 0:30 | |
| 4. Elongation | | 0:40 | |
| 5. Amplification | 72 | 5:00 | 1 |
| 6. Finish | 4 | ∞ | n/a |

Primers:

| Name | Nucleotide Sequence (5' - 3') |
|--------------|-------------------------------|
| 1: 11559-tgF | GCTACATCAAGGCCATCGAC |
| 2: 11559-tgR | AACTCCAGCAGGACCATGTGATCG |

Electrophoresis Protocol:

Agarose: 1.5% V: 90

Estimated Running Time: 90 min.

| Primer Combination | Band | Genotype |
|--------------------|------|------------|
| 1 and 2 | ~1kb | transgenic |

