

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

Protocol Name: B6;129S5-Gpc4^{tm1Lex}/Mmucd MMRRC: 032331-UCD

Protocol:

| Reagent/Constituent | Volume (µL) |
|---|------------------|
| Water | 10.275 |
| 10x Buffer | 2.5 |
| MgCl ₂ (stock concentration is 25mM) | 1.7 |
| Betaine (stock concentration is 5M) <i>Optional</i> | 6.5 |
| dNTPs (stock concentration is 10mM) | 0.5 |
| DMSO <i>Optional</i> | 0.325 |
| Primer 1. (stock concentration is 20µM) | 0.5 |
| Primer 2. (stock concentration is 20µM) | 0.5 |
| Primer 3. (stock concentration is 20µM) | 0.5 |
| Primer 4. (stock concentration is 20µM) | 0.5 |
| Taq Polymerase 5Units/µL | 0.2 |
| DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits" | 1.0 |
| TOTAL VOLUME OF REACTION: | 25.000 µL |

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- 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 and DMSO have been standardized due to high GC content. 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 | |
| 3. Annealing steps 2-3-4 cycle in sequence | 65 to 55 (↓1°C/cycle) | 0:30 | 40x |
| 4. Elongation | 72 | 0:40 | |
| 5. Amplification | 72 | 5:00 | 1 |
| 6. Finish | 15 | ∞ | n/a |

Primers:

Electrophoresis Protocol:

| Name | Nucleotide Sequence (5' - 3') | Agarose: 1.5% | V: 90 |
|-------------|-------------------------------|----------------------------------|------------------|
| 1. DNA078-1 | GTTCGTGAAGACATATGGCC | Estimated Running: Time: 90 min. | |
| 2. DNA078-2 | CATCCCTTGCAACTGCTAAG | Primer Combination | Band (bp) |
| 3. Neo3a | GCAGCGCATCGCCTTCTATC | 1 & 2 | 323 |
| 4. DNA078-4 | CTTAAGAATGGCTACCACGG | 3 & 4 | 403 |
| | | | Genotype |
| | | | Wildtype |
| | | | mutant |