

GENOTYPING BY PCR PROTOCOL

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530-754-MMRRC

Protocol Name: B6;129S5-Nrg4^{Gt(OST98495)Lex}/Mmucd MMRRC: 011746-UCD

Protocol:

| Reagent/Constituent | Volume (µL) |
|---|-------------|
| Water | 10.775 |
| 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 |
| Taq Polymerase 5Units/µL | 0.2 |
| DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits" | 1.0 |
| TOTAL VOLUME | |
| | 24.5 |

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') | Argarose: 1.5% V: 90 | | |
|-------------------|-------------------------------|----------------------|------------------|-----------------|
| 1. 11746-R | ACAAGACTAGGAAGTGCAGAATAGAC | Estimated 9 min. | | |
| 2. 11746-MutF3 | CCAAACCTACAGGTGGGGTCTTTC | Primer | Band (bp) | Genotype |
| 3. Primer 0933-5' | GGTTACCTTTGGAATGAATGAATGCC | 1 & 2 | 228 | mutant |
| | | 1 & 3 | 285 | wildtype |

