

GENOTYPING BY PCR PROTOCOL

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Protocol Name: B6J.129S4-Kif20atm1c(EUCOMM)Hmgu/QuMmucd MMRRC: 050513-UCD

Protocol:

| Reagent/Constituent | Volume (µL) |
|---|-------------|
| Water | 5.6 |
| GoTaq® G2 Colorless Master Mix,2X | 7.5 |
| Primer 1. (stock concentration is 20µM) | 0.45 |
| Primer 2. (stock concentration is 20µM) | 0.45 |
| DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits" | 1.0 |
| TOTAL VOLUME | |
| 15 | |

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.

Strategy:

| Steps | Temp (°C) | Time (m:ss) | # of Cycles |
|--|-----------------|-------------|-------------|
| 1. Initiation/Melting HOT START? <input type="checkbox"/> | 94 | 5:00 | 1x |
| 2. Denaturation | 94 | 0:15 | |
| 3. Annealing steps 2-3-4 cycle in sequence | 65 (↓1°C/cycle) | 0:30 | 10x |
| 4. Elongation | 72 | 0:40 | |
| 5. Denaturation | 94 | 0:15 | |
| 6. Annealing steps 5-6-7 cycle in sequence | 55 | 0:30 | 30X |
| 7. Elongation | 72 | 0:40 | |

Primers:

Electrophoresis Protocol:

| Name | Nucleotide Sequence (5' - 3') | Argarose: 1.5% | V: 90 | | |
|------------|-------------------------------|---------------------------------|------------------|-----------------|--|
| 1. 50513-F | TGTGGGTTCTTTACGTGTCTGT | Estimated Running:Time: 90 min. | | | |
| 2. 50513-R | GTTGGGTGTAAGCATGGGTTTG | Primer Combination | Band (bp) | Genotype | |
| | | 1 & 2 | 460 | mutant | |
| | | 1 & 2 | 212 | wildtype | |

