

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6J.129S1(129X1)-Ryr1tm1.1lnp/Mmucd/Mmucd MMRRC: 042038-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 | |
| | |

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 | 2:00 | 1x |
| 2. Denaturation | 94 | 0:10 | |
| 3. Annealing steps 2-3-4 cycle in sequence | 65 (↓1°C/cycle) | 0:30 | |
| 4. Elongation | 68 | 2:00 | |
| | | | Go to 2 (9x) |
| 5. Denaturation | 94 | 0:15 | |
| 6. Annealing steps 5-6-7 cycle in sequence | 55 | 0:30 | |
| 7. Elongation | 68 | 2:00 (+20s/cycle) | |
| | | | Go to 5 (24x) |

Primers:

Electrophoresis Protocol:

| Name | Nucleotide Sequence (5' - 3') | Argarose: 1.5% | V: 90 | |
|-----------------|-------------------------------|---------------------------------|------------------|-----------------|
| 1. 42036.7-F | TGATTCTCCTCACTCTCACTGTCCATAG | Estimated Running:Time: 90 min. | | |
| 2. 42036.7-mutR | GGCTCGAAGTAGCCAATGAG | Primer Combination | Band (bp) | Genotype |
| | | 1 & 2 | ~550 | mutant |
| | | 1 & 2 | 1748 | wildtype |

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