

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

**Protocol Name:** FVB/NCrl-Rr43em1Axvi/Mmucd **MMRRC: 043913-UCD**

**Protocol:**

| Reagent/Constituent                                                                   | Volume (μL) |
|---------------------------------------------------------------------------------------|-------------|
| Water                                                                                 | 5.15        |
| GoTaq® G2 Colorless Master Mix, 2X                                                    | 7.5         |
| Primer 1. (stock concentration is 20μM)                                               | 0.45        |
| Primer 2. (stock concentration is 20μM)                                               | 0.45        |
| Primer 3. (stock concentration is 20μM)                                               | 0.45        |
| DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits" | 1.0         |
| <b>TOTAL VOLUME</b>                                                                   |             |
|                                                                                       |             |

**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. 43913-comF | CTTGGCATACCATTAGAAATGGAGAGCC  | Estimated Running Time: 90 min. |                  |                 |
| 2. 43913-mutR | GGCCAATACCAGAACTTCACCCTAAT    | <b>Primer Combination</b>       | <b>Band (bp)</b> | <b>Genotype</b> |
| 3. 43913-wtR  | CGGAAGTTGAAAATCCACAGAGAATGCT  | 1 & 2                           | 249              | mutant          |
|               |                               | 1 & 3                           | 169              | wildtype        |
|               |                               |                                 |                  |                 |

