

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

**Protocol Name:** C57BL/6N-Daoem1Ament/Mmucd **MMRRC: 067164-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         |
| <b>TOTAL VOLUME</b>                                                                   |             |
| 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.
- The mutant PCR is a general LacZ PCR. The wild type is specific for this strain.

**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                | 10x         |
| 4. Elongation                                             | 68              | 2:00                |             |
| 5. Denaturation                                           | 94              | 0:15                |             |
| 6. Annealing steps 5-6-7 cycle in sequence                | 55              | 0:30                | 25x         |
| 7. Elongation                                             | 68              | 2:00 (↑20sec/cycle) |             |

**Primers:**

**Electrophoresis Protocol:**

| Name       | Nucleotide Sequence (5' - 3') | Argarose: 1.5% V: 90            |                  |                 |
|------------|-------------------------------|---------------------------------|------------------|-----------------|
| 1. 67164-F | GCTAAACCACTCTCAGATGAGGAAACG   | Estimated Running Time: 90 min. |                  |                 |
| 2. 67164-R | GGCACCCCCCAAGGTCTTCT          | <b>Primer Combination</b>       | <b>Band (bp)</b> | <b>Genotype</b> |
|            |                               | 1 & 2                           | 491              | wildtype        |
|            |                               | Tail digest                     | 330, 161         | mutant          |

**Digest PCR with Tail**

**Alternative**

Sequence with primer 1

Wt TACGGC

Mut TACGTC

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