

**GENOTYPING BY PCR PROTOCOL**  
**MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS**  
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 530-754-MMRRC

**NAME OF PCR:** C57BL/6-Card11<sup>unm</sup>/Mmcd (Unmodulated) **MMRRC #** 011120-UCD

**Protocol:**

| Reagent/ Constituent                            | Volume (μL) |
|-------------------------------------------------|-------------|
| Water                                           | 29.65       |
| 10x Buffer (without 15mM MgCl <sub>2</sub> )    | 5.0         |
| MgCl <sub>2</sub> (stock concentration is 25mM) | 7.0         |
| dNTPs (stock concentration is 2mM)              | 0.5         |
| Primer 1 (stock concentration is 20μM) RevMut   | 1.0         |
| Primer 2 (stock concentration is 20μM) Forward  | 5.0         |
| Primer 3 (stock concentration is 20μM) RevWT    | 0.75        |
| Taq Polymerase 5 Units/μL                       | 0.1         |
| DNA Sample                                      | 1.0         |
| <b>TOTAL VOLUME OF REACTION:</b>                | <b>50μL</b> |

**Comments on protocol:**

MgCl<sub>2</sub> concentration is as given above; each new primer set needs to be titrated as we have had variable results with each new batch of primers.

**Strategy:**

| Steps                                                     | Temp (°C ) | Time (m:ss) | # of Cycles |
|-----------------------------------------------------------|------------|-------------|-------------|
| 1. Initiation/Melting HOT START? <input type="checkbox"/> | 94         | 2:00        | 1           |
| 2. Denaturation                                           | 94         | 0:20        | } 35x       |
| 3. Annealing } steps 2-3-4 will cycle in sequence         | 65         | 0:20        |             |
| 4. Elongation                                             | 72         | 0:30        |             |
| 5. Amplification                                          | 72         | 5:00        | 1           |
| 6. Finish                                                 | 4          | ∞           | n/a         |

**Primers:**

| Primer Name | Nucleotide Sequence (5' - 3')                    |
|-------------|--------------------------------------------------|
| 1: RevMut   | TTGACCAGTTCCTGCCGGCCCTTAGCGCCTCCTTCCGGTCATGTGCCT |
| 2: Forward  | TTCCGCCACGAAATCTTCTGC                            |
| 3: RevWT    | TCTAGCGCCTCCTTCCGGTCATGTTACA                     |

**Electrophoresis Protocol:**

% Agarose: 3 mV: 150

Estimated Running Time (min): \_\_\_\_\_

| Expected Bands | Genotype |
|----------------|----------|
| 190 bp         | MT       |
| 190 / 169 bp   | Het +/-  |
| 169 bp         | WT +/+   |