

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

[mmrrc@ucdavis.edu](mailto:mmrrc@ucdavis.edu)

530-754-MMRRC

Protocol Name: B6N.129-Wisp1<sup>tm1Mfy</sup>/Mmucd

MMRRC: 041571-UCD

**Protocol:**

| Reagent/Constituent   | Volume (µL) |
|---|-------------|
| Water   | 10.275      |
| 10x Buffer  | 2.5         |
| MgCl <sub>2</sub> (stock concentration is 25mM)                                       | 1.7         |
| Betaine (stock concentration is 5M) <i>Optional</i>                                   | 6.5         |
| dNTPs (stock concentration is 10mM)   | 0.5         |
| DMSO <i>Optional</i>  | 0.325       |
| Primer 1. (stock concentration is 20µM)   | 0.5         |
| Primer 2. (stock concentration is 20µM)   | 0.5         |
| Primer 3. (stock concentration is 20µM)   | 0.5         |
| Taq Polymerase 5Units/µL  | 0.2         |
| DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits" | 1.0         |
| <b>TOTAL VOLUME</b>   |             |
| <b>24.5</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.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust MgCl<sub>2</sub> to increase reaction or decrease non-specific amplifications.

**Strategy:**

| Steps  | Temp (°C)             | Time (m:ss) | # of Cycles |
|--|-----------------------|-------------|-------------|
| 1. Initiation/Melting <span style="float: right;">HOT START? <input type="checkbox"/></span> | 94                    | 5:00        | 1           |
| 2. Denaturation  | 94                    | 0:15        |             |
| 3. Annealing <span style="float: right;">steps 2-3-4 cycle in sequence</span>                | 65 to 55 (↓1°C/cycle) | 0:30        | <b>40x</b>  |
| 4. Elongation  | 72                    | 0:40        |             |
| 5. Amplification   | 72                    | 5:00        | 1           |
| 6. Finish  | 15                    | ∞           | n/a         |

**Primers:**

**Electrophoresis Protocol:**

| Name          | Nucleotide Sequence (5' - 3') | Argarose: 1.5%    V: 90 |                  |                 |
|---------------|-------------------------------|-------------------------|------------------|-----------------|
| 1. 41571 comF | ACCCCCACAACAATGACCT           | Estimated 9 min.        |                  |                 |
| 2. 41571 mutR | GTGCTTTACGGTATCGCCGCT         | <b>Primer</b>           | <b>Band (bp)</b> | <b>Genotype</b> |
| 3. 41571 wtR  | AGCTGCTGGGCACATATCTT          | 1 & 2                   | 400              | mutant          |
|               |                               | 1 & 3                   | 150              | wildtype        |

