

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

**Protocol Name:** C57BL/6N-A<sup>tm1Brd</sup>/a Tmem161b<sup>tm2a(EUCOMM)Hmgu</sup>/BcmMmucd **MMRRC: 041541-UCD**

**Protocol:**

| Reagent/Constituent                                                                   | Volume (μL)      |
|---------------------------------------------------------------------------------------|------------------|
| Water                                                                                 | 11.275           |
| 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              |
| Taq Polymerase 5Units/μL                                                              | 0.2              |
| DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits" | 1.0              |
| <b>TOTAL VOLUME OF REACTION:</b>                                                      | <b>25.000 μL</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        | <b>1</b>    |
| 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        | <b>1</b>    |
| 6. Finish                                                                                    | 15                    | ∞           | n/a         |

**Primers:**

**Electrophoresis Protocol:**

| Name          | Nucleotide Sequence (5' - 3') | Argarose: 1.5% V: 90            |                  |                   |
|---------------|-------------------------------|---------------------------------|------------------|-------------------|
| 1. 41541-lacF | GCTACCATTACCAGTTGGTCTGGTGTC   | Estimated Running Time: 90 min. |                  |                   |
| 2. 41541-neoF | GGGATCTCATGCTGGAGTTCTTCG      | <b>Primer Combination</b>       | <b>Band (bp)</b> | <b>Genotype</b>   |
| 3. 41541-loxF | GAGATGGCGCAACGCAATTAATG       | 3 & 5                           | 349              | floxed            |
| 4. 41541-TTR  | TAGTATCCTGTTTCACACCCATTCTTCC  | 2 & 4                           | 592              | PreCre            |
| 5. 41541-R    | AGGAACACCACTTCAGACAGCTTCC     | 1 & 5                           | 647              | PostCre           |
| 6. 41541-F    | ATGTGACATATTGTACGGAGAGCC      | 6 & 4                           | 425              | Wildtype          |
|               |                               | 6 & 4                           | 529              | PostFlp           |
|               |                               | 6 & 5                           | 633              | PostFlp & PostCre |

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