

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

**Protocol Name:** C57BL/6N-Atm1Brd Cyb561d2tm1a(EUCOMM)Wtsi/BayMmucd **MMRRC:** 041554-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        | 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        | 40x         |
| 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. 41554-lacF | GCTACCATTACCAGTTGGTCTGGTGTC   | Estimated Running Time: 90 min. |                  |                   |
| 2. 41554-neoF | GGGATCTCATGCTGGAGTTCTTCG      | <b>Primer Combination</b>       | <b>Band (bp)</b> | <b>Genotype</b>   |
| 3. 41554-loxF | GAGATGGCGCAACGCAATTAATG       | 3 & 5                           | 318              | floxed            |
| 4. 41554-TTR  | CAGGATACAATTTCTTGCAAGAGC      | 2 & 4                           | 597              | PreCre            |
| 5. 41554-SR2  | AATAAAGCGGTTCTTTGAGGTCTGGC    | 1 & 5                           | 616              | PostCre           |
| 6. 41554-F    | CCAGTGTAGACGAGCTGTATGTCC      | 6 & 4                           | 260              | Wildtype          |
|               |                               | 6 & 4                           | 464              | PostFlp           |
|               |                               | 6 & 5                           | 532              | PostFlp & PostCre |

Please note, these primers are auto-designed and may not have been verified by the repository, and as such may require optimization or redesign by your facility.

We recommend running primers singleplex. For screening of pups prior to any Flp or Cre recombination, the Floxed or PreCre primers may be used to identify the mutant mice. The Floxed primers test for the distal LoxP site. The PostCre primers should be used if mutant mice were crossed with a Cre recombinase line (without any FLP recombination). The PostFlp primers should be used if mutant mice were crossed with a Flp recombinase line. The PostFlp & Cre primers should be used if mutant mice were crossed with a Flp recombinase line and then a Cre recombinase line. The wildtype primers should be used for zygosity testing of all mutant mice (pre or post recombination).

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