

# GENOTYPING PROTOCOL

UC Davis Mouse Biology Program

Protocol Name: FVB/NJ-Zfp423em4Haml/Mmucd MMRRC: 051016

Protocol: GoTaq® G2 Colorless Master Mix(Promega)

| Reagent/Constituent                                                                   | Volume (µL)     |
|---------------------------------------------------------------------------------------|-----------------|
| Water                                                                                 | 4.7             |
| GoTaq® G2 Colorless Master Mix, 2X                                                    | 7.5             |
| Primer 1. (stock concentration is 20µM)                                               | 0.45            |
| Primer 2. (stock concentration is 20µM)                                               | 0.45            |
| Primer 3. (stock concentration is 20µM)                                               | 0.45            |
| Primer 4. (stock concentration is 20µM)                                               | 0.45            |
| DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits" | 1.0             |
| <b>TOTAL VOLUME OF REACTION:</b>                                                      | <b>15.00 µL</b> |

## Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Example is run with all 4 primers. It's suggest that the wild type (1, 2, 4) and mutant (1, 2, 3) reactions be run separately.

## Strategy:

| Steps                                                     | Temp (°C )      | Time (m:ss) | # of Cycles |
|-----------------------------------------------------------|-----------------|-------------|-------------|
| 1. Initiation/Melting HOT START? <input type="checkbox"/> | 94              | 5:00        | <b>1x</b>   |
| 2. Denaturation                                           | 94              | 0:15        |             |
| 3. Annealing steps 2-3-4 cycle in sequence                | 65 (↓1°C/cycle) | 0:30        | <b>10x</b>  |
| 4. Elongation                                             | 72              | 0:40        |             |
| 5. Denaturation                                           | 94              | 0:15        |             |
| 6. Annealing steps 5-6-7 cycle in sequence                | 55              | 0:30        | <b>30X</b>  |
| 7. Elongation                                             | 72              | 0:40        |             |
| 8. Finish                                                 | 4               | ∞           | n/a         |

## Primers:

## Electrophoresis Protocol:

| Name          | Nucleotide Sequence (5' - 3') | Agarose: 1.5%             | :                | 90              |
|---------------|-------------------------------|---------------------------|------------------|-----------------|
| 1. 51016-comF | CTCGAGCACCTCGCATTT            | Estimated Running 90 min. |                  |                 |
| 2. 51016-comR | GCCTCATGGCCAAATCATT           | <b>Primers</b>            | <b>Band (bp)</b> | <b>Genotype</b> |
| 3. 51016-mutF | GGCGGGGCGGCTTTGCAGAACT        | 1 & 4                     | 163              | wildtype        |
| 4. 51016-wtR  | GGGGGCGGGGCTCATCGTGTG         | 2 & 3                     | 182              | mutant          |
|               |                               | 1 & 2                     | 302              | control         |
|               |                               |                           |                  |                 |
|               |                               |                           |                  |                 |

