

# GENOTYPING PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR1366 Cyp4v3 EXDEL

Protocol: GoTaq® G2 Colorless Master Mix(Promega)

| Reagent/Constituent   | Volume (µL)     |
|---|-----------------|
| Water   | 3.9             |
| GoTaq® G2 Colorless Master Mix,2X   | 7.5             |
| Primer 1. (stock concentration is 20µM) comF  | 0.6             |
| Primer 2. (stock concentration is 20µM) wtR   | 1.2             |
| Primer 3. (stock concentration is 20µM) mutR  | 0.3             |
| DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits" | 1.5             |
| <b>TOTAL VOLUME OF REACTION:</b>  | <b>15.00 µL</b> |

**Comments on protocol:**

- Protocol may work with other DNA extraction methods.

**Strategy:**

| Steps  | Temp (°C)       | Time (m:ss)         | # of Cycles |
|--|-----------------|---------------------|-------------|
| 1. Initiation/Melting <span style="float: right;">HOT START? <input type="checkbox"/></span> | 94              | 2:00                | <b>1x</b>   |
| 2. Denaturation  | 94              | 0:10                |             |
| 3. Annealing <span style="float: right;">steps 2-3-4 cycle in sequence</span>                | 65 (↓1°C/cycle) | 0:30                | <b>10x</b>  |
| 4. Elongation  | 68              | 2:00                |             |
| 5. Denaturation  | 94              | 0:15                |             |
| 6. Annealing <span style="float: right;">steps 5-6-7 cycle in sequence</span>                | 55              | 0:30                | <b>25x</b>  |
| 7. Elongation  | 68              | 2:00 (↑20sec/cycle) |             |
| 8. Finish  | 4               | ∞                   | n/a         |

**Primers:**

**Electrophoresis Protocol:**

| Name              | Nucleotide Sequence (5' - 3') | Agarose: 1.5%                   | V: 90            |
|-------------------|-------------------------------|---------------------------------|------------------|
| 1. CR_Cyp4v3-comF | GCATGGTAGCAGACATTTTACTGAC     | Estimated Running Time: 90 min. |                  |
| 2. CR_Cyp4v3-wtR  | GCCTCATGAATGTAATGCTCTTTGAAG   | <b>Primer Combination</b>       | <b>Band (bp)</b> |
| 3. CR_Cyp4v3-mutR | GAAGAGCATTTCACAACACGTCC       | 1 & 2, 1 & 3                    | 719,934          |
|                   |                               | 1 & 3                           | 311              |
|                   |                               |                                 | wildtype         |
|                   |                               |                                 | mutant           |

**Allele Description:** Exon 2 [ENSMUSE00000428202](#) and flanking splicing regions were constitutively deleted from the Cyp4v3 gene [ENSMUST00000095328.5](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

