

# GENOTYPING PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11682 Psmg4 EXDEL

Stock # 76003

Protocol: GoTaq® G2 Colorless Master Mix(Promega)

| Reagent/Constituent   | Volume (µL)     |
|---|-----------------|
| Water   | 4.5             |
| GoTaq® G2 Colorless Master Mix,2X   | 7.5             |
| Primer 1. (stock concentration is 20µM) comF  | 0.5             |
| Primer 2. (stock concentration is 20µM) wtR   | 0.5             |
| Primer 3. (stock concentration is 20µM) mutR  | 0.5             |
| 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:**

| Name             | Nucleotide Sequence (5' - 3') | Agarose: 1.5%                   | V: 90            |
|------------------|-------------------------------|---------------------------------|------------------|
| 1. CR_Psmg4_comF | TGTGAAATCAGTCTTAGAAACCATTAGC  | Estimated Running Time: 90 min. |                  |
| 2. CR_Psmg4_wtR* | GCTAGTGTCAAAGCATTAGCCTTGG     | <b>Primer Combination</b>       | <b>Band (bp)</b> |
| 3. CR_Psmg4_mutR | GATTGAAGGGGAGAACCAAGAAGG      | 1 & 2, 1 & 3                    | 345, 566         |
|                  |                               | 1 & 3                           | 263              |
|                  |                               |                                 | <b>Genotype</b>  |
|                  |                               |                                 | wildtype         |
|                  |                               |                                 | mutant           |

**Electrophoresis Protocol:**

**Allele Description:** Exon 2 ENSMUSE00000616641 and flanking splicing regions were constitutively deleted from the Psmg4 Gene ENSMUSG00000071451 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 303bp deletion from Chr 13: 34349936- 34350238 GRCm39 was screened by PCR analysis. The selected founder animal was backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals for establishing and archiving the line.

\*wtR primer untested (ePCR verified)

