# GENOTYPING PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

**Protocol Name:** **CR10223 Fam19a4 EXDEL**

**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 |
| **TOTAL VOLUME OF REACTION:** | **15.00 µL** |

***Comments on protocol:***

- Protocol may work with other DNA extraction methods.

**Strategy:**

| Steps | | Temp (ºC ) | Time (m:ss) | # of Cycles |
|---|---|---|---|---|
| 1. Initiation/Melting | HOT START? ☐ | 94 | 2:00 | **1x** |
| 2. Denaturation | | 94 | 0:10 | |
| 3. Annealing | steps 2-3-4 cycle in sequence | 65 (↓1ºC/cycle) | 0:30 | **10x** |
| 4. Elongation | | 68 | 2:00 | |
| 5. Denaturation | | 94 | 0:15 | |
| 6. Annealing | steps 5-6-7 cycle in sequence | 55 | 0:30 | **25x** |
| 7. Elongation | | 68 | 2:00 (↑20sec/cycle) | |
| 8. Finish | | 4 | ∞ | n/a |

**Primers:**

| Name | Nucleotide Sequence (5' - 3') |
|---|---|
| 1. CR_Fam19a4_comF | CAACCAATCAGCAATCTTAATTTCAGC |
| 2. CR_Fam19a4-wtR* | GGAAGCAGGAACATTTGACCGTC |
| 3. CR_Fam19a4_mutR | CACACACATGAGAGAGAGTCCCCTT |
| | |

**Electrophoresis Protocol:**

Agarose: **1.5%** **V:** **90**

Estimated Running Time: **90** min.

| Primer Combination | **Band** (bp) | **Genotype** |
|---|---|---|
| 1 & 2,1 & 3 | 405,638 | wildtype |
| 1 & 3 | 282 | mutant |

**Allele Description:** Exon 4 ENSMUSE00000380551 had 138 bp deleted from the 116th coding nucleotide through the 253rd coding nucleotide from the Fam19a4 gene ENSMUSG00000046500 using CRISPR Cas9 gene editing technology in mouse zygotes. Splice acceptor was removed. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

*wtR primer untested (ePCR verified)