

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR1166 Tas2r139 exdel

MMRRC: 42227-UCD

Protocol: GoTaq® Long PCR Master Mix(Promega)

| Reagent/Constituent | Volume (µL) |
|---|-----------------|
| Water | 5.6 |
| GoTaq® Long PCR 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 |
| TOTAL VOLUME OF REACTION: | 15.00 µL |

Comments on protocol:

- Protocol may work with other DNA extraction methods.

Strategy:

| Steps | Temp (°C) | Time (m:ss) | # of Cycles |
|--|-----------------|---------------------|-------------|
| 1. Initiation/Melting HOT START? <input type="checkbox"/> | 94 | 2:00 | 1x |
| 2. Denaturation | 94 | 0:10 | |
| 3. Annealing steps 2-3-4 cycle in sequence | 65 (↓1°C/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_Tas2r139-comF | AATCTAGGGAGAGGACTTGAAAGCG |
| 2. CR_Tas2r139-wtR | CAGGGCATTAAATTCAGAAATTCTCC |
| 3. CR_Tas2r139-mutR | TTCTTGTGTGTACTTCTGCATTTCAC |

Electrophoresis Protocol:

| Agarose: 1.5% | V: 90 | |
|---------------------------------|-----------|----------|
| Estimated Running Time: 90 min. | | |
| Primer Combination | Band (bp) | Genotype |
| 1 & 2, 1 & 3 | 654, 1828 | wildtype |
| 1 & 3 | 477 | mutant |

Allele Description: Exon 1 [ENSMUSE00000404314](#) and flanking splicing regions were constitutively deleted from the Tas2r139 gene [ENSMUST00000057686.4](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

