

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR1578 Mief1 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 (°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:

Electrophoresis Protocol:

| Name | Nucleotide Sequence (5' - 3') | Agarose: 1.5% | V: 90 |
|------------------|-------------------------------|---------------------------------|------------------|
| 1. CR_Mief1-comF | TAAAAAAGGAGAAAGGGCAAACC | Estimated Running Time: 90 min. | |
| 2. CR_Mief1-wtR* | AAGGCTCAAGGAGTTCCTCACC | Primer Combination | Band (bp) |
| 3. CR_Mief1-mutR | GGGCAGAATGTATCTGCCAAGG | 1 & 2, 1 & 3 | 618,799 |
| | | 1 & 3 | 465 |
| | | | wildtype |
| | | | mutant |

Allele Description: Exon 4 [ENSMUSE00000126304](#) and flanking splicing regions were constitutively deleted from the Mief1 gene [ENSMUST00000023048.10](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

*wtR primer untested (ePCR verified)

