# GENOTYPING PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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**Protocol Name:** **CR11774 Msantd4 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_Msantd4_comF | CAGTAGATTAGAGATCCTTGGGAGC |
| 2. CR_Msantd4_wtR* | CACGTTTATTGTTGTGTTGAGCTGC |
| 3. CR_Msantd4_mutR | CAATTCATTGGGTTCCACATCATGG |
| | |

**Electrophoresis Protocol:**

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

Estimated Running Time: **90** min.

| Primer Combination | **Band** (bp) | **Genotype** |
|---|---|---|
| 1 & 2,1 & 3 | 931, 3387 | wildtype |
| 1 & 3 | 871 | mutant |

**Allele Description:** Exon 2-3 (ENSMUSE00001393601, ENSMUSE00002196404) and flanking splicing regions were constitutively deleted from the 5'UTR through the 3'UTR from Msantd4 Gene ENSMUSG00000041124 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 2516bp deletion from Chr 9:4,383,469-4,385,984 GRCm39 was sequence verified. 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)