

# GENOTYPING PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR10952 Thop1 EXDEL Stock #: 067400

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:

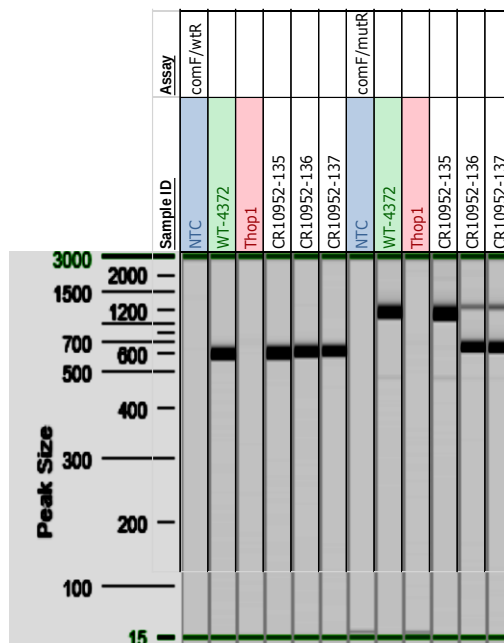
| Steps                                                     | Temp (°C )      | Time (m:ss)         | # of Cycles |
|-----------------------------------------------------------|-----------------|---------------------|-------------|
| 1. Initiation/Melting HOT START? <input type="checkbox"/> | 94              | 2:00                | <b>1x</b>   |
| 2. Denaturation                                           | 94              | 0:10                |             |
| 3. Annealing steps 2-3-4 cycle in sequence                | 65 (↓1°C/cycle) | 0:30                | <b>10x</b>  |
| 4. Elongation                                             | 68              | 2:00                |             |
| 5. Denaturation                                           | 94              | 0:15                |             |
| 6. Annealing steps 5-6-7 cycle in sequence                | 55              | 0:30                | <b>25x</b>  |
| 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_Thop1_comF | CCTCCATCCTTTAGCAACTTCACAC     | Estimated Running Time: 90 min. |                  |
| 2. CR_Thop1_wtR  | GCTCATCTCTACATCAAACCTCCGAGAG  | <b>Primer Combination</b>       | <b>Band (bp)</b> |
| 3. CR_Thop1_mutR | GGACACAGAGACACCGACCTGTGA      | 1 & 2, 1 & 3                    | 632, 1239        |
|                  |                               | 1 & 3                           | 708              |
|                  |                               |                                 | wildtype         |
|                  |                               |                                 | mutant           |

**Allele Description:** Exon 3 [ENSMUSE00001287362](#) and flanking splicing regions were constitutively deleted from the Thop1 gene [ENSMUSG00000004929.12](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.



PCR protocol developed by MMRRC at University of California, Davis