

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: CR1256 Zbtb48 Exdel

Protocol:

| Reagent/Constituent | Volume (μL) |
|---|------------------|
| Water | 9.975 |
| 10x Buffer | 2.5 |
| MgCl ₂ (stock concentration is 25mM) | 1.7 |
| Betaine (stock concentration is 5M) <i>Optional</i> | 6.5 |
| dNTPs (stock concentration is 10mM) | 0.5 |
| DMSO <i>Optional</i> | 0.325 |
| 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 |
| Taq Polymerase 5Units/μL | 0.2 |
| DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits" | 1.0 |
| TOTAL VOLUME OF REACTION: | 25.000 μL |

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65°C decreasing in temperature by 1.0°C; next 30 cycles anneal at 55°C.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust MgCl₂ to increase reaction or decrease non-specific amplifications.

Strategy:

| Steps | Temp (°C) | Time (m:ss) | # of Cycles |
|--|-----------------------|-------------|-------------|
| 1. Initiation/Melting HOT START? <input type="checkbox"/> | 94 | 5:00 | 1 |
| 2. Denaturation | 94 | 0:15 | |
| 3. Annealing steps 2-3-4 cycle in sequence | 65 to 55 (↓1°C/cycle) | 0:30 | 40x |
| 4. Elongation | 72 | 0:40 | |
| 5. Amplification | 72 | 5:00 | 1 |
| 6. Finish | 15 | ∞ | n/a |

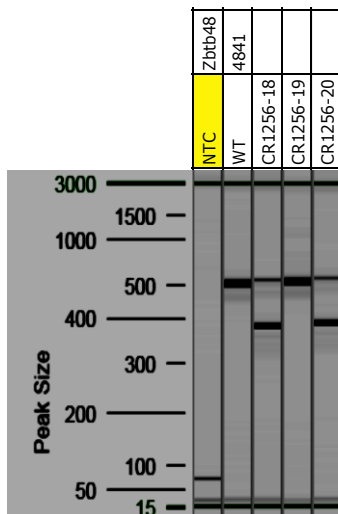
Primers:

Electrophoresis Protocol:

| Name | Nucleotide Sequence (5' - 3') | Agarose: 1.5% V: 90 | | |
|-------------------|-------------------------------|---------------------------------|-----------|----------|
| 1. CR_Zbtb48-comF | GCTTGTGGGAGTTGTTTTCTTCACC | Estimated Running Time: 90 min. | | |
| 2. CR_Zbtb48-wtR | GGGACACTCGAAGGGTTTCTCC | Primer Combination | Band (bp) | Genotype |
| 3. CR_Zbtb48-mutR | AAACGCTGCTGGTAAGTGTCTGC | 1 & 2, 1 & 3 | 501,1078* | wildtype |
| | | 1 & 3 | 374 | mutant |

Allele Description: Exon 4 [ENSMUSE00001289765](#) and flanking splicing regions were constitutively deleted from the Zbtb48 gene [ENSMUST00000155389.7](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

*May not see larger wildtype band



PCR protocol developed by MMRRC at University of California, Davis