# GENOTYPING BY PCR PROTOCOL
## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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**Protocol Name: CR10258 Abhd17c iDex**

**Protocol:**

| Reagent/Constituent | Volume (µL) |
|---|---|
| Water | 10.775 |
| 10x Buffer | 2.5 |
| $MgCl_2$ (stock concentration is 25mM) | 1.7 |
| Betaine (stock concentration is 5M) *Optional* | 6.5 |
| dNTPs (stock concentration is 10mM) | 0.5 |
| DMSO *Optional* | 0.325 |
| 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 |
| 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 65ºC decreasing in temperature by 1.0ºC; next 30 cycles anneal at 55ºC.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non-specific amplifications.

**Strategy:**

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

**Primers:**

| Name | Nucleotide Sequence (5' - 3') |
|---|---|
| 1. CR_Abhd17c-comF2 | CAGGATGAACGGCTTCTCGCT |
| 2. CR_Abhd17c_wtR | GAGAAGAAGACCTCGACGGCGT |
| 3. CR_Abhd17c-mutR2 | GCCGATGTAGAAGCTACACATCTGG |
| | |

**Electrophoresis Protocol:**

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

Estimated Running:Time: **90** min.

| Primer Combination | **Band** (bp) | Genotype |
|---|---|---|
| 1 & 2, 1 & 3 | 291,418 | wildtype |
| 1 & 3 | 105 | mutant |

**Allele Description:** Exon 1 ENSMUSE00000307637 had 313bp deleted from the 70th coding nucleotide through the 382nd coding nucleotide from the Abhd17c gene ENSMUSG00000038459 using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.