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

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**Protocol Name:** **CR10782 Pramef12 iDex**

**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_Pramef12_comF | AGAAGGAGCATAGGAGGAGGAAAGTG |
| 2. CR_Pramef12_wtR | AGGACAGGCATGTATCCAAGGCTC |
| 3. CR_Pramef12_mutR | GCATCTCCTTTCCCCACTGTCAA |
| | |

**Electrophoresis Protocol:**

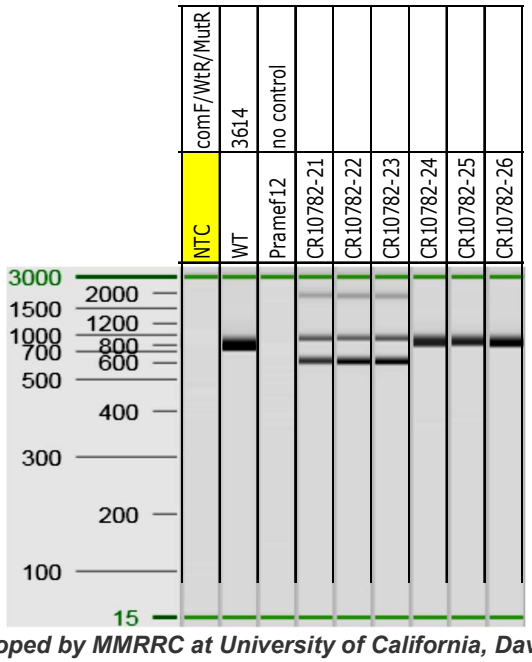
Agarose: **1.5%** **V:** **90**

Estimated Running Time: **90** min.

| Primer Combination | **Band** (bp) | **Genotype** |
|---|---|---|
| 1 & 2,1 & 3 | 449, 941 | wildtype |
| 1 & 3 | 640 | mutant |

**Allele Description**: 302bp were deleted from Pramef12, deleting 233bp from the 353rd coding nucleotide to the 585th coding nucleotide of Exon 3 ENSMUSE00000180478 plus some flanking sequence. The Pramef12 gene ENSMUST00000030326.9 was modified 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)




*PCR protocol developed by MMRRC at University of California, Davis*