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

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**Protocol Name:** **STOCK *Dlx1*$^{tm2.1Jlr}$/Mmucd** **MMRRC: 037640-UCD**

**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) | 0.5 |
| Primer 2. (stock concentration is 20µM) | 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** |
| | **24.5** |

***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:**

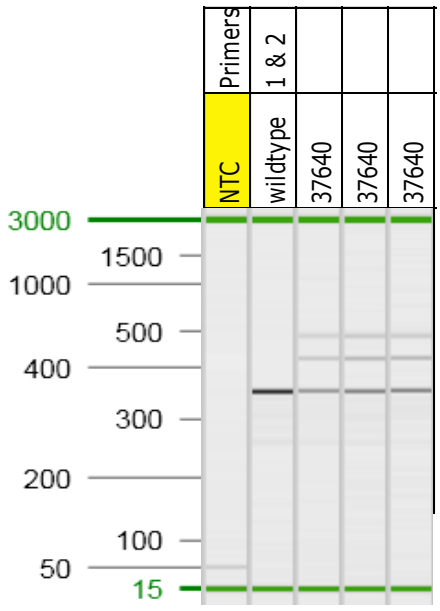
| Name | Nucleotide Sequence (5' - 3') |
|---|---|
| 1. 37640-F2 | GGGGACTGGAGCTCTCTTCTGG |
| 2. 37640-R2 | CAGTTTCAAAATTCACCGAATCTCC |
| | |
| | |

**Electrophoresis Protocol:**

Argarose: **1.5%** V: **90**

Estimated **9** min.

| Primer | Band (bp) | Genotype |
|---|---|---|
| 1 & 2 | 361 | wildtype |
| 1 & 2 | 420, 480 | mutant |




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