# GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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## Protocol:

**NAME OF PCR _____STOCK *Dlx1$^{tm1Jlr}$* #30264-UCD_____**

| Reagent/ Constituent | Volume (µL) |
|---|---|
| Water | 10.275 |
| 10x Buffer | 2.5 |
| 25 mM $MgCl_2$ | 1.7 |
| 5M Betaine | 6.5 |
| 10 mM dNTPs | 0.5 |
| DMSO | 0.325 |
| Primer **1:** Neo-F2 (20 µM) | 0.5 |
| Primer **2:** Neo-R2 (20 µM) | 0.5 |
| Primer **3:** 30264-wtF (20 µM) | 0.5 |
| Primer **4:** 30264-wtR (20 µM) | 0.5 |
| Taq Polymerase-5 Units/µl | 0.2 |
| DNA Sample | 1.0 |
| **TOTAL VOLUME OF REACTION:** | **25 µl** |

***Comments on protocol:*** Use Touch-Down cycling protocol-first 10 cycles anneal at 65° C decreasing in temperature by 1.0° C; final 30 cycles anneal at 55° C. *Concentration of $MgCl_2$ may be adjusted to reduce non-specific banding.

## Cycling:

| Steps | Temp (°C ) | Time (m:ss) | # of Cycles |
|---|---|---|---|
| 1. Initiation/Melting | 95 | 5:00 | **1** |
| 2. Denaturation | 94 | 0:20 | \ |
| 3. Annealing } steps 2-3-4 will cycle in sequence | 65 to 55 | 0:30 | > **x 40** |
| 4. Elongation | 72 | 0:30 | / |
| 5. Final Extension | 72 | 5:00 | **1** |
| 6. Finish | 25 | Hold | -- |

## Primers:

| Primer Name | Nucleotide sequence (5'– 3') |
|---|---|
| **1 Neo-F2** | CGGGAAGGGACTGGCTGCTA |
| **2 Neo-R2** | GTCAAGAAGGCGATAGAAGGCGAT |
| **3 30264-wtF** | AGGGAGACGGGCAGGAAGCG |
| **4 30264-wtR** | AAGGCGGGGCAGCTCTGGAG |

## Electrophoresis Protocol:

**% Agarose: __1.5__ Volts : __90__**

**Estimated Running Time (min): __90__**

| Primer Combinations | Band size (bp) | genotype |
|---|---|---|
| 1 & 2 | 500 | Mutant |
| 3 & 4 | 244 | Wild type |

No Photo Available