# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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**NAME OF PCR:** B6;D2-Tg(Pcp2-cre)22Lfr/Mmucd **MMRRC #** 000192-UCD

### Protocol: (Cre)

| Reagent/ Constituent | Volume (µL) |
|---|---|
| Water | 11.275 |
| 10x Buffer (contains / without 15mM $MgCl_2$) | 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) Cre Up 2 | 0.5 |
| Primer 2 (stock concentration is 20µM) Cre Dn 2 | 0.5 |
| Taq Polymerase 5Units/µL | 0.2 |
| DNA extracted with ☒ NaOH ☐ Proteinase K ☐ Other: | 1.0 |
| **TOTAL VOLUME OF REACTION:** | **25µL** |

### *Comments on protocol:*

- 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/DMSO is standardized due to high GC content in promoter regions. Protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non specific amplifications.

### Strategy:

| Steps | | Temp (°C ) | 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 will cycle in sequence | 65 to 55 (↓1°C/cycle) | 0:30 | **10x** |
| 4. Elongation | | 72 | 0:40 | |
| 5. Denaturation | | 94 | 0:15 | |
| 6. Annealing | steps 5-6-7 will cycle in sequence | 55 | 0:30 | **30x** |
| 7. Elongation | | 72 | 0:40 | |
| 8. Amplification | | 72 | 5:00 | **1** |
| 9. Finish | | 15 | ∞ | n/a |

### Primers:

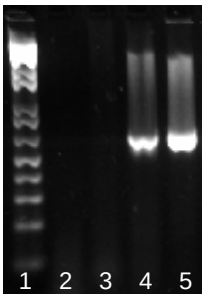
| Name | Nucleotide Sequence (5' - 3') |
|---|---|
| 1: Cre Up 2 | GAT CTC CGG TAT TGA AAC TCC AGC |
| 2: Cre Dn 2 | GCT AAA CAT GCT TCA TCG TCG G |

### Electrophoresis Protocol:

**Agarose:** 2% **mV:** 100

**Estimated Running Time:** 60 **min.**

| Expected Bands | Genotype |
|---|---|
| No band | wild-type |
| 650 bp | Cre present |




Lanes:
1. 1 Kb+ Ladder
2. $H_2O$
3. B6
4. Cre positive control
5. Cre positive sample