# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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**NAME OF PCR:** STOCK Tg(Crym-Ncre, Crym-Ccre)RL89Gsat/Mmucd **MMRRC #** 036627-UCD

## Protocol:

| Reagent/ Constituent | Volume (µL) |
|---|---|
| Water | 11.275 |
| 10x Buffer | 2.5 |
| $MgCl_2$ (stock concentration is 25mM) | 1.7 |
| Betaine (stock concentration is 5M) | 6.5 |
| dNTPs (stock concentration is 10mM) | 0.5 |
| DMSO | 0.325 |
| Primer F (stock concentration is 20µM) | 0.5 |
| Primer R (stock concentration is 20µM) | 0.5 |
| Taq Polymerase (5Units/µL) | 0.2 |
| DNA extracted with ☐ NaOH ☒ Proteinase K ☐ Other: | 1.0 |
| **TOTAL VOLUME OF REACTION:** | **25.000µL** |

## *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 and 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 | **40x** |
| 4. Elongation | | 72 | 0:40 | |
| 5. Amplification | | 72 | 5:00 | **1** |
| 6. Finish | | 4 | ∞ | n/a |

## Primers:

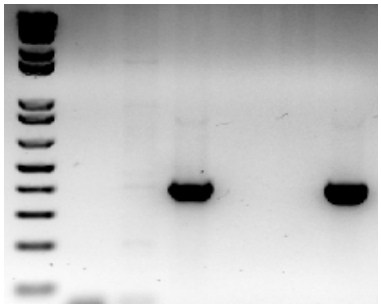
| Name | Nucleotide Sequence (5' - 3') |
|---|---|
| 1: Crym (36627) F2 | GCTACTCAGGCAGTCCGCTCATT |
| 2: GS-CCRE-R1 | ATGTCCCTCACATCCTCAGGTTCAGCAG |
| 3: GS-NCRE-R1 | ATCCCTGAACATGTCCATCAGGTTC |

## Electrophoresis Protocol:

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

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

| Primer Combination | Band | Genotype |
|---|---|---|
| 1 and 2 | 400 bp | transgenic |
| | | |



1 2 3 4 5 6 7

Lanes
1. 1 kb+ ladder
(Invitrogen, Cat. #10787-026)
2. Non-template control
3. Wild-type
4. Crym Ccre (Primer 1 & 2)
5. Non-template control
6. Wild-type
7. Crym Ncre (Primer 1 & 3)