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

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530-754-MMRRC

**NAME OF PCR:** **C57BL/6N-*Hprt*$^{tm1(CMV-cre)Wtsi}$/Mmucd** **MMRRC #** **034412-UCD**

### Protocol: srPCR

| Reagent/ Constituent | Volume (µL) |
|---|---|
| Water | 15.2 |
| 10x Buffer | 2.0 |
| $MgCl_2$ (stock concentration is 50mM) | 0.6 |
| dNTPs (stock concentration is 100mM) | 0.2 |
| Primer 1 (stock concentration is 10µM) | 0.4 |
| Primer 2 (stock concentration is 10µM) | 0.4 |
| PtTaq (Platinum Taq (Invitrogen)) | 0.2 |
| DNA (50-100 ng/µL) | 1.0 |
| **TOTAL VOLUME OF REACTION:** | **20µL** |

### *Comments on protocol:*

- Homozygotes and heterozygotes can be distinguished by Southern blot (Su *et al.* (2002)), Neo count qPCR http://www.knockoutmouse.org/kb/entry/91/, or by a quantitative PCR (qPCR) assay designed to the Cre gene (protocol on next page).

### Strategy:

| Steps | | Temp (°C ) | Time (m:ss) | # of Cycles |
|---|---|---|---|---|
| 1. Initiation/Melting HOT START? ☐ | | 94 | 5:00 | **1** |
| 2. Denaturation | | 94 | 0:30 | |
| 3. Annealing | steps 2-3-4 will cycle in sequence | 58 | 0:30 | **35x** |
| 4. Elongation | | 72 | 0:45 | |
| 5. Amplification | | 72 | 5:00 | **1** |
| 6. Finish | | 12 | ∞ | n/a |

### Primers:

| Name | Nucleotide Sequence (5' - 3') |
|---|---|
| 1: Hprt1_F | CTTTCCTCATGCCCCAAAATCTTAC |
| 2: Hprt1_Mut_R | GCTATCAGGACATAGCGTTGGCTAC |

### Electrophoresis Protocol:

**Agarose:** 1.5% **V:** 90 **Estimated Running Time:** 90 **min.**

| Primer Combination | Expected Bands | Genotype |
|---|---|---|
| 1 and 2 | 700 bp | Mutant |
| 1 and 2 | 311 bp | Wild-type |

For Southern blot analysis please see:
A targeted X-linked CMV-Cre line. Hong Su, Alea A. Mills, Xiaozhong Wang and Allan Bradley (2002). Genesis 32:187-188

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**NAME OF PCR:** **C57BL/6N-*Hprt*$^{tm1(CMV\text{-}cre)Wtsi}$/Mmucd** **MMRRC #** **034412-UCD**

### Protocol: qPCR

| Reagent/ Constituent | Volume (µL) |
|---|---|
| 2x GTXpress™ buffer | 5.0 |
| Cre 20x assay | 0.5 |
| Water | 3.0 |
| Tfrc 20x assay | 0.5 |
| DNA (50-100 ng/µL) | 1.0 |
| **TOTAL VOLUME OF REACTION:** | **10µL** |

### *Comments on protocol:*

- The number of copies of the Cre allele can be detected using a FAM-labelled custom qPCR TaqMan® assay. These are multiplexed with a VIC® labelled endogenous control assay (for example TaqMan® Copy Number Reference Assay, Mouse, Tfrc; Applied Biosystems part #4458366). Reference DNA controls of known genotypes should also be included to facilitate correct analysis.
- Reactions are performed in a 10µl volume using an Applied Biosystems 7900HT Fast Real-Time PCR System with DNA prepared using the Sample-to-SNPTM kit (Applied Biosystems) from mouse ear biopsies. GTXpressTM buffer is also used (Applied Biosystems).

### Strategy:

| Steps | | Temp (°C ) | Time (m:ss) | # of Cycles |
|---|---|---|---|---|
| 1. Initiation/Melting | | 95 | 0:20 | **1** |
| 2. Denaturation | | 95 | 0:10 | |
| 3. Annealing | steps 2-3-4 will cycle in sequence | 58 | 0:30 | **35x** |
| 4. Elongation | | 72 | 0:45 | |

### Primers:

| Name | Nucleotide Sequence (5' - 3') |
|---|---|
| 1: CRE-2_F | ACGTACTGACGGTGGGAGAA |
| 2: CRE-2_R | GTGCTAACCAGCGTTTTCGTT |
| 3: CRE-2_M; Reporter Dye: FAM | CTGCCAATATGGATTAACA |

### Analysis of Results:

- The number of copies of the Cre allele can be detected using a FAM-labelled custom qPCR TaqMan® assay. These are multiplexed with a VIC® labelled endogenous control assay (for example TaqMan® Copy Number Reference Assay, Mouse, Tfrc; Applied Biosystems part #4458366). Reference DNA controls of known genotypes should also be included to facilitate correct analysis.