Wellcome Trust Sanger Institute
Wellcome Trust Genome Campus
Hinxton Cambridge CB10 1SA, U.K.

**MGPgenotyping@sanger.ac.uk**
**www.sanger.ac.uk**

## CMV-Cre (CRET)

**Allele:** *Hprt1* $^{Tg(CMV\text{-}Cre)Brd}$

## Genotyping Information

Please note the short range PCR (srPCR) assay will not discriminate between heterozygotes and homozygotes as the wildtype PCR assay was originally designed amplifying a section of exon 3 (Su *et al.* (2002)). The assay detects the presence of the insertion.
Homozygotes and heterozygotes can be distinguished by Southern blot (Su *et al.* (2002)), Neo count qPCR (www.knockoutmouse.org/kb/entry/91/), or by a quantitative PCR (qPCR) assay designed to the Cre gene.

## srPCR Assay

- **PCRs primer pairs and expected size bands**

| PCR type | Forward primer | Reverse primer | Expected size band (bp) |
|---|---|---|---|
| Mutant PCR | Hprt1_F | Hprt1_Mut_R | 700 |
| Wild type PCR | Hprt1_F | Hprt1_WT_R | 311 |

- **Primer sequences**

| Primer name | Primer sequence (5' > 3') |
|---|---|
| Hprt1_F | CTTTCCTCATGCCCCAAAATCTTAC |
| Hprt1_Mut_R | GCTATCAGGACATAGCGTTGGCTAC |
| Hprt1_WT_R | ATGTAATCCAGCAGGTCAGCAAGA |



**Last updated: 27/07/2011**

Wellcome Trust Sanger Institute
Wellcome Trust Genome Campus
Hinxton Cambridge CB10 1SA, U.K.

**MGPgenotyping@sanger.ac.uk**
**www.sanger.ac.uk**

## ➢ Reaction

| **Reagent** | **µl** |
|---|---|
| DNA (~50-100 ng) | 1.0 |
| 10x Buffer | 2.0 |
| $MgCl_2$ (50 mM) | 0.6 |
| PtTaq (Platinum® Taq (Invitrogen)) | 0.2 |
| dNTPs (100 mM) | 0.2 |
| Primer 1 (10 µM) | 0.4 |
| Primer 2 (10 µM) | 0.4 |
| $H_20$ | 15.2 |
| **Total** | **20.0** |

## ➢ Cycling conditions

### Wild type and mutant PCRs

| Cycle | | |
|---|---|---|
| 1 | 94 °C | 5 min |
| 2 | 94 °C | 30 sec |
| 3 | 58 °C | 30 sec |
| 4 | 72 °C | 45 sec |
| 5 | Go to '2' + 34 cycles | |
| 6 | 72 °C | 5 min |
| 7 | 12 °C | forever |

Su, H., Mills, A. A., Wang, X., and Bradley, A. (2002). A targeted X-linked CMV-Cre line. *Genesis* **32**:187-188.



**Last updated: 27/07/2011**

**Genome Research Limited**
Registered Office 215 Euston Road London NW1 2BE. A company registered in England No. 2742969 and a charity No. 1021457 registered in England

Wellcome Trust Sanger Institute
Wellcome Trust Genome Campus
Hinxton Cambridge CB10 1SA, U.K.

**MGPgenotyping@sanger.ac.uk**
**www.sanger.ac.uk**

## qPCR Assay (designed to the Cre allele)

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.

| Forward Primer Name | Forward Primer Seq. |
|---|---|
| CRE-2_F | ACGTACTGACGGTGGGAGAA |

| Reverse Primer Name | Reverse Primer Seq. |
|---|---|
| CRE-2_R | GTGCTAACCAGCGTTTTCGTT |

| Reporter 1 Name | Reporter 1 Sequence | Reporter 1 Dye |
|---|---|---|
| CRE-2_M | CTGCCAATATGGATTAACA | FAM |

### ➢ Reaction setup

Reactions are performed in a 10µl volume using an Applied Biosystems 7900HT Fast Real-Time PCR System with DNA prepared using the Sample-to-SNP™ kit (Applied Biosystems) from mouse ear biopsies. GTXpress™ buffer is also used (Applied Biosystems)

| | Volume µl |
|---|---|
| **2x GTXpress™ buffer** | 5 |
| **Cre 20x assay** | 0.5 |
| **water** | 3 |
| **Tfrc 20x assay** | 0.5 |
| **DNA** | 1 |



**Last updated: 27/07/2011**

Wellcome Trust Sanger Institute
Wellcome Trust Genome Campus
Hinxton Cambridge CB10 1SA, U.K.

**MGPgenotyping@sanger.ac.uk**
**www.sanger.ac.uk**

- **qPCR Cycling conditions**

| Cycle | | |
|---|---|---|
| 1 | 95°C | 20sec |
| 2 | 95°C | 10sec |
| 3 | 60°C | 30sec |
| 4 | Go to '2' + 34 cycles | |

Results can be analysed using CopyCaller™ software (Applied Biosystems) or RQ Manager (Applied Biosystems). Both packages use the comparative Ct (ddCt) method to perform the analysis.
Other instrument systems will have their own analysis software – please see the manufacturer's guidelines for information about your system.



**Last updated: 27/07/2011**