# KOMP PCR Design

Mouse PCR Protocol (version 1)

Design ID: 119276
Project ID: CSD48005
Selection Cassette: L1L2_Pgk_P

MMRRC @ UCDavis
Mutant Mouse Resource & Research Centers supported by NIH

MMRRC Stock #: 048065-UCD
C57BL/6N-Atm1Brd Hip1rtm2a(KOMP)Wtsi/
BayMmucd

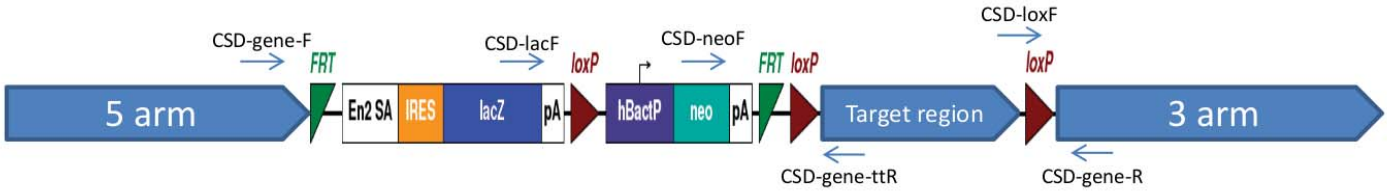
## Suggested DNA Prep: DNeasy®Tissue Kit

| Reagent | 1X ( µL) |
|---|---|
| water (biological grade) | 10.725 |
| betain 5M (Sigma) | 6.5 |
| DMSO (Sigma) | 0.325 |
| 10X buffer w\o $MgCl_2$ (AB) | 2.5 |
| 25 mM $MgCl_2$ (AB) | 1.75 |
| 10 mM dNTPs (Invitrogen) | 0.5 |
| primers (20 µM each) | 0.5 |
| Taq 5U/ µL (AmpliTaq, AB) | 0.2 |
| total cocktail | 23 |
| template | 2 |
| reaction volume | 25 |

**Cycling Parameters**

| Temperature °C | Time | |
|---|---|---|
| 94 | 5 min | |
| 94 | 15 sec | |
| 65 | 30 sec | **10X (decrease 1°C/cycle)** |
| 72 | 40 sec | |
| 94 | 15 sec | |
| 55 | 30 sec | **30X** |
| 72 | 40 sec | |
| 72 | 5 min | |
| 4 | finished | |

## Primer Strategy




| Cassette Primers | |
|---|---|
| CSD-lacF: | GCTACCATTACCAGTTGGTCTGGTGTC |
| CSD-neoF: | GGGATCTCATGCTGGAGTTCTTCG |
| CSD-loxF: | GAGATGGCGCAACGCAATTAATG |

| Gene Specific Primers | |
|---|---|
| CSD-Hip1r-R: | GAAGGTACAGCCCAGAACAATCACC |
| CSD-Hip1r-ttR: | AGCTTCCCCTCCTTAACCTCATTCC |
| CSD-Hip1r-F: | GTCTTCTTGACTTGGAACAGGTGGC |

| Genotype | Forward Primer | Reverse Primer | Amplicon size (bp) |
|---|---|---|---|
| Floxed | CSD-loxF | CSD-Hip1r-R | 384 |
| PreCre | CSD-neoF | CSD-Hip1r-ttR | 661 |
| PostCre | CSD-lacF | CSD-Hip1r-R | 682 |
| Wildtype | CSD-Hip1r-F | CSD-Hip1r-ttR | 587 |
| PostFlp | CSD-Hip1r-F | CSD-Hip1r-ttR | 765 |
| PostFlp & Cre | CSD-Hip1r-F | CSD-Hip1r-R | 812 |

Please note, these primers are auto-designed and may not have been verified by the repository, and as such may require optimization or redesign by your facility.

We recommend running primers singleplex. For screening of pups prior to any Flp or Cre recombination, the Floxed or PreCre primers may be used to identify the mutant mice. The Floxed primers test for the distal LoxP site. The PostCre primers should be used if mutant mice were crossed with a Cre recombinase line (without any FLP recombination). The PostFlp primers should be used if mutant mice were crossed with a Flp recombinase line. The PostFlp & Cre primers should be used if mutant mice were crossed with a Flp recombinase line and then a Cre recombinase line. The wildtype primers should be used for zygosity testing of all mutant mice (pre or post recombination).