

# KOMP PCR Design

Mouse PCR Protocol (version 1BW)

Design ID: 96285

Project ID: CSD26920

Selection Cassette: L1L2\_Bact\_P\_



MMRRC Stock #: 050416-UCD

C57BL/6N-Atm1Brd Zbed4tm1(KOMP)Mbp/

MbpMmucd

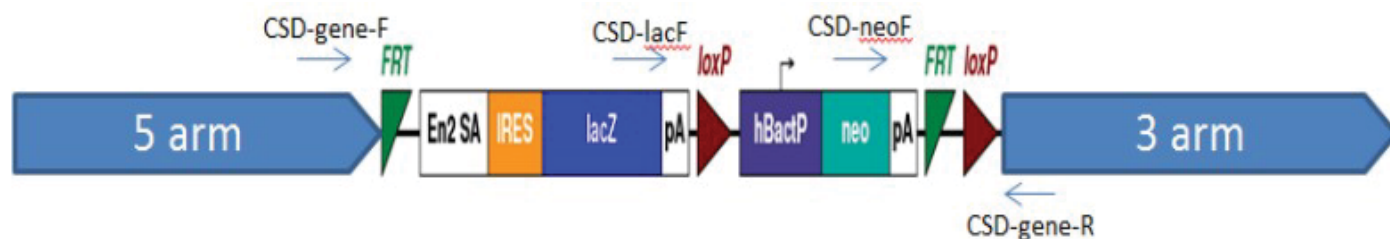
## Suggested DNA Prep: DNeasy®Tissue Kit

| Reagent                               | 1X (μL) |
|---------------------------------------|---------|
| water (biological grade)              | 10.725  |
| betain 5M (Sigma)                     | 6.5     |
| DMSO (Sigma)                          | 0.325   |
| 10X buffer w/o MgCl <sub>2</sub> (AB) | 2.5     |
| 25 mM MgCl <sub>2</sub> (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   | 10X (decrease 1°C/cycle) |
| 65             | 30 sec   |                          |
| 72             | 40 sec   |                          |
| 94             | 15 sec   | 30X                      |
| 55             | 30 sec   |                          |
| 72             | 40 sec   |                          |
| 72             | 5 min    |                          |
| 4              | finished |                          |

## Primer Strategy



## Cassette Primers

CSD-lacF: GCTACCATTACCAGTTGGTCTGGTGTC  
CSD-neoF: GGGATCTCATGCTGGAGTTCTTCG

## Gene Specific Primers

CSD-R: CTTCTGAAAACGCACCTTGACACC  
CSD-F: TTCTCCTATATATTTAGGCCGATCCC  
CSD-wtR: CTTGTATAAACCAATGCGTGCAGC

| Genotype | Forward Primer | Reverse Primer | Amplicon size (bp) |
|----------|----------------|----------------|--------------------|
| PreCre   | CSD-neoF       | CSD-R          | 565                |
| PostCre  | CSD-lacF       | CSD-R          | 516                |
| Wildtype | CSD-F          | CSD-R          | 183                |
| PostFlp  | CSD-F          | CSD-R          | 397                |

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 PreCre primers may be used to identify the mutant mice. The PostCre primers should be used if mutant mice were crossed with a Cre recombinase line. The PostFlp primers should be used if mutant mice were crossed with a Flp recombinase line. The wildtype primers should be used for zygosity testing of all mutant mice (pre or post recombination).