

# KOMP PCR Design

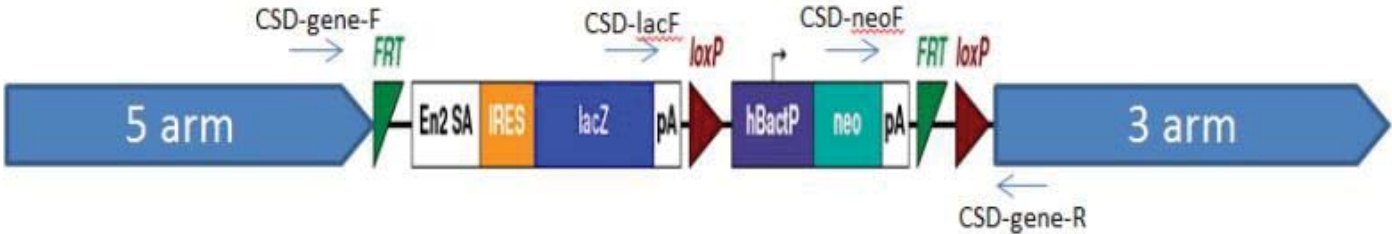
Mouse PCR Protocol (version 1BW)

Design ID: 237955  
Project ID: CSD67589  
Selection Cassette: - -

## Suggested DNA Prep: DNeasy®Tissue Kit

| Reagent                               | 1X (μL) | Cycling Parameters |          |                          |
|---------------------------------------|---------|--------------------|----------|--------------------------|
| water (biological grade)              | 10.725  | Temperature °C     | Time     |                          |
| betain 5M (Sigma)                     | 6.5     | 94                 | 5 min    |                          |
| DMSO (Sigma)                          | 0.325   | 94                 | 15 sec   | 10X (decrease 1°C/cycle) |
| 10X buffer w/o MgCl <sub>2</sub> (AB) | 2.5     | 65                 | 30 sec   |                          |
| 25 mM MgCl <sub>2</sub> (AB)          | 1.75    | 72                 | 40 sec   |                          |
| 10 mM dNTPs (Invitrogen)              | 0.5     | 94                 | 15 sec   | 30X                      |
| primers (20 μM each)                  | 0.5     | 55                 | 30 sec   |                          |
| Taq 5U/μL (AmpliTaq, AB)              | 0.2     | 72                 | 40 sec   |                          |
| total cocktail                        | 23      | 72                 | 5 min    |                          |
| template                              | 2       | 4                  | finished |                          |
| reaction volume                       | 25      |                    |          |                          |

## Primer Strategy



## Cassette Primers

CSD-lacF: GCTACCATTACCAGTTGGTCTGGTGTC  
CSD-neoF: GGGATCTCATGCTGGAGTTCTTCG

## Gene Specific Primers

CSD-R: GTAATGGCTCACACCTGGAGTCTGC  
CSD-F: CAATAGGTTGTCCAAGGTAGCCTTG  
CSD-wtR: ATGGTTCTAAGTGAATTGCTTAAGTGC

| Genotype | Forward Primer | Reverse Primer | Amplicon size (bp) |
|----------|----------------|----------------|--------------------|
| PreCre   | CSD-neoF       | CSD-R          | 574                |
| PostCre  | CSD-lacF       | CSD-R          | 595                |
| Wildtype | CSD-F          | CSD-wtR        | 345                |
| PostFlp  | CSD-F          | CSD-R          | 551                |

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).