

# KOMP PCR Design

Mouse PCR Protocol (version 1BG)

Design ID: 10020

Project ID: VG10020

Selection Cassette: ZEN-Ub1



MMRRC Stock #: 047083-UCD

C57BL/6N-Ch25htm1(KOMP)Vlbg/Mmucd

## 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

Reg-NeoF: GCAGCCTCTGTTCCACATACACTTCA  
Reg-LacF: ACTTGCTTTAAAAACCTCCCACA

### Gene Specific Primers

Reg-Ch25h-R: AAATCAGGTCTTCTCTCAACATGTGAGG  
Reg-Ch25h-wtF: CTCGTCCAGCTCCTAAGTCACG  
Reg-Ch25h-wtR: AGCGAAAGCAGCTCCCAGAAGC

| Genotype | Forward Primer | Reverse Primer | Amplicon size (bp) |
|----------|----------------|----------------|--------------------|
| PreCre   | Reg-NeoF       | Reg- Ch25h-R   | 354                |
| PostCre  | Reg-LacF       | Reg- Ch25h-R   | 554                |
| Wildtype | Reg- Ch25h-wtF | Reg- Ch25h-wtR | 185                |

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 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 wildtype primers should be used for zygosity testing of all mutant mice (pre or post recombination).