

# KOMP PCR Design

Mouse PCR Protocol (version 1)

Design ID: 10755

Project ID: VG10755

Selection Cassette: ZEN-Ub1



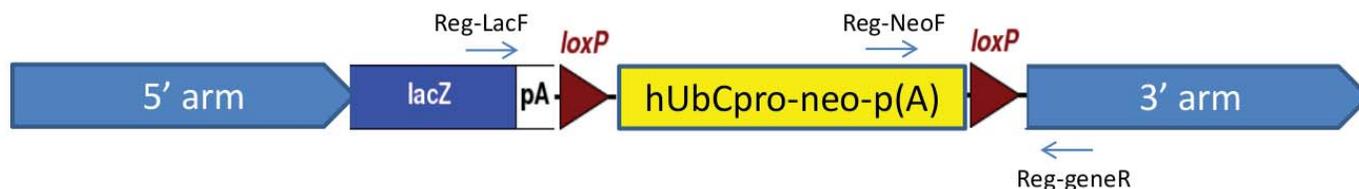
MMRRC Stock #: 047117-UCD

C57BL/6N-Clcf1tm1.1(KOMP)Vlcg/Mmucd

## Suggested DNA Prep: DNeasy®Tissue Kit

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

## Primer Strategy



### Cassette Primers

Reg-NeoF: GCAGCCTCTGTTCCACATACACTTCA  
Reg-LacF: ACTTGCTTTAAAAACCTCCCACA

### Gene Specific Primers

Reg-Clcf1-R: ACATGAGGGACTGAGTGAGTACTGG  
Reg-Clcf1-wtR: TGTAACAGAGGAGACTTCCCCAGC  
Reg-Clcf1-wtF: TCCAGAAAACCTATGACCTCACCCG

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
PreCre	Reg-NeoF	Reg-Clcf1-R	404
PostCre	Reg-LacF	Reg-Clcf1-R	604
Wildtype	Reg-Clcf1-wtF	Reg-Clcf1-wtR	348

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).