

KOMP PCR Design

Mouse PCR Protocol (version 2BW)

Design ID: 12573

Project ID: VG12573

Selection Cassette: ZEN-Ub1



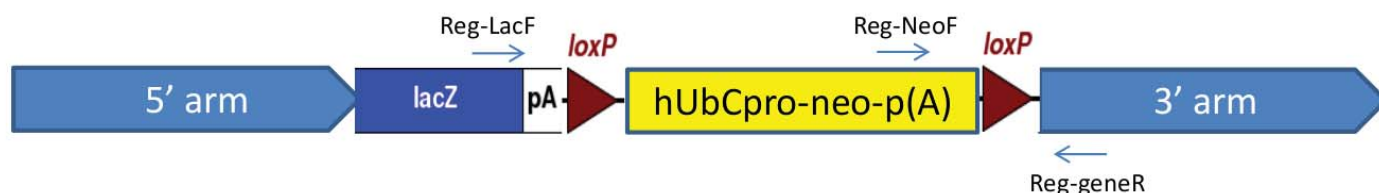
MMRRC Stock #: 050050-UCD

C57BL/6N-Thpotm1.1(KOMP)Vlcy/MbpMmucd

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 ₂ (AB)	2.5	65	30 sec	
25 mM MgCl ₂ (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 (AmpliTa, AB)	0.2	72	40 sec	
total cocktail	23	72	5 min	
template	2	4	finished	
reaction volume	25			

Primer Strategy



Cassette Primers

Reg-NeoF: GCAGCCTCTGTTCCACATACACTTCA
Reg-LacF: ACTTGCTTTAAAAACCTCCCACA

Gene Specific Primers

Reg-Thpo-R: GGTGCTCCCAGTTATTCTCTTACAGG
Reg-Thpo-wtR2: TGGACTTCTGAGCAGGCTTCAGG
Reg-Thpo-wtF: CTGAAGTGAGGTTCCAGCAAAGAGC

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
PreCre	Reg-NeoF	Reg-Thpo-R	699
PostCre	Reg-LacF	Reg-Thpo-R	899
Wildtype	Reg-Thpo-wtF	Reg-Thpo-wtR	157

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).