

KOMP PCR Design

Mouse PCR Protocol (version 1JE)

Design ID: 12970

Project ID: VG12970

Selection Cassette: ZEN-Ub1



MMRRC Stock #: 046882-UCD

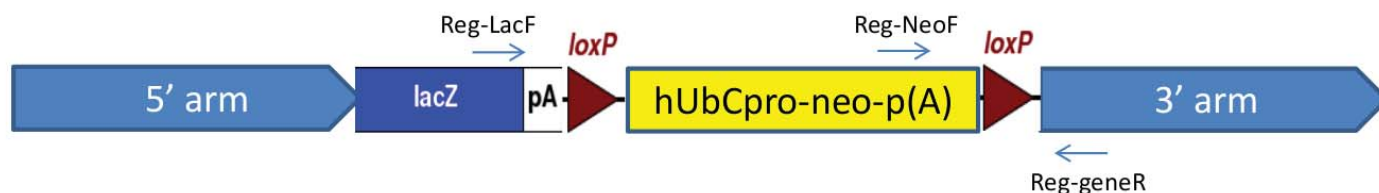
C57BL/6N-Calhm1tm1.1(KOMP)Vlclg/Mmucd

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 ₂ (AB)	2.5
25 mM MgCl ₂ (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
65	30 sec
72	40 sec
10X (decrease 1°C/cycle)	
94	15 sec
55	30 sec
72	40 sec
30X	
72	5 min
4	finished

Primer Strategy



Cassette Primers

Reg-NeoF: GCAGCCTCTGTTCCACATACACTTCA
Reg-LacF: ACTTGCTTTAAAAACCTCCCACA

Gene Specific Primers

Reg-R: GGCTGCCCTCAGTTTCTTGAGC
Reg-wtF: GATGGCAAGTGCTTTCTCTGTGC

Reg-wtR: CATGGGCTCTGCTCTGAGTCTTAGC

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
PreCre	Reg-NeoF	Reg-R	457
PostCre	Reg-LacF	Reg-R	657
Wildtype			