KOMP PCR Design

Mouse PCR Protocol (version 1JE)

Design ID: 15800
Project ID: VG15800
Selection Cassette: ZEN-Ub1

MMRRC @ UCDavis
Mutant Mouse Resource & Research Centers supported by NIH

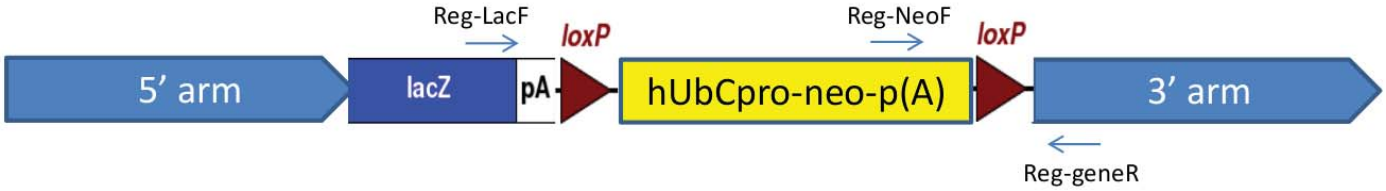
MMRRC Stock #: 050096-UCD
C57BL/6N-Tmem114tm1.1(KOMP)Vlcg/MbpMmucd

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	**10X (decrease 1°C/cycle)**
72	40 sec	
94	15 sec	
55	30 sec	**30X**
72	40 sec	
72	5 min	
4	finished	

Primer Strategy



Cassette Primers

Reg-NeoF: GCAGCCTCTGTTCCACATACACTTCA
Reg-LacF: ACTTGCTTTAAAAAACCTCCCACA

Gene Specific Primers

Reg-R: CAGGGTGAGGCAGAGCTCAA
Reg-wtF: TTCCAGGGTCCGTGTTCTCACC

Reg-wtR: CTCTGGACTGATGGGAGGAAGG

Geneotype	Forward Primer	Reverse Primer	Amplicon size (bp)
PreCre	Reg-NeoF	Reg-R	415
PostCre	Reg-LacF	Reg-R	615
Wildtype	Reg-wtF	Reg-wtR	289

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).