

Mouse PCR protocol
Design ID: MAID 11377
selection cassette: ZEN-Ub1

Suggested DNA Prep: DNeasy® Tissue Kit

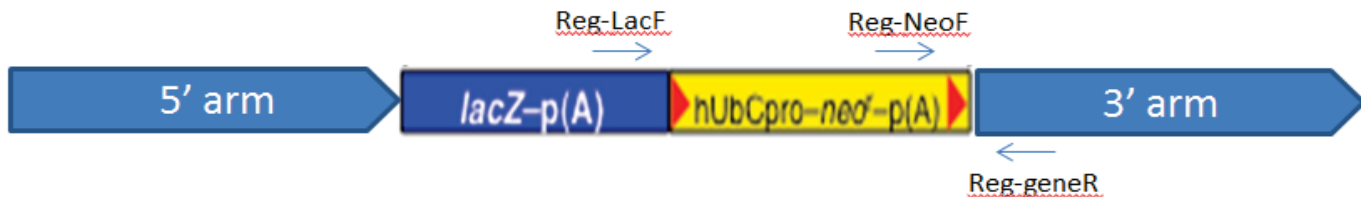
MMRRC @ UCDavis
 Mutant Mouse Resource & Research Centers supported by NIH
 MMRRC Stock #: 046460-UCD
 C57BL/6NTac-Acvr1btm1.1(KOMP)Vlclg/Mmucd

Reagents	1X (uL)
Water (biology 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 (20uM each)	0.5
taq 5U/uL (AmpliTaq, AB)	0.2
total cocktail	23
template	2
reaction volume	25

Cycling Parameters

Temp C	Time	
94	5 min	
94	15 sec	10X (decrease 1C/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: acttgctttaaaaaacctcccacac

Gene Specific Primers

Reg-Acvr1b-R: GGCATTGAGTTGTCTACATGATTTGC

Reg-Acvr1b-wtF: TTCAGTGGAGCAGTGTCAGACACC

Reg-Acvr1b-wtR: TTGTGGGAGACCACTTCCTCTGG

Genotypes	Primer pairs	amplicon size (bp)
PreCre	neoF & -R	358
PostCre	lacF & -R	558
Wildtype	-wtF & -wtR	238

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).