

**Mouse PCR protocol**

Design ID: MAID 11377

selection cassette: ZEN-Ub1

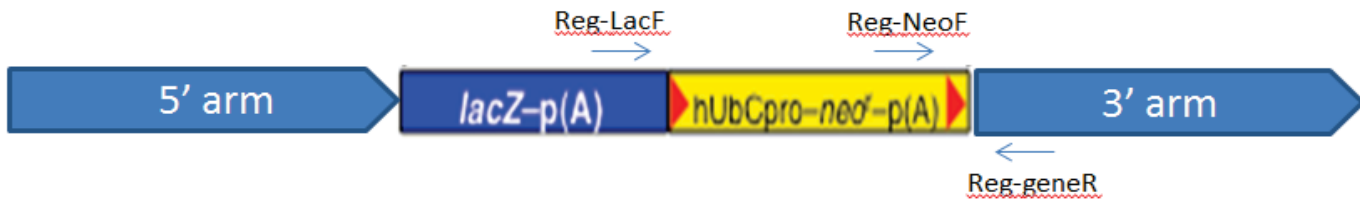
Suggested DNA Prep: DNeasy® Tissue Kit

Reagents	1X (uL)
Water (biology grade)	10.725
betain 5M (Sigma)	6.5
dmsO (Sigma)	0.325
10X Buffer w/o MgCl <sub>2</sub> (AB)	2.5
25 mM MgCl <sub>2</sub> (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	
65	30 sec	10X (decrease 1C/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: acttgctttaaaaaacctcccacac

**Gene Specific Primers**

Reg-Acvr1b-R: GGCATTGAGTTGTCTACATGATTTGC

Reg-Acvr1b-wtF: TTCAGTGGAGCAGTGTCTAGACACC

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).