

KOMP PCR Design

Mouse PCR Protocol (version 2BG)

Design ID: 14622

Project ID: VG14622

Selection Cassette: ZEN-Ub1



MMRRC Stock #: 048254-UCD

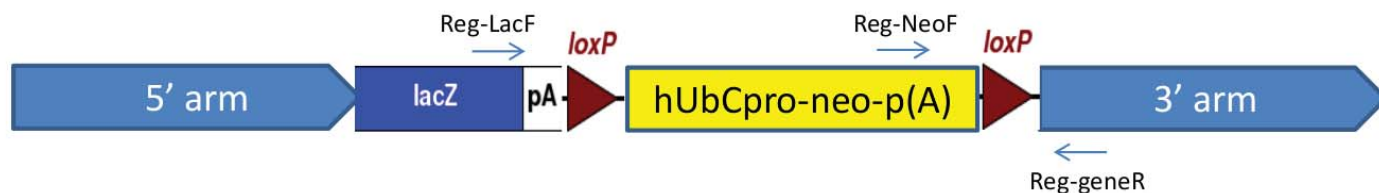
C57BL/6N-Itsn2tm1.1(KOMP)Vlclg/Mmucd

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-Itsn2-R: GCCGGTCTTAAACTCTGTAGGTTGC
Reg-Itsn2-wtR: AGCTTACCACTGCTTCCAGTTCC
Reg-Itsn2-wtF: AAAACAGGAGCTTGAGAGACAACG

Genotype Forward Primer Reverse Primer Amplicon size (bp)

PreCre	Reg-NeoF	Reg-Itsn2-R	546
PostCre	Reg-LacF	Reg-Itsn2-R	746
Wildtype	Reg-Itsn2-wtF	Reg-Itsn2-wtR	152

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).