

KOMP PCR Design

Mouse PCR Protocol (version 1)

Design ID: 12547

Project ID: VG12547

Selection Cassette: ZEN-Ub1



MMRRC Stock #: 047499-UCD

C57BL/6N-Efna4tm1.1(KOMP)Vlclg/JMmucd

Suggested DNA Prep: DNeasy®Tissue Kit

Reagent	1X (µL)	Cycling Parameters	
water (biological grade)	10.725	Temperature °C	Time
betain 5M (Sigma)	6.5	94	5 min
DMSO (Sigma)	0.325	94	15 sec
10X buffer w/o MgCl ₂ (AB)	2.5	65	30 sec
25 mM MgCl ₂ (AB)	1.75	72	40 sec
10 mM dNTPs (Invitrogen)	0.5	94	15 sec
primers (20 µM each)	0.5	55	30 sec
Taq 5U/ µL (AmpliTaq, AB)	0.2	72	40 sec
total cocktail	23	72	5 min
template	2	4	finished
reaction volume	25		

Primer Strategy



Cassette Primers

Reg-NeoF: GCAGCCTCTGTTCCACATACACTTCA
Reg-LacF: ACTTGCTTTAAAAACCTCCCACA

Gene Specific Primers

Reg-Efna4-R: CCATGGCTTCTCCTTTGCTTCTTGG
Reg-Efna4-wtR: TGGCTCCTGATGCTTAGAGAGTTGG
Reg-Efna4-wtF: ATGGCTCTCAGCATTTCCCTAGTCC

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
PreCre	Reg-NeoF	Reg-Efna4-R	534
PostCre	Reg-LacF	Reg-Efna4-R	734
Wildtype	Reg-Efna4-wtF	Reg-Efna4-wtR	118

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).