

KOMP PCR Design

Mouse PCR Protocol (version 1)

Design ID: 10916

Project ID: VG10916

Selection Cassette: ZEN-Ub1



Mutant Mouse Resource & Research Centers supported by NIH

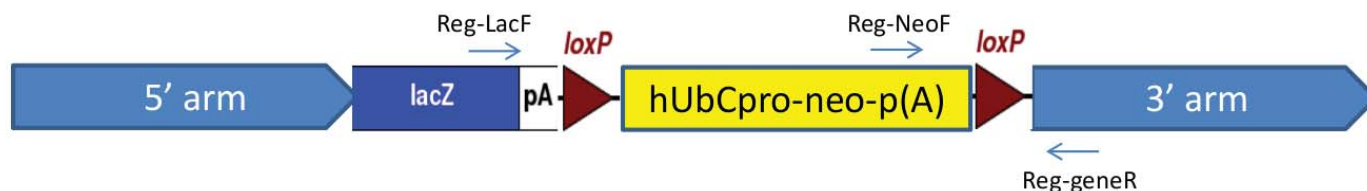
MMRRC Stock #: 047874-UCD

C57BL/6N-Glycam1^{tm1}(KOMP)Vl^{cg}/Mmucd

Suggested DNA Prep: DNeasy[®]Tissue Kit

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

Primer Strategy



Cassette Primers

Reg-NeoF: GCAGCCTCTGTTCCACATACACTTCA
Reg-LacF: ACTTGCTTTAAAAACCTCCCACA

Gene Specific Primers

Reg-Glycam1-R: TGGGAGAGCAACTATAATGGGCTGG
Reg-Glycam1-wtR: TCATGCTGTTGGCCTTAATTCTGCC
Reg-Glycam1-wtF: GCTCTTGGGTATGAAATGCTGTCCC

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
PreCre	Reg-NeoF	Reg-Glycam1-R	542
PostCre	Reg-LacF	Reg-Glycam1-R	742
Wildtype	Reg-Glycam1-wtF	Reg-Glycam1-wtR	133

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).