

KOMP PCR Design

Mouse PCR Protocol (version 2BW)

Design ID: 43233
Project ID: CSD41673
Selection Cassette: L1L2_Bact_P

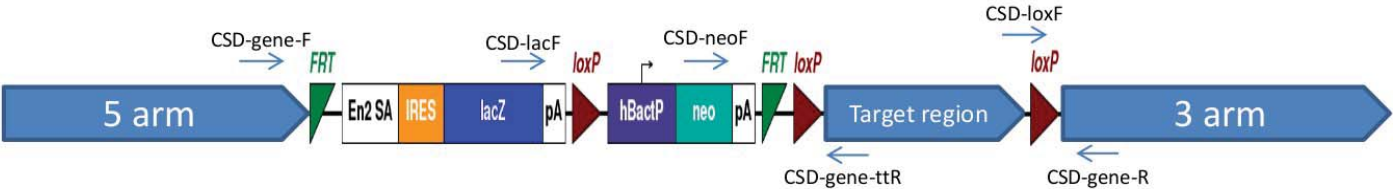


MMRRC Stock #: 050484-UCD
C57BL/6N-Atm1Brd Zfyve16tm2a(KOMP)Wtsi/
MbpMmucd

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 (decrease 1°C/cycle)
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	30X
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

CSD-lacF: GCTACCATTACCAGTTGGTCTGGTGTG
CSD-neoF: GGGATCTCATGCTGGAGTTCTTCG
CSD-loxF: GAGATGGCGCAACGCAATTAATG

Gene Specific Primers

CSD-Zfyve16-R: GTCACAGTAGCCCCAACTCACATGC
CSD-Zfyve16-ttR: TTAAGCTCAACTCAAGGCTTTCGCC
CSD-Zfyve16-F2: AAGGATGTTGCATTCCCTAGACTGC

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
Floxed	CSD-loxF	CSD-Zfyve16-R	399
PreCre	CSD-neoF	CSD-Zfyve16-ttR	596
PostCre	CSD-lacF	CSD-Zfyve16-R	697
Wildtype	CSD-Zfyve16-F2	CSD-Zfyve16-ttR	491
PostFlp	CSD-Zfyve16-F2	CSD-Zfyve16-ttR	636
PostFlp & Cre	CSD-Zfyve16-F2	CSD-Zfyve16-R	786

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 Flp or Cre recombination, the Floxed or PreCre primers may be used to identify the mutant mice. The Floxed primers test for the distal LoxP site. The PostCre primers should be used if mutant mice were crossed with a Cre recombinase line (without any FLP recombination). The PostFlp primers should be used if mutant mice were crossed with a Flp recombinase line. The PostFlp & Cre primers should be used if mutant mice were crossed with a Flp recombinase line and then a Cre recombinase line. The wildtype primers should be used for zygosity testing of all mutant mice (pre or post recombination).