

KOMP PCR Design

Mouse PCR Protocol (version 2BW)

Design ID: 220962

Project ID: CSD29330

Selection Cassette: L1L2_Bact_P



MMRRC Stock #: 048551-UCD

C57BL/6N-Atm1Brd Mcamt1(KOMP)Wtsi/

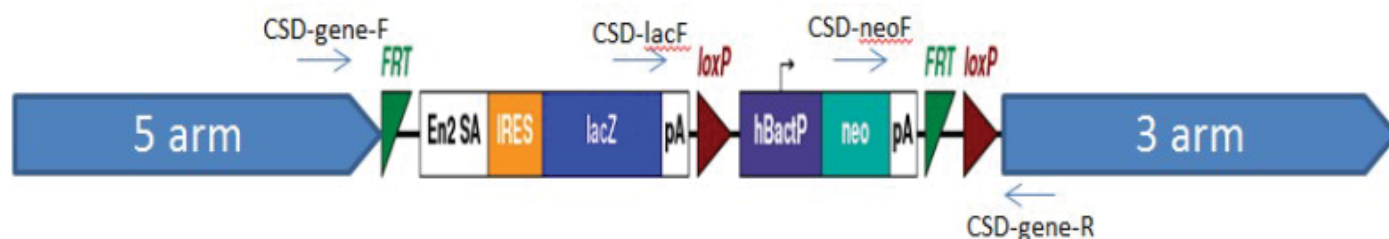
Mmucd

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

CSD-lacF: GCTACCATTACCAGTTGGTCTGGTGTGTC
CSD-neoF: GGGATCTCATGCTGGAGTTCTTCG

Gene Specific Primers

CSD-Mcam-R: AGGAAGGGAGAATTGGGGAAGATGG
CSD-Mcam-F: ATCATAGCCTCTGGCTAGAGTTGGC
CSD-Mcam-wtF: gtggctgtgatagtgtacctgg

Genotype Forward Primer Reverse Primer Amplicon size (bp)

PreCre	CSD-neoF	CSD-Mcam-R	613
PostCre	CSD-lacF	CSD-Mcam-R	564
Wildtype	CSD-Mcam-wtF	CSD-Mcam-R	287
PostFlp	CSD-Mcam-F	CSD-Mcam-R	807

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 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 PostFlp primers should be used if mutant mice were crossed with a Flp recombinase line. The wildtype primers should be used for zygosity testing of all mutant mice (pre or post recombination).