

KOMP PCR Design

Mouse PCR Protocol (version 1)

Design ID: 82706

Project ID: CSD79975

Selection Cassette: L1L2_Bact_P

MMRRC @ UC Davis
Mutant Mouse Resource & Research Centers supported by NIH

MMRRC Stock #: 047369-UCD

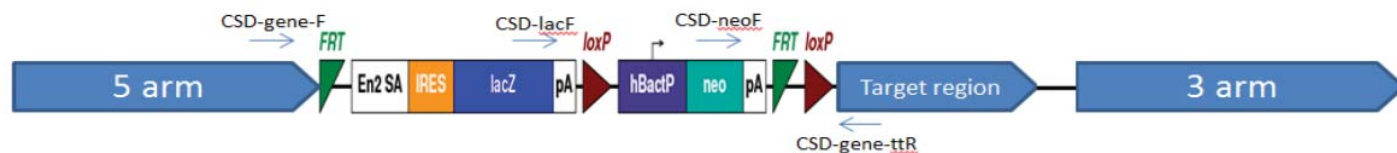
C57BL/6N-Atm1Brd Dip2atm1e(KOMP)Wtsi/

MbpMmucd

Suggested DNA Prep: DNeasy® Tissue Kit

Reagent	1X (µL)	Cycling Parameters	
		Temperature °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 µM each)	0.5	72	40 sec
Taq 5U/µL (AmpliTaq, AB)	0.2	72	5 min
total cocktail	23	4	finished
template	2		
reaction volume	25		

Primer Strategy



Cassette Primers

CSD-lacF: GCTACCATTACCAGTTGGTCTGGTGTC
CSD-neoF: GGGATCTCATGCTGGAGTTCTTCG

Gene Specific Primers

CSD-Dip2a-ttR: ATCGTTTACACTGAGTCCTGTCTGG
CSD-Dip2a-F: TGTGATGTGAGTGAGGGTCTTGCC

Genotype Forward Primer Reverse Primer Amplicon size (bp)

PreCre	CSD-neoF	CSD-Dip2a-ttR	517
PostCre	CSD-lacF	CSD-Dip2a-ttR	519
Wildtype	CSD-Dip2a-F	CSD-Dip2a-ttR	419
PostFlp	CSD-Dip2a-F	CSD-Dip2a-ttR	561

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).