

KOMP PCR Design

Mouse PCR Protocol (version 1)

Design ID: 103919

Project ID: CSD120312

Selection Cassette: L1L2_Bact_P



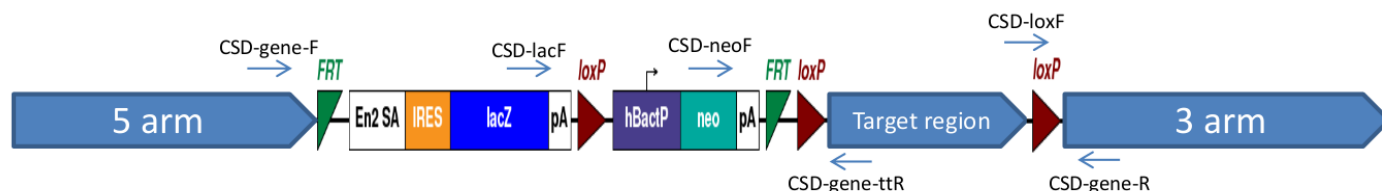
MMRRC Stock #: 050700-UCD

C57BL/6N-Atm1Brd Cnbptm1a(KOMP)Wtsi/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

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

Gene Specific Primers

CSD-Cnbp-R: ACTAGTTCCAAGCCCAAAACATCAGC
 CSD-Cnbp-ttR: ACGCCATATATGCTTACTGCTCAAGG
 CSD-Cnbp-F: GTGGTATGCTGCAGTGACTCAAAGG

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
Floxed	CSD-loxF	CSD-Cnbp-R	365
PreCre	CSD-neoF	CSD-Cnbp-ttR	586
PostCre	CSD-lacF	CSD-Cnbp-R	663
Wildtype	CSD-Cnbp-F	CSD-Cnbp-ttR	571
PostFlp	CSD-Cnbp-F	CSD-Cnbp-ttR	680
PostFlp & Cre	CSD-Cnbp-F	CSD-Cnbp-R	752