

KOMP PCR Design

Mouse PCR Protocol (version 1JE)

Design ID: 234768

Project ID: CSD28041

Selection Cassette: L1L2_Bact_P



MMRRC Stock #: 049470-UCD

C57BL/6N-Atm1Brd Rln3tm1(KOMP)Mbp/

MbpMmucd

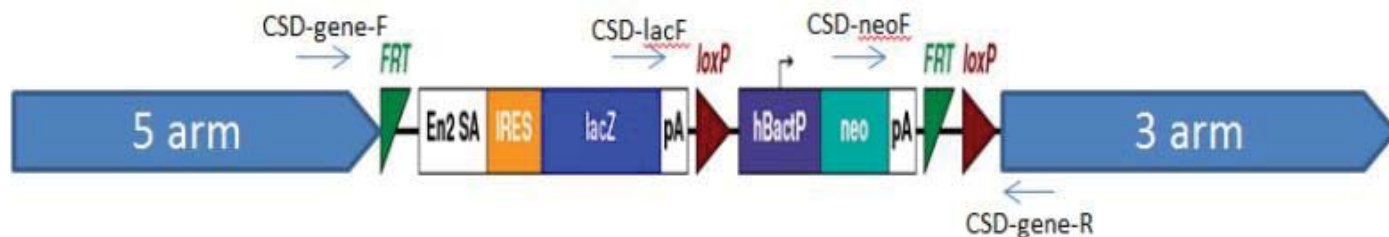
Suggested DNA Prep: DNeasy®Tissue Kit

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	10X (decrease 1°C/cycle)
65	30 sec	
72	40 sec	
94	15 sec	30X
55	30 sec	
72	40 sec	
72	5 min	
4	finished	

Primer Strategy



Cassette Primers

CSD-lacF: GCTACCATTACCAGTTGGTCTGGTGTC
CSD-neoF: GGGATCTCATGCTGGAGTTCTTCG

Gene Specific Primers

CSD-R: CCTGTAGGAATGCTCTTGAACATCG
CSD-F: CATGAACTCAGTGACAGGAAAGTCC

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
PreCre	CSD-neoF	CSD-R	555
PostCre	CSD-lacF	CSD-R	506
Wildtype	CSD-F	CSD-R	484
PostFlp	CSD-F	CSD-R	428