

KOMP PCR Design

Mouse PCR Protocol (version 1)

Strain ID: Cd47^{tm1a(KOMP)}Mbp

Design ID: 233459

Project ID: CSD44832

Selection Cassette: L1L2_Bact_P

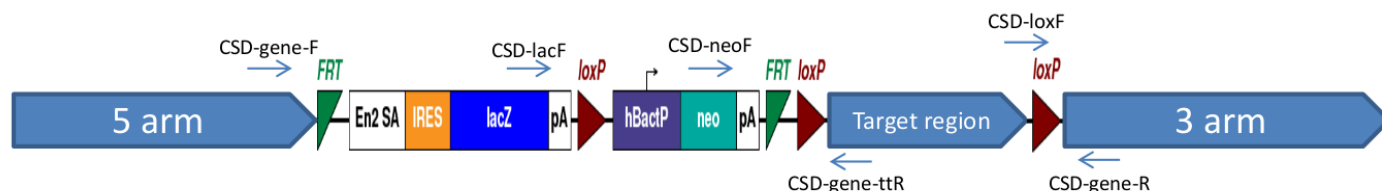


MMRRC Stock #: 065322-UCD

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-Cd47-R: TAATTCAGCAGTCCTGATCCCACCC
 CSD-Cd47-ttR: CTGTCTCTGTGCTCTCTGGCTAAGG
 CSD-Cd47-F: TCTACACTAAACTCAGCTGGCCTGG

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
Floxed	CSD-loxF	CSD-Cd47-R	312
PreCre	CSD-neoF	CSD-Cd47-ttR	603
PostCre	CSD-lacF	CSD-Cd47-R	610
Wildtype	CSD-Cd47-F	CSD-Cd47-ttR	445
PostFlp	CSD-Cd47-F	CSD-Cd47-ttR	658
PostFlp & Cre	CSD-Cd47-F	CSD-Cd47-R	660