

KOMP PCR Design

Mouse PCR Protocol (version 1)

Design ID: 45894

Project ID: CSD24761

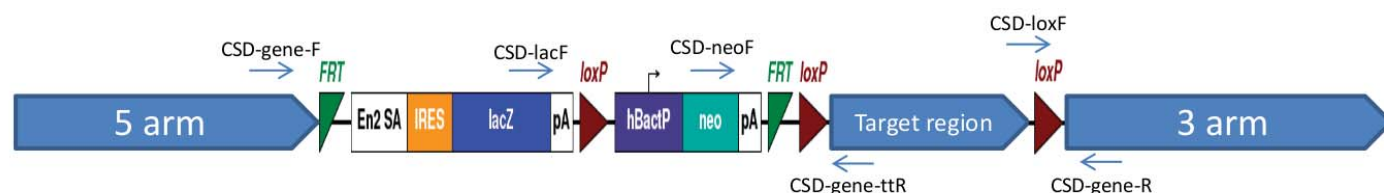
Selection Cassette: L1L2_Bact_P

MMRRC @ UC Davis
 Mutant Mouse Resource & Research Centers supported by NIH
 MMRRC Stock #: 049442-UCD
 C57BL/6N-Rhoutm1a(KOMP)Wtsi/Mmucd

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-Rhou-R: CAAGAATGACCAAACAGAGTGGCCC
 CSD-Rhou-ttR: GTCCAGAGATAGGGTGAGAGCAAGG
 CSD-Rhou-F: GTTACAGCAAGTTCTCAAGGGTGGG

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
Floxed	CSD-loxF	CSD-Rhou-R	340
PreCre	CSD-neoF	CSD-Rhou-ttR	524
PostCre	CSD-lacF	CSD-Rhou-R	638
Wildtype	CSD-Rhou-F	CSD-Rhou-ttR	380
PostFlp	CSD-Rhou-F	CSD-Rhou-ttR	520
PostFlp & Cre	CSD-Rhou-F	CSD-Rhou-R	629

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 Floxed or PreCre primers may be used to identify the mutant mice. The Floxed primers test for the distal LoxP site. The PostCre primers should be used if mutant mice were crossed with a Cre recombinase line (without any FLP recombination). The PostFlp primers should be used if mutant mice were crossed with a Flp recombinase line. The PostFlp & Cre primers should be used if mutant mice were crossed with a Flp recombinase line and then a Cre recombinase line. The wildtype primers should be used for zygosity testing of all mutant mice (pre or post recombination).