

KOMP PCR Design

Mouse PCR Protocol (version 2JE)

Design ID: 45718

Project ID: CSD22703

Selection Cassette: L1L2_Bact_P



MMRRC Stock #: 046578-UCD

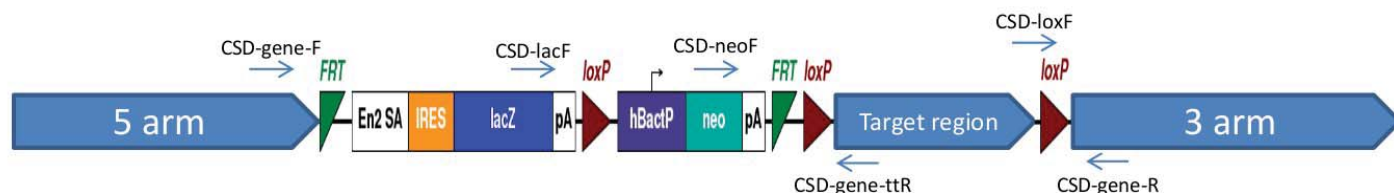
C57BL/6N-Amfrtm1a(KOMP)Wtsi/Mmucd

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

Primer Strategy



Cassette Primers

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

Gene Specific Primers

CSD-Amfr-R:	ACCTCCTGAGTGCTGGGATTAAAGG
CSD-Amfr-ttR:	CTATGGACAGAGTTCCAGGACAGCC
CSD-Amfr-F:	TTACTCAGGAGAGGTTGTGACAGTGG

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
Floxed	CSD-loxF	CSD-Amfr-R	327
PreCre	CSD-neoF	CSD-Amfr-ttR	639
PostCre	CSD-lacF	CSD-Amfr-R	625
Wildtype	CSD-Amfr-F	CSD-Amfr-ttR	486
PostFlp	CSD-Amfr-F	CSD-Amfr-ttR	572
PostFlp & Cre	CSD-Amfr-F	CSD-Amfr-R	607

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).