Mouse PCR protocol
Design ID: 47323
selection cassette: L1L2 Bact P

MMRRC @ UCDavis
Mutant Mouse Resource & Research Centers supported by NIH

MMRRC Stock #: 048309-UCD

C57BL/6N-Kctd15tm1b(EUCOMM)Wtsi/Mmucd

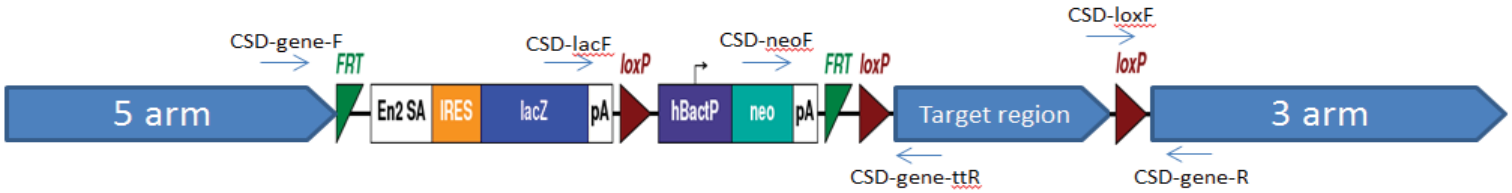
Suggeseted DNA Prep: DNeasy® Tissue Kit

Reagents	1X (uL)
Water (biology grade)	10.725
betain 5M (Sigma)	6.5
dmso (Sigma)	0.325
10X Buffer w/o MgCl2 (AB)	2.5
25 mM MgCl2 (AB)	1.75
10 mM dNTPs (invitrogen)	0.5
Primers (20uM each)	0.5
taq 5U/uL (amplitaq, AB)	0.2
total cocktail	23
template	2
reaction volume	25

Cycling Parameters

Temp C	Time	
94	5 min	
94	15 sec	10X (decrease 1C/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

CSD-loxF: gagatggcgcaacgcaattaatg

Gene Specific Primers

CSD-Kctd15-R: TGTCCTGCCATTGCTAATTCATCG

CSD-Kctd15-ttR: TCCAGGGGCATAAAACAAACTGC

CSD-Kctd15-F: ccctctttgctcttttctagggtacc

Genotypes	Primer pairs	amplicon size (bp)
Floxed	loxF & -R	250
PreCre	neoF & -ttR	607
PostCre	lacF & -R	548
Wildtype	-F & -ttR	340
PostFlp	-F & -ttR	
PostFlp & Cre	-F & -R	

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).