KOMP PCR Design

Mouse PCR Protocol (version 1)

Design ID: 42823
Project ID: CSD34685
Selection Cassette: L1L2_Bact_P

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

MMRRC Stock #: 047251-UCD
C57BL/6N-Ctshtm1a(KOMP)Wtsi/BayMmucd

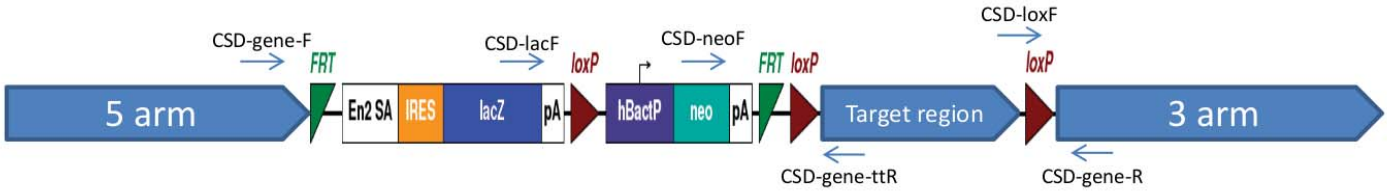
Suggested DNA Prep: DNeasy®Tissue Kit

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	**10X (decrease 1°C/cycle)**
72	40 sec	
94	15 sec	
55	30 sec	**30X**
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-Ctsh-R:	TGGCACAGTACAGCTTTATGCATGG
CSD-Ctsh-ttR:	CAAGGAACTGTGCTAGAGGGAGAGG
CSD-Ctsh-F:	GCCATCCCTTAAAGAACAGACCACC

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
Floxed	CSD-loxF	CSD-Ctsh-R	289
PreCre	CSD-neoF	CSD-Ctsh-ttR	564
PostCre	CSD-lacF	CSD-Ctsh-R	587
Wildtype	CSD-Ctsh-F	CSD-Ctsh-ttR	479
PostFlp	CSD-Ctsh-F	CSD-Ctsh-ttR	660
PostFlp & Cre	CSD-Ctsh-F	CSD-Ctsh-R	678

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).