

KOMP PCR Design

Mouse PCR Protocol (version v1 PK)

Design ID: 41211

Project ID: CSD25784

Selection Cassette: L1L2_Bact_P



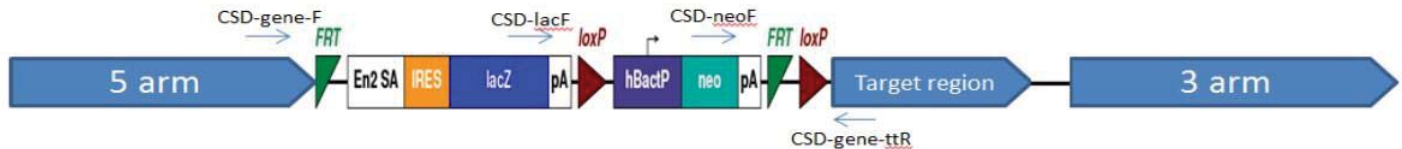
MMRRC Stock #: 049677-UCD

C57BL/6N-Slc1a4tm1e(KOMP)Wtsi/MbpMmucd

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	10X (decrease 1°C/cycle)	
primers (20 μM each)	0.5		
Taq 5U/μL (AmpliTaq, AB)	0.2		
total cocktail	23	94	15 sec
template	2	55	30 sec
reaction volume	25	72	40 sec
		72	5 min
		4	finished
		30X	

Primer Strategy



Cassette Primers

CSD-lacF: GCTACCATTACCAGTTGGTCTGGTGTC
CSD-neoF: GGGATCTCATGCTGGAGTTCTTCG

Gene Specific Primers

CSD-ttR: GAGGTTTCAGAGGGAGAAGCACTGAG
CSD-F: atgacagagattccagcttctctg

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
PreCre	CSD-neoF	CSD-ttR	667
PostCre	CSD-lacF	CSD-ttR	618
Wildtype	CSD-F	CSD-ttR	398
PostFlp	CSD-F	CSD-ttR	601

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 PreCre primers may be used to identify the mutant mice. The PostCre primers should be used if mutant mice were crossed with a Cre recombinase line. The PostFlp primers should be used if mutant mice were crossed with a Flp recombinase line. The wildtype primers should be used for zygosity testing of all mutant mice (pre or post recombination).