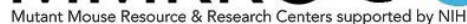


Mouse PCR Protocol (version 1)

Selection Cassette: L1L2_Bact_P



C57BL/6N-Atm1Brd Hic2tm1a(KOMP)Mbp/
Mmucd

Reagent	1X (μL)
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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

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	

Schematic diagram of the CSD gene targeting strategy. The diagram shows a linear DNA construct. On the left is the '5 arm' (blue arrow). This is followed by a green triangle labeled 'FRT'. Then is a cassette: 'En2 SA' (white box), 'IRES' (orange box), 'lacZ' (blue box), and 'pA' (white box). Above this cassette is 'CSD-gene-F' with a blue arrow pointing to the FRT site, and 'CSD-lacF' with a blue arrow pointing to the lacZ gene. This is followed by a red triangle labeled 'loxP'. Then is another cassette: 'hBacP' (purple box), 'neo' (green box), and 'pA' (white box). Above this cassette is 'CSD-neoF' with a blue arrow pointing to the neo gene. This is followed by another green triangle labeled 'FRT' and a red triangle labeled 'loxP'. Then is a blue box labeled 'Target region'. Below this box is 'CSD-gene-ttR' with a blue arrow pointing to the target region. This is followed by a red triangle labeled 'loxP'. Then is a blue box labeled '3 arm'. Above this box is 'CSD-loxF' with a blue arrow pointing to the loxP site. Below this box is 'CSD-gene-R' with a blue arrow pointing to the 3 arm.

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

CSD-Hic2-R:	CTGCAAAGACACAAGCACACATGC
CSD-Hic2-ttR:	CAAATACAGGTGCCAAGCAAAACCC
CSD-Hic2-F:	TGTGTGTGTGTGTGTGTGTAAGTGTGG

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
Floxed	CSD-loxF	CSD-Hic2-R	289
PreCre	CSD-neoF	CSD-Hic2-ttR	597
PostCre	CSD-lacF	CSD-Hic2-R	587
Wildtype	CSD-Hic2-F	CSD-Hic2-ttR	372
PostFlp	CSD-Hic2-F	CSD-Hic2-ttR	574
PostFlp & Cre	CSD-Hic2-F	CSD-Hic2-R	559

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).