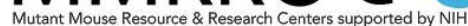


Mouse PCR Protocol (version 1)

Selection Cassette: L1L2_Bact_P



C57BL/6N-Atm1Brd Rnm1tm1a(KOMP)Mbp/
JMmucd

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

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 construct consists of a 5' arm, a targeting cassette, a target region, and a 3' arm. The targeting cassette includes a *lacZ* gene flanked by *FRT* sites and a *neo* gene flanked by *loxP* sites. The *lacZ* gene is used for initial identification, and the *neo* gene is removed. The target region is the CSD gene. The 3' arm contains a *neo* gene flanked by *loxP* sites, which is removed. The final construct contains the 5' arm, the *lacZ* gene, the target region, and the 3' arm.

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

CSD-Rnmt-R: AGACAGACAGACAAGCTCTGTGACC
CSD-Rnmt-ttR: GCCGCTACATTTTAGGATCAGTGGG
CSD-Rnmt-F: AACGGATACTGAGGCTGAATCTGGG

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
Floxed	CSD-loxF	CSD-Rnmt-R	356
PreCre	CSD-neoF	CSD-Rnmt-ttR	498
PostCre	CSD-lacF	CSD-Rnmt-R	698
Wildtype	CSD-Rnmt-F	CSD-Rnmt-ttR	162
PostFlp	CSD-Rnmt-F	CSD-Rnmt-ttR	621
PostFlp & Cre	CSD-Rnmt-F	CSD-Rnmt-R	720

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).