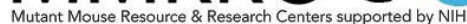


Mouse PCR Protocol (version 1)

Selection Cassette: L1L2_Pgk_P



C57BL/6N-Padi6tm1b(KOMP)Wtsi/MbpMmucd

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	10X (decrease 1°C/cycle)
65	30 sec	
72	40 sec	
94	15 sec	30X
55	30 sec	
72	40 sec	
72	5 min	
4	finished	

The diagram illustrates the targeting strategy for the CSD locus. It shows the 5' arm, a targeting cassette, the Target region, and the 3' arm. The targeting cassette contains the En2 SA, IRES, lacZ, pA, hBacP, neo, and pA. Various CSD genes (CSD-gene-F, CSD-lacF, CSD-neoF, CSD-gene-ttR, CSD-gene-R) are shown with arrows indicating their locations and orientations. Red loxP sites are also indicated.

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

CSD-Padi6-R: ACTCTCCATCCTGCATCAGACATCC
CSD-Padi6-ttR: CTCTTACCCACTGAGCCATCTCTCC
CSD-Padi6-F: CTTCTCATTCTCATTGCTGCTGGG

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
Floxed	CSD-loxF	CSD-Padi6-R	316
PreCre	CSD-neoF	CSD-Padi6-ttR	603
PostCre	CSD-lacF	CSD-Padi6-R	614
Wildtype	CSD-Padi6-F	CSD-Padi6-ttR	439
PostFlp	CSD-Padi6-F	CSD-Padi6-ttR	605
PostFlp & Cre	CSD-Padi6-F	CSD-Padi6-R	642

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).