

Mouse PCR Protocol (version 1)

Selection Cassette: L1L2_Bact_P



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	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	

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

CSD-Gm13119-R: AACACATACGGCTACATGCCAAAGG
CSD-Gm13119-ttR: CACATAGCAACTCAAGCCTTCTGGG
CSD-Gm13119-F: ATGACAGTATTGCAAGCTGTGCTGG

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
Floxed	CSD-loxF	CSD-Gm13119-R	346
PreCre	CSD-neoF	CSD-Gm13119-ttR	594
PostCre	CSD-lacF	CSD-Gm13119-R	644
Wildtype	CSD-Gm13119-F	CSD-Gm13119-ttR	404
PostFlp	CSD-Gm13119-F	CSD-Gm13119-ttR	603
PostFlp & Cre	CSD-Gm13119-F	CSD-Gm13119-R	648

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).