

Mouse PCR Protocol (version 1)

Selection Cassette: L1L2_Bact_P



C57BL/6N-Dusp26tm1a(KOMP)Wtsi/Mmucd

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

CSD-Dusp26-R: GTGGAAGTCAAGCAATACAGAGGGC
CSD-Dusp26-ttR: CCAAACCAAACCAAACCAACCAACC
CSD-Dusp26-F: TTCTTAACCACTGAGCCGTCTCTCC

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
Floxed	CSD-loxF	CSD-Dusp26-R	311
PreCre	CSD-neoF	CSD-Dusp26-ttR	537
PostCre	CSD-lacF	CSD-Dusp26-R	609
Wildtype	CSD-Dusp26-F	CSD-Dusp26-ttR	502
PostFlp	CSD-Dusp26-F	CSD-Dusp26-ttR	540
PostFlp & Cre	CSD-Dusp26-F	CSD-Dusp26-R	607

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).