

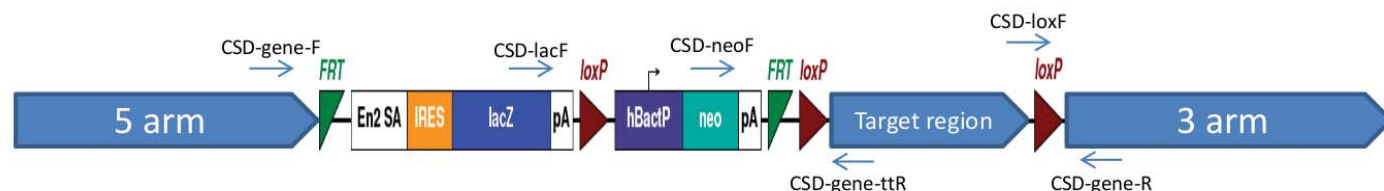
Mouse PCR Protocol (version 1)

Selection Cassette: L1L2_Bact_P



Reagent	1X (μ L)
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 μ M each)	0.5
Taq 5U/ μ L (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	



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

CSD-Tmc3-R: AGAGAGGACAATCCACTCGCCTAGC
CSD-Tmc3-ttR: GGCTTTGGGTATTCTGATAGCAGCC
CSD-Tmc3-F: TTGAAGCAGGGTCCTTGAAGGTAGG

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
Floxed	CSD-loxF	CSD-Tmc3-R	239
PreCre	CSD-neoF	CSD-Tmc3-ttR	513
PostCre	CSD-lacF	CSD-Tmc3-R	537
Wildtype	CSD-Tmc3-F	CSD-Tmc3-ttR	313
PostFlp	CSD-Tmc3-F	CSD-Tmc3-ttR	504
PostFlp & Cre	CSD-Tmc3-F	CSD-Tmc3-R	523

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