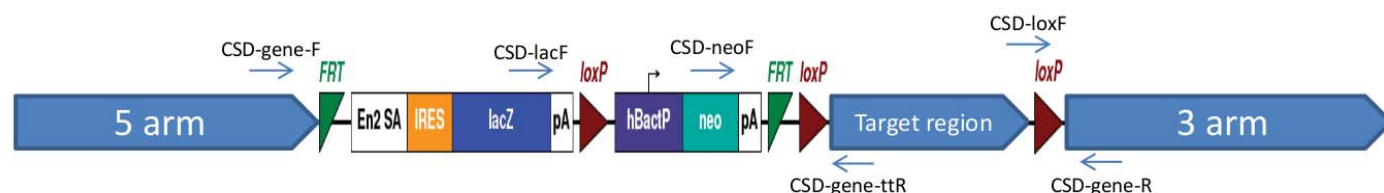


Mouse PCR Protocol (version 1)

Selection Cassette: L1L2_Bact_P



Reagent	1X (μL)	Cycling Parameters	
water (biological grade)	10.725	Temperature °C	Time
betain 5M (Sigma)	6.5	94	5 min
DMSO (Sigma)	0.325	94	15 sec
10X buffer w\o MgCl ₂ (AB)	2.5	65	30 sec
25 mM MgCl ₂ (AB)	1.75	72	40 sec
10 mM dNTPs (Invitrogen)	0.5	94	15 sec
primers (20 μM each)	0.5	55	30 sec
Taq 5U/ μL (AmpliTaq, AB)	0.2	72	40 sec
total cocktail	23	72	5 min
template	2	4	finished
reaction volume	25		



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

CSD-Ppt2-R: AACTCCTGGGCTCAAGTGATCTTGC
CSD-Ppt2-ttR: GTTGCTGGGAATTGAACTCTGGACC
CSD-Ppt2-F: AGGCTAGTTTTTCAGGGTACTGCTCC

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
Floxed	CSD-loxF	CSD-Ppt2-R	245
PreCre	CSD-neoF	CSD-Ppt2-ttR	661
PostCre	CSD-lacF	CSD-Ppt2-R	543
Wildtype	CSD-Ppt2-F	CSD-Ppt2-ttR	651
PostFlp	CSD-Ppt2-F	CSD-Ppt2-ttR	850
PostFlp & Cre	CSD-Ppt2-F	CSD-Ppt2-R	727

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).