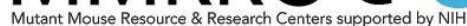


Mouse PCR Protocol (version 1)

Selection Cassette: L1L2-Bact-P



Mmucd

reaction volume 25

4 finished

The diagram illustrates the CSD gene targeting strategy. It shows a linear DNA construct with the following components from left to right: a 5' arm, a selection cassette (En2 SA, IRES, lacZ, pA), a loxP site, a hBactP promoter, a neo cassette (neo, pA), another loxP site, a Target region, and a 3' arm. Various CSD gene constructs are indicated by arrows: CSD-gene-F (green arrow) points to the first FRT site; CSD-lacF (blue arrow) points to the lacZ gene; CSD-neoF (blue arrow) points to the neo gene; CSD-gene-ttR (blue arrow) points to the Target region; and CSD-gene-R (blue arrow) points to the 3' arm. The FRT sites are marked with green triangles, and the loxP sites are marked with red triangles.

CSD-loxF: GAGATGGCGCAACGCAATTAATG

CSD-Nes-F: ATGCAGTTGTCAATCAGAAGGTGCC

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
Floxed	CSD-loxF	CSD-Nes-R	333
PreCre	CSD-neoF	CSD-Nes-ttR	487
PostCre	CSD-lacF	CSD-Nes-R	687
Wildtype	CSD-Nes-F	CSD-Nes-ttR	316
PostFlp	CSD-Nes-F	CSD-Nes-ttR	684
PostFlp & Cre	CSD-Nes-F	CSD-Nes-R	753

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).