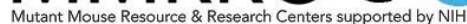


Mouse PCR Protocol (version 1)

Selection Cassette: L1L2_Bact_P

C57BL/6N-Rab36^{tm1b(KOMP)Wtsi/J}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

Schematic diagram of the CSD gene targeting strategy. The construct consists of a 5' arm, a targeting cassette, a target region, and a 3' arm. The targeting cassette includes a *lacZ* gene flanked by *FRT* sites, a *neo* gene flanked by *loxP* sites, and a *hBactP* promoter. The targeting cassette is flanked by *loxP* sites. The target region is flanked by *loxP* sites. The 3' arm is flanked by *loxP* sites. The targeting cassette is flanked by *loxP* sites.

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

CSD-Rab36-R: TGTGTGATGGTTTGTATATGCTGGGC
CSD-Rab36-ttR: TCCTGGGATGCAGGGCAGATATGG
CSD-Rab36-F: CCTATGGATTCTGTGCGAAAGCTGG

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
Floxed	CSD-loxF	CSD-Rab36-R	229
PreCre	CSD-neoF	CSD-Rab36-ttR	576
PostCre	CSD-lacF	CSD-Rab36-R	527
Wildtype	CSD-Rab36-F	CSD-Rab36-ttR	609
PostFlp	CSD-Rab36-F	CSD-Rab36-ttR	814
PostFlp & Cre	CSD-Rab36-F	CSD-Rab36-R	760

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).