

KOMP PCR Design

Mouse PCR Protocol (version 2JE)

Design ID: 35262
Project ID: CSD34496
Selection Cassette: L1L2_gt0



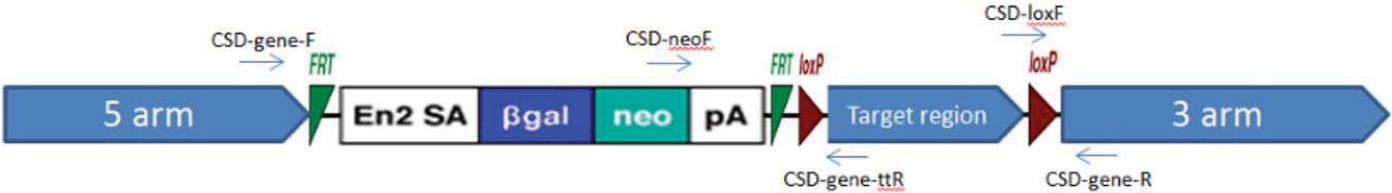
MMRRC Stock #: 049801-UCD

C57BL/6N-Snx5tm1a(KOMP)Wtsi/Mmucd

Suggested ^{35262_KO_PI} DNA Prep: DNeasy® Tissue Kit Parameters

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

Primer Strategy



Cassette Primers

CSD-neoF: GGGATCTCATGCTGGAGTTCTTCG
CSD-loxF: GAGATGGCGCAACGCAATTAATG

Gene Specific Primers

CSD-Snx5-R: GACATGAGCACACTGTTAGTGCAGG
CSD-Snx5-ttR: CTAAGACAATAAAACCCACCGGGCG
CSD-Snx5-F: GCTTTGCCTCTGATTTGGATCTCC

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
Floxed	CSD-loxF	CSD-Snx5-R	386
PreCre	CSD-neoF	CSD-Snx5-ttR	641
PostCre	CSD-neoF	CSD-Snx5-R	733
Wildtype	CSD-Snx5-F	CSD-Snx5-ttR	405
PostFlp	CSD-Snx5-F	CSD-Snx5-ttR	556
PostFlp & Cre	CSD-Snx5-F	CSD-Snx5-R	648

Please note, these primers are auto-designed and may not have been verified by the repository, and as such may require optimization or redesign by your facility.

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).