

KOMP PCR Design

Mouse PCR Protocol (version 1BW)

Design ID: 345

Selection Cassette: L1L2.gt0



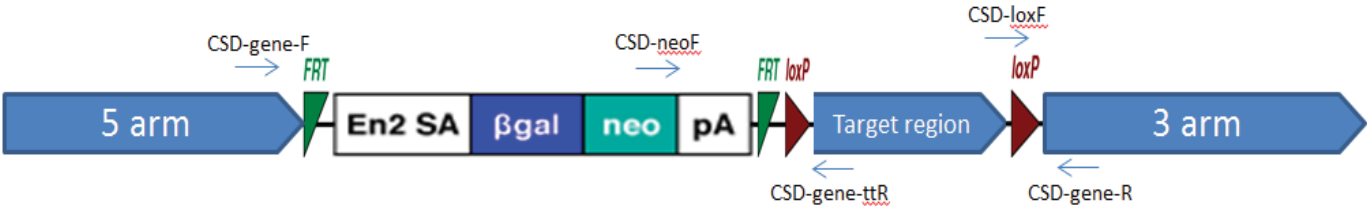
MMRRC Stock #: 048881-UCD

C57BL/6N-Nxntm1a(EUCOMM)Wtsi/JMmucd

Suggested DNA Prep: DNeasy®Tissue Kit

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	10X (decrease 1°C/cycle)	
primers (20 μM each)	0.5		
Taq 5U/μL (AmpliTaq, AB)	0.2		
total cocktail	23	94	15 sec
template	2	55	30 sec
reaction volume	25	72	40 sec
		72	5 min
		4	finished
		30X	

Primer Strategy



Cassette Primers

CSD-neoF: GGGATCTCATGCTGGAGTTCTTCG
CSD-loxF: GAGATGGCGCAACGCAATTAATG

Gene Specific Primers

CSD-R: CATTGTGTATCCCTGGCTAGTGTGG
CSD-ttR: GGAGTGCAATGAGACCTTAACCAG
CSD-F: CTGGGCCTCTAATAGTTGCACTGC

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
Floxed	CSD-loxF	CSD -R	232
PreCre	CSD-neoF	CSD -ttR	604
PostCre	CSD-neoF	CSD -R	579
Wildtype	CSD -F	CSD -ttR	323
PostFlp	CSD -F	CSD -ttR	506
PostFlp & Cre	CSD -F	CSD -R	481

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).