

# KOMP PCR Design

Mouse PCR Protocol (version 1)

Design ID: 48601

Project ID: CSD37568

Selection Cassette: L1L2\_Bact\_P

**MMRRC @ UC Davis**  
Mutant Mouse Resource & Research Centers supported by NIH

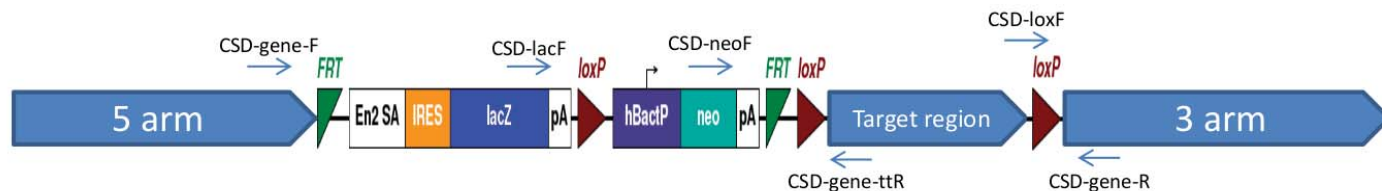
MMRRC Stock #: 046536-UCD

C57BL/6N-Agptm1a(KOMP)Wtsi/Mmucd

## Suggested DNA Prep: DNeasy®Tissue Kit

Reagent	1X (µL)	Cycling Parameters	
		Temperature °C	Time
water (biological grade)	10.725	94	5 min
betain 5M (Sigma)	6.5	94	15 sec
DMSO (Sigma)	0.325	65	30 sec
10X buffer w/o MgCl <sub>2</sub> (AB)	2.5	72	40 sec
25 mM MgCl <sub>2</sub> (AB)	1.75	94	15 sec
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-lacF: GCTACCATTACCAGTTGGTCTGGTGTC  
 CSD-neoF: GGGATCTCATGCTGGAGTTCTTCG  
 CSD-loxF: GAGATGGCGCAACGCAATTAATG

### Gene Specific Primers

CSD-Agps-R: AATGGAGATGAGAATGGGAGGTGGG  
 CSD-Agps-ttR: AAGGAACCCATGCTGGCTATAGTGG  
 CSD-Agps-F: TGAATCAGGATCCAAAGAGTCACCCC

### Genotype Forward Primer Reverse Primer Amplicon size (bp)

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
Floxed	CSD-loxF	CSD-Agps-R	366
PreCre	CSD-neoF	CSD-Agps-ttR	570
PostCre	CSD-lacF	CSD-Agps-R	664
Wildtype	CSD-Agps-F	CSD-Agps-ttR	490
PostFlp	CSD-Agps-F	CSD-Agps-ttR	679
PostFlp & Cre	CSD-Agps-F	CSD-Agps-R	768

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).