

# KOMP PCR Design

Mouse PCR Protocol (version 1)

Design ID: 82550

Project ID: CSD36669

Selection Cassette: L1L2\_Bact\_P



Mutant Mouse Resource & Research Centers supported by NIH

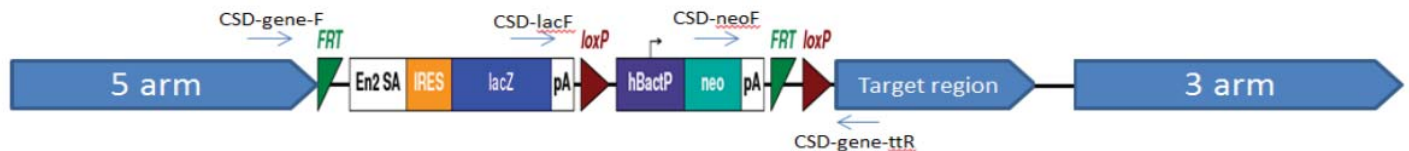
MMRRC Stock #: 050311-UCD

C57BL/6N-Vangl1<sup>tm1e(KOMP)Wtsi/Mmucd</sup>

## 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

### Gene Specific Primers

CSD-Vangl1-ttR: CATCCCTCGAATTGAAAAGTTCTGG  
CSD-Vangl1-F: ATGAGCTGAGACTCCATCTGTGTCC

Genotype	Forward Primer	Reverse Primer	Amplicon size (bp)
PreCre	CSD-neoF	CSD-Vangl1-ttR	549
PostCre	CSD-lacF	CSD-Vangl1-ttR	500
Wildtype	CSD-Vangl1-F	CSD-Vangl1-ttR	623
PostFlp	CSD-Vangl1-F	CSD-Vangl1-ttR	782

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 PreCre primers may be used to identify the mutant mice. The PostCre primers should be used if mutant mice were crossed with a Cre recombinase line. The PostFlp primers should be used if mutant mice were crossed with a Flp recombinase line. The wildtype primers should be used for zygosity testing of all mutant mice (pre or post recombination).