

KOMP PCR Design

Mouse PCR Protocol (version 1BW)

Design ID: 211660

Project ID: CSD47151

Selection Cassette: - -



MMRRC Stock #: 049467-UCD

C57BL/6N-Atm1Brd Ripk3tm1.1(KOMP)Wtsi/

MbpMmucd

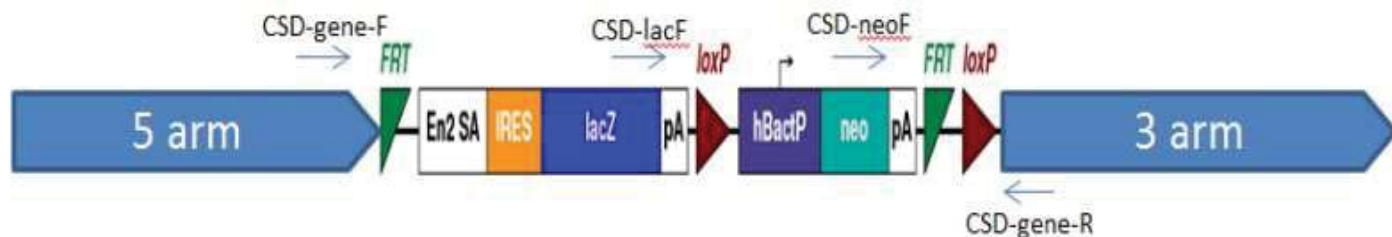
Suggested DNA Prep: DNeasy®Tissue Kit

Reagent	1X (μL)
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

Cycling Parameters

Temperature °C	Time	
94	5 min	
94	15 sec	10X (decrease 1°C/cycle)
65	30 sec	
72	40 sec	
94	15 sec	30X
55	30 sec	
72	40 sec	
72	5 min	
4	finished	

Primer Strategy



Cassette Primers

CSD-lacF: GCTACCATTACCAGTTGGTCTGGTGTC
CSD-neoF: GGGATCTCATGCTGGAGTTCTTCG

Gene Specific Primers

CSD-R: GAGAGTCATCCAAGTGAGAGGTGAGG
CSD-F: AGTGTGGTCACGAGCCTGAGTGG
CSD-wtR: CCACTGGAGGTCCTCAGTGACC

Genotype Forward Primer Reverse Primer Amplicon size (bp)

PreCre	CSD-neoF	CSD-R	527
PostCre	CSD-lacF	CSD-R	478
Wildtype	CSD-F	CSD-wtR	171
PostFlp	CSD-F	CSD-R	334

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).