

## pMCSG29

COOH-terminal His Tag with TEV recognition site, COOH-terminal MBP and TVMV recognition site; LIC cloning site identical to MCSG28

LIC Region:

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          Xba I                               rbs                               G
5' tCT AGA AAT AAT TTT GTT TAA CTT TAA GAA GGA qtc tct ccc GGG E N L Y F Q S A G H H H H H H H H H H H H H
3' aga tcT TTA TTA AAA CAA ATT GAA ATT CTT CCT CAG AGA GGG ccc ctc ttg gAC ATG AAG GTT AGG CGG CCC GTG GTG GTG GTA GTA GTA GTA GTG GTA GTG
                                     Sma I
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          TVMV site                               MBP->
E T V R F Q S K I E E
GAA ACC GTG CGT TTC CAG TCT AAA ATC GAA GAA 3'
CTT TGG CAC GCA AAG GTC AGA TTT TAG CTT CTT 5'
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Vector: digest with Sma I and treat with T4 polymerase and dATP

PCR Products: treat with T4 polymerase and dTTP

Forward Primer: GTC TCT CCC **ATG** . . . (ATG is start codon for the gene of interest followed by gene-specific sequences)

Reverse Primer: TG GTG GTG CCC AXN . . . (followed by gene-specific sequence [reverse complement of final codons]) **Note:** If the final codon of your gene ends in "U" then you only need the extra Gly residue; otherwise a second spacer codon is needed. We normally use Ala (GCT) [see below].

Options for Reverse Primer:

codons for additional spacer amino acids can be added if desired

reverse primer (Gly-Ala): G GTT CTC CCC AGC ...  
← L N E G A