

## pMCSG28

COOH-terminal His Tag with TEV site (constructed from pMCSG26)  
forward primer identical to pMCSG26; reverse primer specific for pMCSG28 (and pMCSG29)

LIC Region:

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      Xba I                               rbs                               G   TEV site
5' tCT AGA AAT AAT TTT GTT TAA CTT TAA GAA GGA qtc tct ccc GGG GAG AAC CTG TAC TTC CAA TCC GGC GGG CAC CAC CAT CAT CAT CAT TAA CGg atc c 3'
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 GTA GTA GTA GTA ATT GCC TAG g 5'
                                     Sma I
                                     BamH I
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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: G GTT CTC 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].

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