pMCSG27

COOH-terminal His Tag with COOH-terminal MBP and TVMV recognition site; LIC cloning site identical to MCSG26

LIC Region:

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                                                                                                                     TVMV site             MBP->
    Xba I                                 rbs                G   H   H   H   H   H   H   H   H   H   H   E   T   V   R   F   Q   S   K   I   E
5' tCT AGA AAT AAT TTT GTT TAA CTT TAA GAA GGA gtc tct ccc GGG CAC CAC CAT CAT CAT CAT CAC CAC CAT CAC GAA ACC GTG CGT TTC CAG TCT AAA ATC GAA 3'
3' aga tcT TTA TTA AAA CAA ATT GAA ATT CTT CCT CAG AGA GGG ccc gtg gtg gtA GTA GTA GTA GTG GTG GTA GTG CTT TGG CAC GCA AAG GTC AGA TTT TAG CTT 5'
                                                        Sma I
```

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 AXX . . . (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
a TEV (or other) cleavage site can be added if desired

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reverse primer (Gly-Ala):         TG GTG GTG CCC AGC ...
                                ≤ H   H   H   G   A

reverse primer (Gly-Ala-TEV):     TG GTG GTG CCC AGC GGA TTG GAA GTA CAG GTT CTC ...
                                ≤ H   H   H   G   A   S   Q   F   Y   L   N   E
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