pMCSG26

COOH-terminal His Tag constructed from pMCSG

LIC Region:

```
    Xba I                                  rbs                G   H   H   H   H   H   H   ·
5′ tCT AGA AAT AAT TTT GTT TAA CTT TAA GAA GGA gtc tct ccc 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 gtg gtg gtA GTA GTA GTA ATT GCC TAG g 5′
                                                    Sma I                                BamH 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
additional His codons could be added although the Gly residue will separate the new codons from the original six His residues
a TEV (or TVMV) 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
```