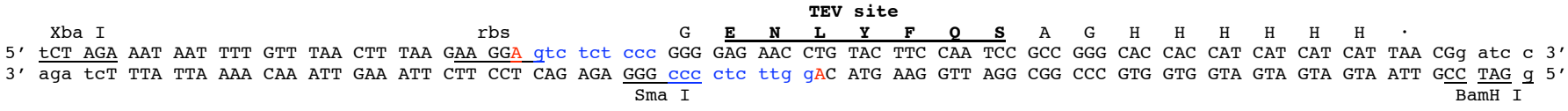
pMCSG28

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

LIC Region:



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 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].

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