Bioinformatics question (see last page for vector):

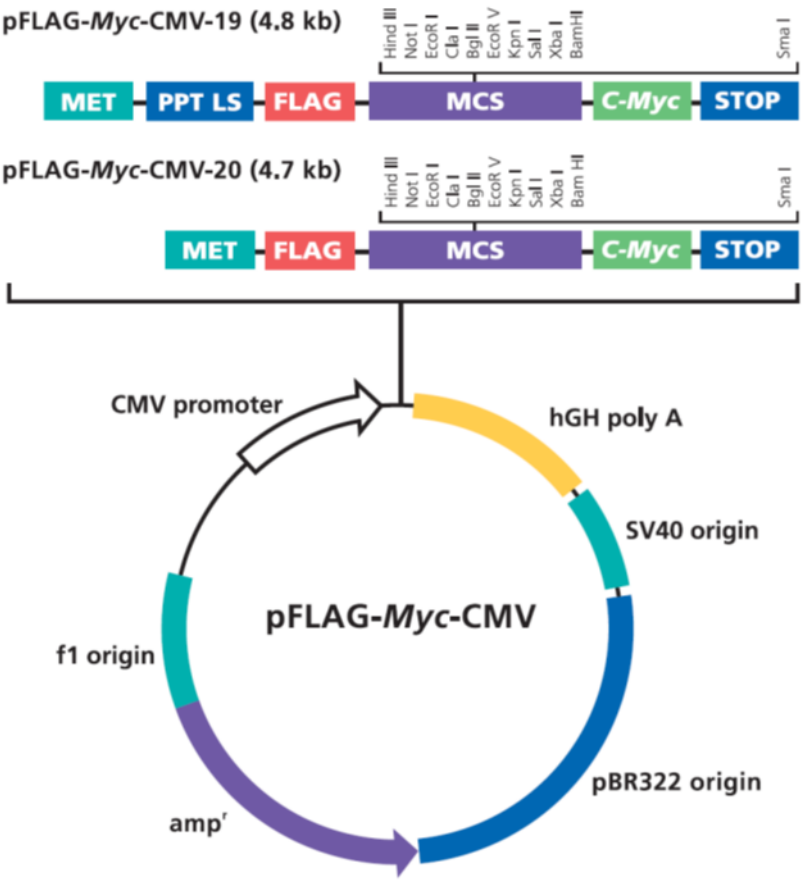
a Here are the forward and reverse primers for a hypothetical gene X.

5’ – ATG GCA ATC CAA TCA ATA GGT CG – 3’

5’ – TTA ACC CTT GAT GAT CGT TCT CC – 3’

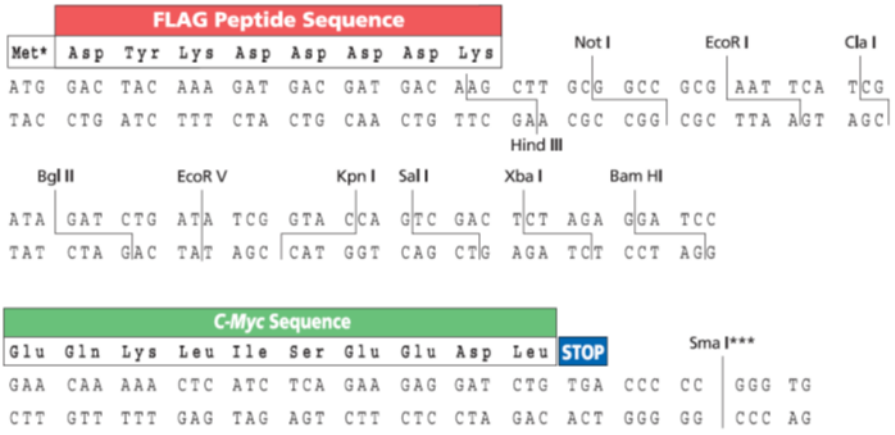
As per the map in page 2,modify the primers above in a way that will allow directional cloning of the gene X to include the FLAG tag upstream and c-myc protein tag downstream of gene X. **After you are finished, the vector should express ONLY the one form of the labeled protein, specifically both tags flanking protein X at the appropriate ends.** You can use any of the enzymes **except Sal I and Xba I**. You may modify the primer sequences by adding and removing (cross-out) the necessary nucleotides to satisfy the conditions of this task.

b. Why can you NOT use Sma I only to clone in the gene?



Multiple Cloning Site

(pFLAG-Myc-CMV-19* and pFLAG-Myc-CMV-20**)



*For pFLAG-*Myc*-CMV-19, the Met-preprotrypsin leader sequence (PPT LS) precedes the FLAG coding sequence.

**pFLAG-*Myc*-CMV-20 has two less C-G base pairs just 5' of the Sma I site.

***Using the Sma I site with another restriction site in the MCS for directional cloing will result in loss of the *c-myc* tag.