

Bioinformatics question (see last page for vector):

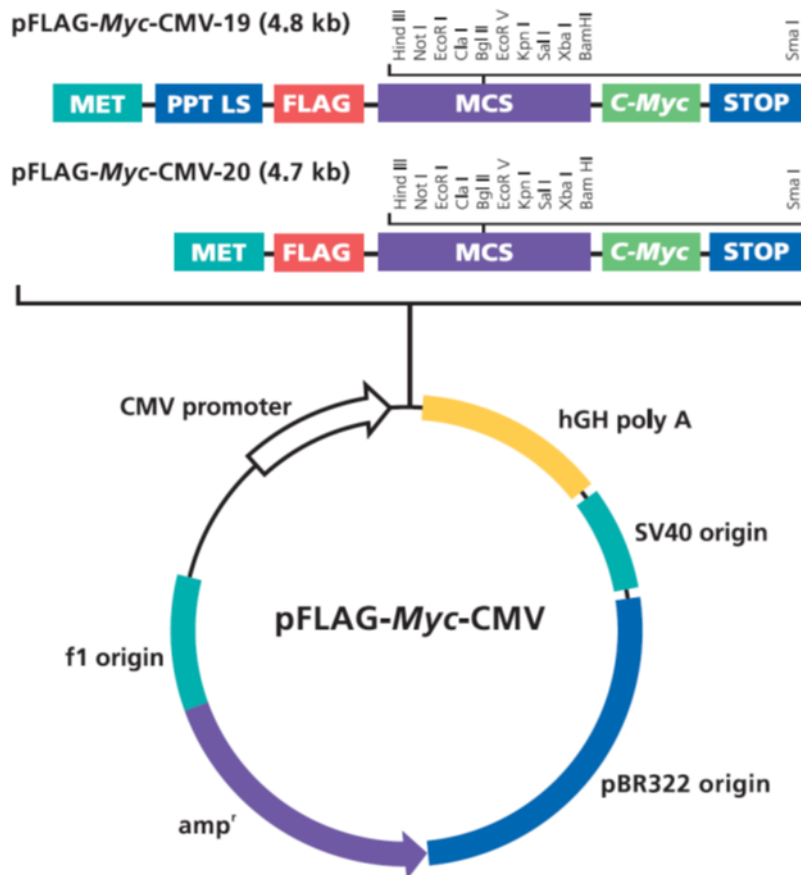
a Here are the forward and reverse primers for a hypothetical gene X.

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**)

FLAG Peptide Sequence																
Met*	Asp	Tyr	Lys	Asp	Asp	Asp	Asp	Lys		Not I	EcoR I	Cla I				
ATG	GAC	TAC	AAA	GAT	GAC	GAT	GAC	AG	CTT	GCG	GCC	GCG	AAT	TCA	TCC	
TAC	CTG	ATC	TTT	CTA	CTG	CAA	CTG	TTC	GAA	CGC	CGG	CGC	TTA	AGT	AGC	
										Hind III						
										Bgl II	EcoR V	Kpn I	Sal I	Xba I	Bam HI	
ATA	GAT	CTG	ATA	TCG	GTA	CCA	GTC	GAC	TCT	AGA	GGA	TCC				
TAT	CTA	GAC	TAT	AGC	CAT	GGT	CAG	CTG	AGA	TCT	CCT	AGG				

C-Myc Sequence															
Glu	Gln	Lys	Leu	Ile	Ser	Glu	Glu	Asp	Leu	STOP					
GAA	CAA	AAA	CTC	ATC	TCA	GAA	GAG	GAT	CTG	TGA	CCC	CC	GGG	TG	
CTT	GTT	TTT	GAG	TAG	AGT	CTT	CTC	CTA	GAC	ACT	GGG	GG	CCC	AG	

*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 cloning will result in loss of the c-myc tag.