

Bioinformatics question (see last page for vector):

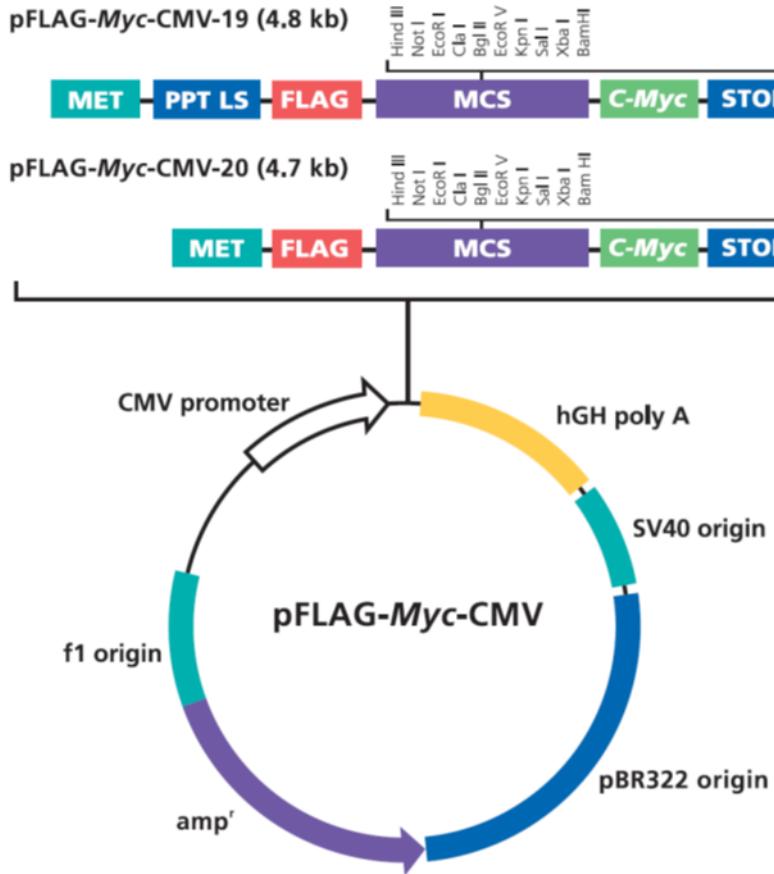
a Here are the forward and reverse primers for a hypothetical gene X.

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\*\*) (100 µg)

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