HOMEWORK #10

You would like to express a cloned DNA fragment as a fusion protein with glutathione-S-transferase (GST). Your DNA fragment is currently in another plasmid and the reading frame of the insert fragment is indicated. The reading frame of GST in the pGEX-5X-3 vector is indicated.

Describe how you would go about making a recombinant construct expressing the cloned DNA fragment as a fusion protein with GST. Discuss the resolution of any potential problems such as orientation. Show the nucleic acid and protein sequences of the final construct. (pdf file for easy printing)

Not sure how to get started? See diagram below.

Some Tips:

- link to text file of the sequences (or cut-and-paste sequences from below)
- use courier, letter gothic or other non-proportional font
- in MSWord hold down alt-key while dragging mouse to block 'columns'

Plasmid containing insert DNA:

```
..| XhoI || SalI | ...| ClaI||NsiI| ..................| SalI|
CCC TCG AGG TCG ACG GTA TCG ATG CAT .....insert.......GTC GAC CAC
GGG AGC TCC AGC TGC CAT AGC TAC GTA ..................CAG CTG GTG
Pro Ser Arg Ser Thr Val Ser Met His ..................Val Asp His

......|EcoRI || PstI || SmaI || BamHI|| SpeI || XbaI |
CCA TCG AAT TCC TGC AGC CCG GGG GAT CCA CTA GTT CTA GAG
GGT AGC TTA AGG ACG TCG GGC CCC CTA GGT GAT CAA GAT CTC
Pro Ser Asn Ser Cys Ser Pro Gly Asp Pro Leu Val Leu Glu
```

pGEX-5X-3:

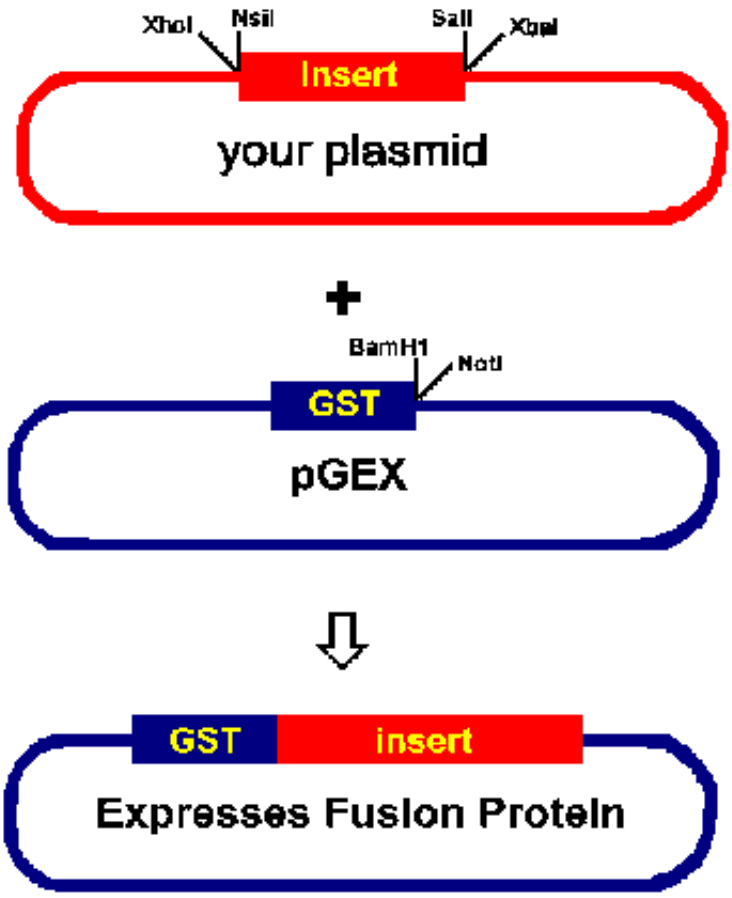
```
..........................|BamH1 | ....|EcoR1 ||SmaI |SalI | XhoI | NotI |
...GST... ATC GAA GGT CGT GGG ATC CCC AGG AAT TCC CGG GTC GAC TCG AGC GGC CGC
......... TAG CTT CCA GCA CCC TAG GGG TCC TTA AGG GCC CAG CTG AGC TCG CCG GCG
......... Ile Glu Gly Arg Gly Ile Pro Arg Asn Ser Arg Val Asp Ser Ser Gly Arg
..........| Factor Xa |

ATC GTG ACT GAC TGA
TAG CAC TGA CTG ACT
Ile Val Thr Asp Stop
```

Restriction Sites	
*Bam*HI	G↓GATCC
*Cla*I	AT↓CGAT

*Eco*RI	G↓AATTC	
*Not*I	GC↓GGCCGC	
*Nsi*I	ATGCA↓T	compatible with PstI
*Pst*I	CTGCA↓G	compatible with *Nsi*I
*Sal*I	G↓TCGAC	compatible with *Xho*I
*Spe*I	A↓CTAGT	compatible with *Xba*I
*Sma*I	CCC↓GGG	blunt ends
*Xba*I	T↓CTAGA	compatible with *Spe*I
*Xho*I	C↓TCGAG	compatible with *Sal*I

Hint

The goal is to identify restriction enzymes that will allow you to subclone (i.e., move) the 'insert' fragment from its current vector into the expression vector pGEX so that the insert is in the correct orientation and in the correct reading frame as illustrated schematically in the following figure:



Try to identify restriction site(s) which will remove the insert and allow that insert to be in frame with the GST. The outermost restriction sites flanking the insert and the multiple cloning site of pGEX are shown in the figure. See above for the complete restriction sites.