

## HOMEWORK #10

Due April 21, 2008

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.** (A text file of the sequences and some hints are available at <http://www.tulane.edu/~wiser/methods/homework/hw10.html> for persons wishing to use a word processor in solving this problem.)

### Plasmid containing insert DNA:

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| XhoI || SalI | | ClaI || NsiI | | SalI | | EcoRI || PstI || SmaI || BamHI || SpeI || XbaI |
CCC TCG AGG TCG ACG GTA TCG ATG CAT .....insert.....GTC GAC CAC CCA TCG AAT TCC TGC AGC CCG GGG GAT CCA CTA GTT CTA GAG
GGG AGC TCC AGC TGC CAT AGC TAC GTA .....CAG CTG GTG GGT AGC TTA AGG ACG TCG GGC CCC CTA GGT GAT CAA GAT CTC
Pro Ser Arg Ser Thr Val Ser Met His .....Val Asp His Pro Ser Asn Ser Cys Ser Pro Gly Asp Pro Leu Val Leu Glu

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### pGEX-5X-3:

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...GST... ATC GAA GGT CGT GGG ATC CCC AGG AAT TCC CGG GTC GAC TCG AGC GGC CGC ATC GTG ACT GAC TGA
..... TAG CTT CCA GCA CCC TAG GGG TCC TTA AGG GCC CAG CTG AGC TCG CCG GCG TAG CAC TGA CTG ACT
..... Ile Glu Gly Arg Gly Ile Pro Arg Asn Ser Arg Val Asp Ser Ser Gly Arg Ile Val Thr Asp Stop
| Factor Xa |

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### Restriction sites

BamH1	G↓GATCC	
ClaI	AT↓CGAT	
EcoRI	G↓AATTC	
NotI	GC↓GGCCGC	
NsiI	ATGCA↓T	compatible with PstI
PstI	CTGCA↓G	compatible with NsiI
SalI	G↓TCGAC	compatible with XhoI
SpeI	A↓CTAGT	compatible with XbaI
SmaI	CCC↓GGG	blunt ends
XbaI	T↓CTAGA	compatible with SpeI
XhoI	C↓TCGAG	compatible with SalI