Your Turn Assignment - Design your own insert.

You have a sequence of a portion of a protein you wish to clone into a fusion protein called Glutathione S Transferase (GST) using a fictional GST plasmid.

- Which of the pGSTplasmids do you use? a, b or c?
- You are going to cut in with BamHI and EcoRI.
- Ensure you have the right reading frame for start and a stop at the end of the inserted sequence. Remember that GST is 5' to your insert. That is where the "#1 bp" is located
- Google search for "Restriction Sequence Translator" to find a program to map for where the RE cuts sites are
- Sequence (DNA and amino acid)

NOTE The DNA sequence does NOT start with the first coding bp

GGATCCTGTAGATCTGCTGGCAGTTAAGAAGAAGCAGGAAACCAAACGTAGCATCAATGAGGAGATTCATACC
CAGTTCCTGGATCATCTGCTGACTGGCATCGAGGACATCTGCGGTCACTATGGTCACCATCACGAATTC

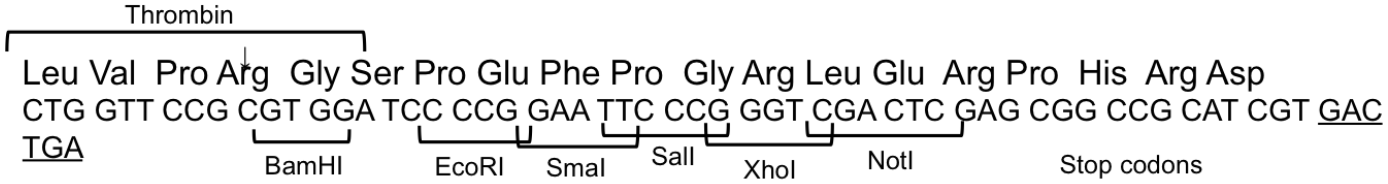
VDLLAVKKKQ ETKRSINEEI HTQFLDHLLT GIEDICGHYG HHH

- Find the map and defend your choice!
- Explain where GST lies in terms of your insert and the N to C terminus of your fusion protein.

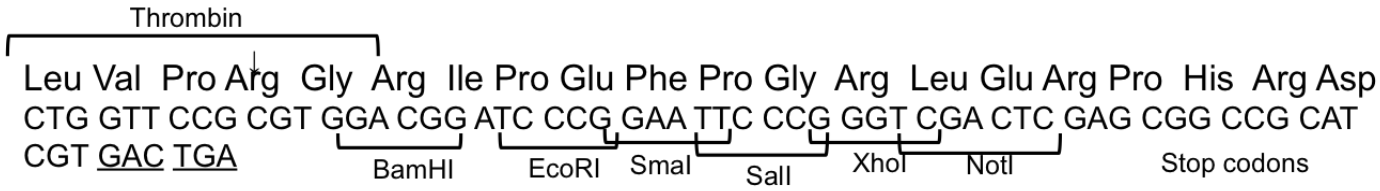
HINTS

- Use the DNA to amino acid translator to find the coded amino acid sequence in the DNA sequence.
- Where is the first coding base pair? Is it the same as the first bp in the DNA sequence?
- What is the reading frame for the DNA sequence?
- Use an amino acid to DNA translator to find the DNA sequence and double check your work.
- You may want to use the codon table to double check yourself
- Look up the restriction site sequence AND find where they cut the DNA sequence for BOTH the insert AND the vector.
- DON'T just rely on a program that gives the bp number, convince yourself by looking for the sequence to confirm things…

pGST-Xa



pGST-Xb



pGST-Xc