*Type (word process) and submit as a* ***PDF*** *file on Bb*

*Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*

1. You were given a plasmid (pGEX4T1) containing a plant clone for a protein called Rubisco. The protein has an affinity tag on the N terminus. Look up the plasmid and determine the appropriate antibiotic to use. What strain of bacteria should you use? What is the affinity tag and the basic properties of the tag?
2. Your first and second attempt to purify the affinity tagged Rubisco protein, very little soluble protein is found. Thankfully not much was located in the inclusion bodies. You have a friend with a mass spec and found that some of the N terminus of the protein is expressed but it seems the production was paused and truncated, leaving a smaller version of the protein. What do you think is going on? Email me for a hint if you are totally stuck.
3. Explain what the pET vectors need in terms of “helpers” for expression. Something about the polymerase...
4. What is IPTG in terms of recombinant protein expression? Outline the process of expression and where IPTG is used in the culture and expression of recombinant proteins. (I am purposefully being a little vague here – show me what you’ve learned)
5. Research the affinity His Tag. Is it really just 6 His amino residues in a row? How does imidazole cause a His-tagged protein to elute from a nickel column?
6. How do you know which and how many fractions to pool after a recombinant protein column chromatography?