1. Did we use SDS-PAGE to purify or verify purification of our recombinant DHFR protein? Explain your answer. (3pt)

2. Imagine we were to use an anti-His tag primary antibody for our Western Blot analysis. Based on the data your lab section obtained this semester, indicate your expected results by completing the chart below: (3pt)

| | **anti-His** | |
|---|---|---|
| **Sample** | **Number of bands** | **Size of each band (kDa)** |
| *GST-DHFR-His | | |
| His-tagged DHFR | | |
| Myc-Flag-tagged DHFR lysate | | |
| Control lysate | | |

   *Which specific data obtained provided rationale for your above expected result for our GST-DHFR-His sample and why? List and briefly explain all that apply. (2pt)

3. We anticipate our recombinant DHFR protein to be functional for use in future research applications. However, if you had been able to test functionality and found that your recombinant GST-DHFR-His protein was not functional, what would you propose is one potential issue you would investigate to troubleshoot further. In other words, what is one thing that could have gone wrong to result in non-functional protein from the method used and results obtained this semester? Please be specific with the issue, where in the procedure it would have occurred, and explain your rationale. (4pt)

4. Use the DHFR fusion protein nucleotide sequence provided below. Hint: the DHFR fusion protein sequence you have been provided with here only contains one tag.
   a. Which reading frame (5'3') did you use to translate the sequence: 1, 2, or 3? _____ (2pt)

   b. Where is the tag located: At the N-terminus or C-terminus of DHFR? _____ (2pt)

   c. **Briefly describe** how you found the tag location in (b.) above. (2pt)

d. What is the tag used: Myc, FLAG, GST, or His? _____ (2pt)

e. What is the calculated molecular weight of this fusion protein? _____kDa (2pt)

**DHFR fusion protein nucleotide sequence:**

ATGGTTGGTTCGCTAAACTGCATCGTCGCTGTGTCCCAGAACATGGGCATCGGCAAGAACGGGGACCTGCCCTGGCCACCGCTCAGGAA
TGAATTCAGATATTTCCAGAGAATGACCACAACCTCTTCAGTAGAAGGTAAACAGAATCTGGTGATTATGGGTAAGAAGACCTGGTTCTCC
ATTCCTGAGAAGAATCGACCTTTAAAGGGTAGAATTAATTTAGTTCTCAGCAGAGAACTCAAGGAACCTCCACAAGGAGCTCATTTTCTTT
CCAGAAGTCTAGATGATGCCTTAAAACTTACTGAACAACCAGAATTAGCAAATAAAGTAGACATGGTCTGGATAGTTGGTGGCAGTTCTGT
TTATAAGGAAGCCATGAATCACCCAGGCCATCTTAAACTATTTGTGACAAGGATCATGCAAGACTTTGAAAGTGACACGTTTTTTCCAGAA
ATTGATTTGGAGAAATATAAACTTCTGCCAGAATACCCAGGTGTTCTCTCTGATGTCCAGGAGGAGAAAGGCATTAAGTACAAATTTGAAG
TATATGAGAAGAATGATCATCACCATCACCATCACTAA

5. Once you have expressed and purified your DHFR fusion protein from the sequence provided above, you want to determine the concentration of the purified protein sample for further analysis. Use the following information to answer question parts a-d below:

   **Standard curve equation:** y = 0.0024x + 0.018

   **Dilution of purified protein sample:** 1:5

   **Absorbance value of protein dilution:** 0.474 (can assume this value is within range of the curve)

   a. What is the concentration of the dilution analyzed? _____ug/mL (2pt)

   b. What is the concentration of your original (undiluted) sample? _____ug/mL (2pt)

   c. Convert your protein concentration above in (b.) from ug/mL to ug/uL: _____ug/uL (1pt)

   d. Complete the following table to prep your DHFR fusion protein sample for SDS-PAGE. You are looking to load 15ug in a total volume of 40uL. (3pt)

| **Protein Concentration (ug/uL) From (c.) above** | **Concentration of sample + 2X Laemmli (ug/uL) (Bringing Laemmli to 1X)** | **Volume (uL) of sample+Laemmli mix needed for 15ug** | **Volume (uL) of 1X Laemmli solution to bring total vol to 40ul** |
|---|---|---|---|
| | | | |

6. Imagine you produce, purify, and analyze this DHFR fusion protein from question 4 & 5 above by Western Blot using an anti-DHFR primary antibody. You obtain the following unexpected result: There is no band

present from your DHFR fusion protein sample nor is there a band present from the purified His-tagged DHFR control sample. Would any of the following errors likely explain this unexpected result? Yes or No and why for each? (6pt)

a. The transfer apparatus was connected to the wrong electrodes (electrical current was provided in the wrong direction) and proteins did not transfer onto the nitrocellulose membrane

b. You accidentally added an anti-FLAG antibody instead of an anti-DHFR antibody at the primary antibody incubation step

c. You forgot to perform the wash steps during purification, so your DHFR sample was contaminated with other proteins.

7. When screening for new DHFR inhibitor molecules to treat bacterial infections, do you want the molecule to specifically target prokaryotic DHFR, eukaryotic DHFR, or both? Briefly explain your rationale. (2pt)

8. Which Labster simulation (Molecular Cloning, Protein Synthesis, Signal Transduction, ELISA) do you feel was most helpful to your understanding of our recombinant DHFR project procedures **and why**? (2pt)

   *(For a reminder of the content and skills covered in each, you can visit the Simulation page summary for each in the Week 2, 3, 7, and 10 Canvas modules.)*