Fall '13 **CH370** Name _____

Hackert **HW- 1 (20 pts) – Due Sept. 24 (8:00 am)** UT eID _____

(Complete this graded homework independently, place all answers on this page, show work below, on back, or on attached pages. No credit for late work)

1. Terminology:

(2) a) Which amino acid's side chain would have an expected pKa around 8? _____

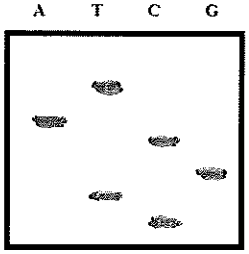
b) Name two differences in conformational characteristics between B-DNA and Z-DNA:

_____/_____

2. Estimate the pI of the oligopeptide: P – R – Y – I – C – A – T – E **(pI ~ _____)**

(2)

3. Given the following dideoxy sequencing gel result, what is the sequence (5'→ 3') of the original



Template? (2) _____

4. What is the role of luciferase and name of the sequencing technique that utilizes this protein?

(2) _____

5. Size, charge, polarity and affinity are all characteristics of proteins that can be exploited to purify a protein, name one separation technique for each of these characteristics:

Property:	Size	Charge	Polarity	Affinity
(4) Technique:	_____	_____	_____	_____

6. What is the percentage yield for a purification step that started with 47 mL of solution at 1.1 mg/mL with a specific activity of 356 and ended with 14 mL of solution at 2.4 mg/mL with a specific activity of 511? _____

(2)

7. Consider a "gel filtration" column that is 100 cm in length and 2.50 cm in diameter. It is packed with spherical beads that are on average 0.22 mm in diameter with a density of 1.33 g/cm^3. Assume that V_o is 36% of V_{tot}. The column is calibrated with trypsin inhibitor (~21.5 kD) and β-galactosidase (~116 kD) which gave Ve /Vo values of 2.50 and 1.50, respectively. What is the best estimate of **molecular mass (kD)** for an **unknown** protein with **Ve /Vo = 2.15?**

(M ~ _____ kD)

(3)

8. Proteins A, B, C, and D with MWs corresponding to 25kDa, 185 kDa, 67kDa, and 85kDa and pIs

(1) corresponding to 5.3, 7.6, 4.4, 9.6, respectively, are added onto a CMC (carboxy methyl cellulose) column at pH 6.9 and then eluted with an increasing salt gradient. Which protein would you predict to be off the column **last**? _____

9. (1) Which of the above proteins would come off **first** when all are loaded on a G-200 column? ____

10. (1) Two common affinity tags used to purify a protein are the His6 tag and the Maltose Binding Protein tag, once bound, the protein is eluted with _____ and _____, respectively for these two tags.

I hereby declare that I did this work independently: _____