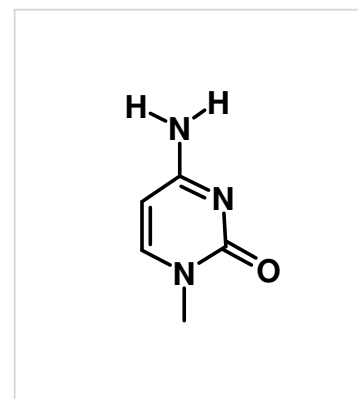


This exam has **14 pages** and is out of **300 points**. On questions with choices all of your attempts will be graded and you will receive the best grade. Use the space provided, or the back of the preceding page.

1. (6 pts) Provide a definition for a hydrogen bond. Identify and label a hydrogen bond donor and a hydrogen bond acceptor on the nucleobase shown on the right.

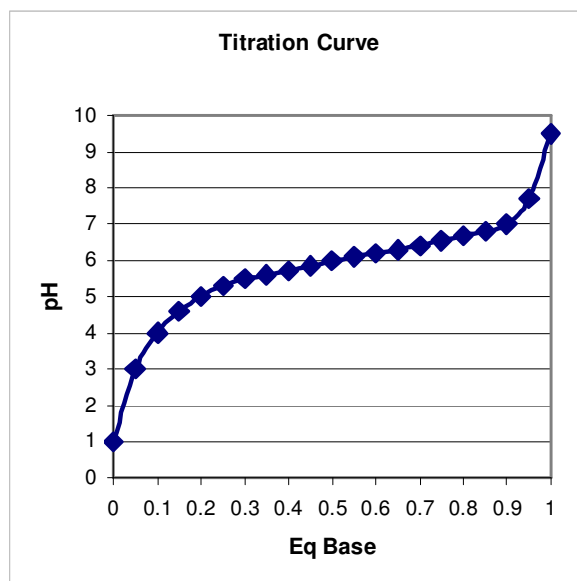


2. (15 pts) The diagram to the right is a titration curve of the side-chain of an amino acid. Please answer the following questions:

i) What is the  $pK_a$  for this ionizable group (2 pts)?

ii) What pH range could this amino acid be used as a buffer? (2 pts)

iii) How does the side-chain buffer the solution if a strong acid or a strong base were added (5 pts)?



- iv) Approximately how many equivalents of a strong base would be required to make a buffer solution at  $pH=7.0$ , assuming you would be starting with the fully protonated compound (consider only ionization of the sidechain) (6 pts).

3. (25 pts) Draw a tri-peptide using any three non-ionizable amino acids (except Ser, Ile, and Val), one of which absorbs UV light. Assume pH=7.0 for your diagram. (6 pts for diagram).

i) Give the primary structure (sequence of your peptide) (3 pts)

ii) label one peptide bond and briefly discuss how its features differ from other non-peptide bonds in the peptide (6 pts).

iii) What type of ion exchange column could you use to separate this peptide from a mixture of other peptides, assuming a pH = 2.0. Briefly justify your answer (6 pts).

iv) How would you determine the concentration of the peptide after purification (2 pts)?

v) What is "specific activity" and how would it differ before and after purification of your peptide (2 pts).

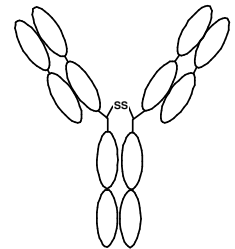
4. (12 pts) Describe or sketch one secondary structure that is commonly found in membrane proteins. What features of the amino acid sequence would you expect to observe in a membrane protein if it adopted the secondary structure you selected? What role do hydrogen bonds play in this structure?
5. (12 pts) Briefly describe the hydrophobic effect and discuss its role in **two** of the following:
- Structure of globular proteins.
  - Membrane stability
  - Stability of double stranded DNA.
6. (8 pts) Briefly describe conformational entropy and discuss its role in **either** protein folding **or** the formation of double stranded (ds) DNA.

7. (10 pts) Briefly describe the role of van der Waals interaction in the **both** of the following and indicate the relative importance of this interaction in stabilizing one structure over another.
- Folding of globular proteins
  - Formation of double stranded DNA.

8. (6 pts) What other significant interaction, besides the hydrophobic effect, conformational entropy, or van der Waals, plays an important role in the stability of double stranded DNA, but not protein stability. Why is this interaction more important for DNA?

9. (10 pts) Please do one of the following two choices.

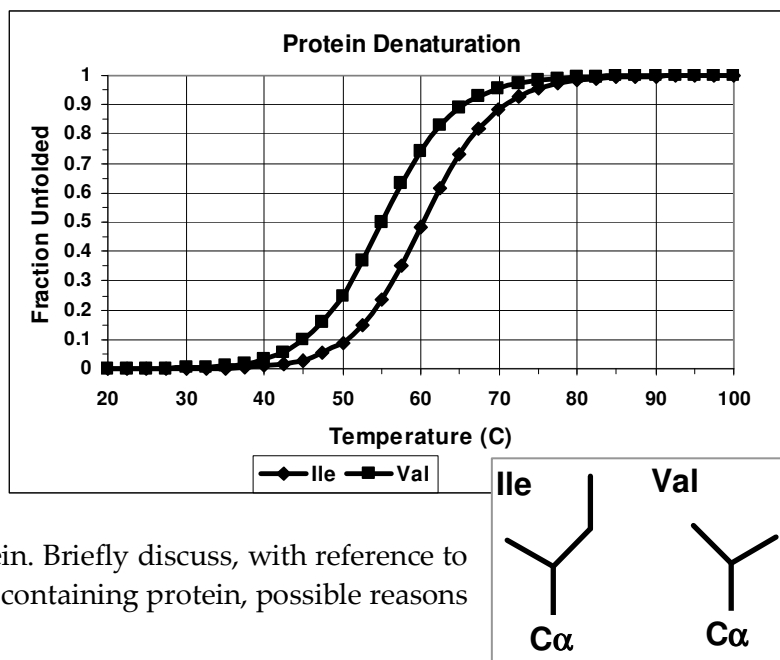
**Choice A:** The quaternary structure of the immunoglobulin was determined by simple techniques 20 years before the X-rays structure of an immunoglobulin was determined. What techniques would you use to determine the quaternary structure? Briefly describe the techniques, the data you would obtain, and how you would use this data to substantiate the structure shown on the right.



**Choice B:** The denaturation curves for a wild type protein and a mutant protein with Ile replaced by Val are shown to the right. The thermodynamic parameters for unfolding of the wild-type protein are:  $\Delta H^\circ = +200$  kJ/mol,  $\Delta S^\circ = +600$  J/mol-deg.

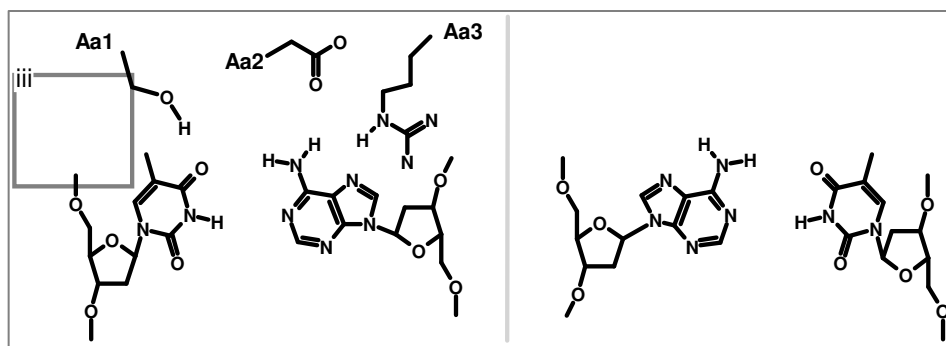
i) Assuming that  $\Delta S^\circ$  of unfolding is the same for both the wild-type and the Valine containing protein, calculate the  $\Delta H^\circ$  for the unfolding for the Val mutant [Hint: No van't Hoff analysis is required,  $\Delta H^\circ = T_M \Delta S^\circ$ ].

ii) Compare your  $\Delta H^\circ$  value with the value obtained for the wild-type protein. Briefly discuss, with reference to the structure of the wild-type and Val containing protein, possible reasons for this difference in  $\Delta H^\circ$ .



10. (12 pts) Allosteric effects play a predominate role in many aspects of biochemistry. Briefly define the term allosteric, and discuss the role of allosteric effects in any **one** of the following.
- Oxygen transport.
  - Regulation of glycolysis
  - Regulation of glycogen levels.
  - Regulation of mRNA synthesis.

11. (21 pts) A protein binds to a specific sequence of double stranded nucleic acid. Part of the interaction between the protein and the nucleic acid is shown on the left side of the diagram. The amino acid sidechains from the protein are



labeled Aa1, Aa2, Aa3. The reversal of the two bases is shown on the right part of the diagram; this part of the diagram will be helpful for parts *viii* and *ix*.

- Label the 5' and 3' carbons of left-most base (2 pt).
- Identify the glycosidic bond on the left-most base (1 pt).
- Place the appropriate missing atoms in the box labeled "iii" that would be required to connect this residue to the previous residue (2 pts).
- Indicate the "Watson-Crick" hydrogen bonds on the right-most base pair (2 pts).
- Is this protein binding to DNA or RNA? Why? (3 pts).

*vi*) Is this protein binding in the major groove or the minor groove? How did you determine this? (2 pts).

*vii*) What is the principle energetic interaction between the protein and the DNA, based on this diagram? (1 pt)

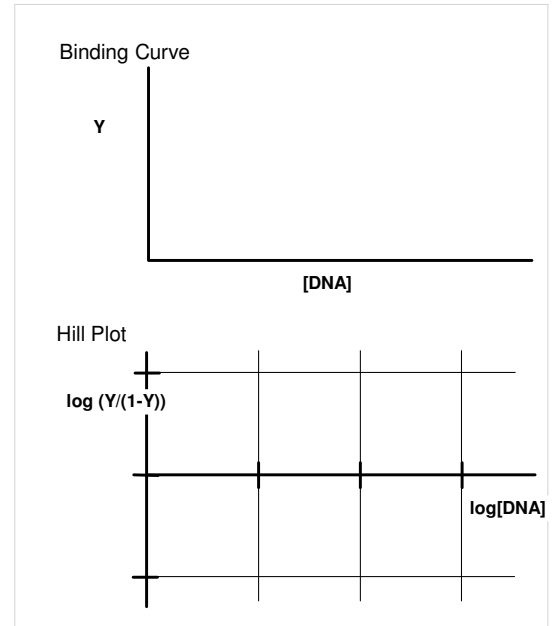
*viii*) How would the binding affinity change if the protein bound to the reversed basepair (shown on the right)? You should assume that the structure of the protein does not change. (4 pts)

*ix*) Would your answer to part *viii* change if the protein used a similar type of interaction in the other groove? (4 pts)

12. (19 pts) A protein binds to DNA and the binding is sensitive to the concentration of NaCl in solution.

i) What functional groups are likely to be involved on both the protein and the DNA? (5 pts)

ii) Sketch, in the space to the right, the binding curve and the Hill plot that you would expect to observe if you measured binding at low salt and at high salt. Assume that the  $K_D$  at low salt is  $10^{-6}$  M and the  $K_D$  is changed by a factor of 10 at high salt (i.e. 10 fold lower or 10 fold higher). Be sure to justify your answer, in particular whether you feel the  $K_D$  is increased or decreased at the higher salt concentration (5 pts).



iii) What assumption did you make about the type of binding when you sketched these curves? (2 pts).

iv) Which kinetic parameter, the on-rate or the off-rate, would you expect to change as the salt concentration is changed? Why? (3 pts).

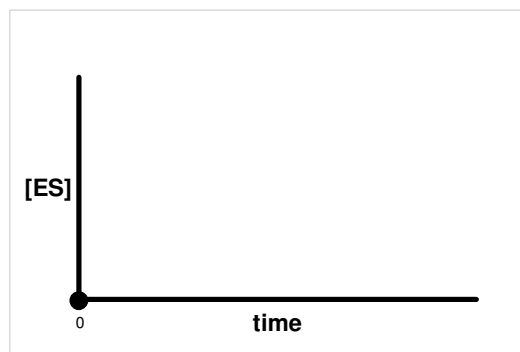
v) Based on your answer to part *i*, how would you expect pH to affect the binding? You can answer this question with either a discussion or a sketch of  $K_D$  versus pH. Be sure to explain the major features of your sketch (4 pts).

13. (8 pts) Please do **one** of the following three choices.

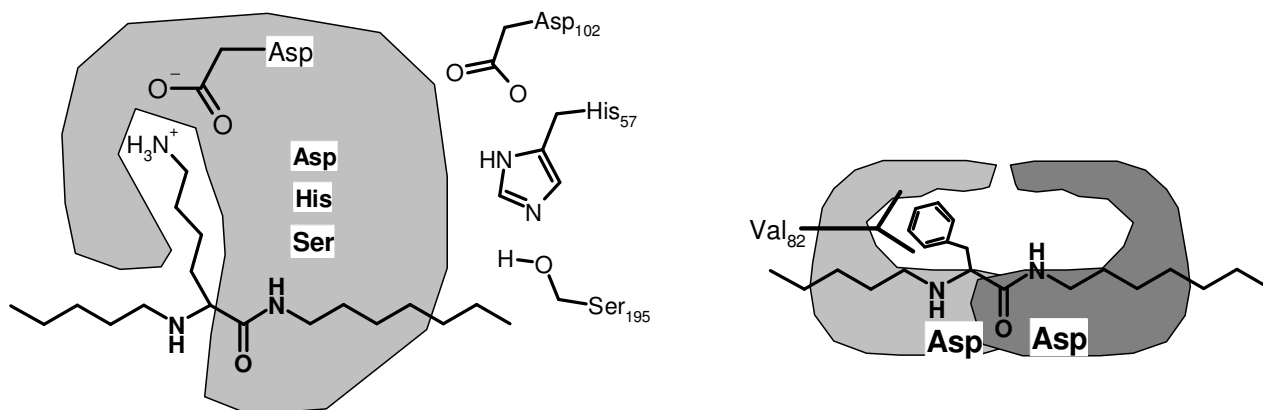
**Choice A:** Proteases (e.g. serine protease, HIV protease) and the Potassium channel both increase the rate of a reaction. What are the common features of the mechanism of rate enhancement? How do the enzymes differ?

**Choice B:** Sketch a graph of [ES] versus time, beginning with  $t=0$  when the substrate is mixed with the enzyme. Which time period on your sketch would measurements of product formation allow you to represent your data using the formula to the right.

$$v = \frac{[E_t]k_{CAT}[S]}{K_M + [S]}$$



**Choice C:** The diagram below shows a “cartoon” diagram of either trypsin (left) or HIV protease (right). You can choose either enzyme to answer

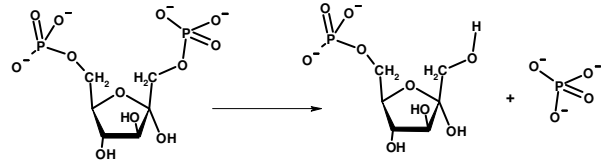


the following questions.

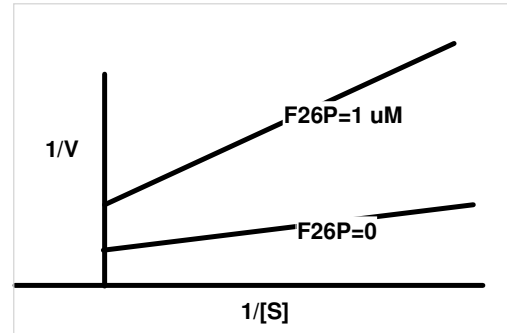
- Which residue would be altered in chymotrypsin (left) or altered in HIV protease mutations (right) that affect drug binding. Circle the residue and briefly justify your answer (2 pts).
- Select one residue that is responsible for catalytic activity and describe how replacement of that residue with glycine (i.e. removal of the sidechain) would affect the catalytic mechanism (4 pts)
- Which steady-state kinetic parameter would be altered due to the change you made in the protein in part ii? Briefly justify your answer (2 pts).



14. (18 pts) The enzyme kinetics of fructose 1,6 bisphosphatase were measured in the presence of different levels of F26P and these data are present on the right. The reaction catalyzed is also shown on the right.



i) Is F26P acting as a competitive or mixed type inhibitor? Briefly justify your answer (5 pts).



ii) Based on your answer to ii, where on the enzyme is the F26P binding (active site or elsewhere?) (2 pts).

iii) Briefly describe how you would obtain the  $K_I$  (and if appropriate,  $K_I'$ ) values from these data? (4 pts).

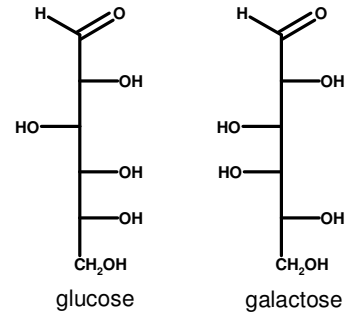
iv) Sketch, on the graph, the effect you would expect to observe if the reaction was performed in the presence of high levels of Fructose-6-phosphate. Justify your answer, using the back of the previous page if necessary. (4 pts).

v) What regulatory method would best describe how the enzyme is regulated by fructose-6-phosphate? (3 pts)

15. (10 pts) Please do **one** of the following choices.

**Choice A:** The structure of glucose and galactose are shown on the right.

- i) Are these sugars aldoses or ketoses? (2 pts)
- ii) Draw  $\beta$ -glucose in its pyranose form using the reduced Haworth representation. (2 pts)
- iii) Indicate the location of the new chiral centers on the pyranose ring of glucose, what is this new center called? (2 pts)
- iv) Sketch, or describe, any **one** of the following carbohydrates (4 pts)
  - a) lactose
  - b) sucrose
  - c) glycogen
  - d) cellulose



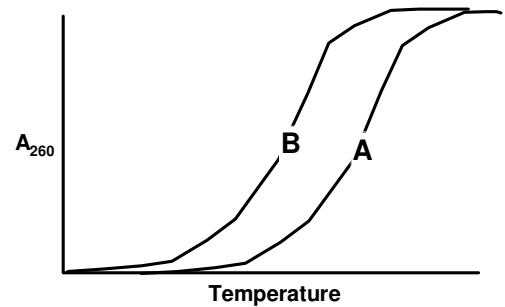
**Choice B:** In what way is a biological membrane similar to a membrane composed of only phospholipids, i.e. what is a general description of their structure? In what way do they differ? List the additional components that are found in a biological membrane and briefly state their role in the function of the membrane.

16. (12 pts) Please do **one** of the following questions.

**Choice A:** Pretend it's next Sunday and you just finished the Pittsburgh marathon. As a consequence, your glycogen levels and ATP levels in the liver are quite low. Discuss the process, with the major focus on regulation in your answer, by which your glycogen levels and ATP levels are restored as you eat lots of carbohydrates after the race.

**Choice B:** Pretend it's next Sunday and you just finished the Pittsburgh marathon by sprinting the last  $\frac{1}{2}$  mile. As a consequence of low oxygen in your leg muscles considerable lactic acid was produced. Why was the lactic acid produced in the muscle? After it leaves the muscle, where does it go? When it gets there, discuss the process by which it is converted back to glucose.

17. (8 pts) The plot on the right show two melting curves, labeled A and B. What kind of difference in **either** the DNA **or** the solution conditions would cause the melting temperature to shift from A to B. Briefly justify your choice.



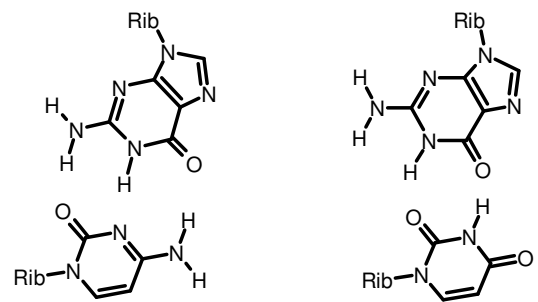
18. (10 pts) The following DNA sequence:  $\begin{array}{l} \text{GCGAATTCCCG} \\ \text{CGCTTAAGGGC} \end{array}$   
was treated with the restriction endonuclease EcoR1 ( $G^{\wedge}AATTC$ ).  
i) Draw the expected products of the reaction (4 pts)

ii) Explain why the recognition sequence is the same on both the upper and lower strand (6 pts).

19. (12 pts) Please do **one** of the following choices.

**Choice A:** Describe the basic reaction mechanism for a typical DNA polymerase. Discuss why the Gibbs energy for the overall reaction is negative and also comment on the fidelity of the reaction, or why the polymerase is more likely to incorporate the correct base (Note: do not discuss removal of an incorrect base, see following question).

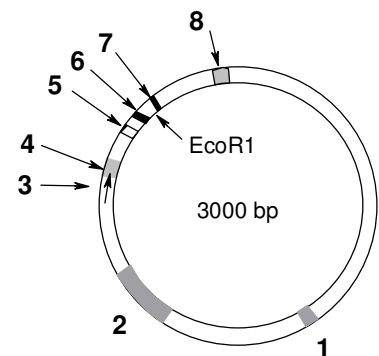
**Choice B:** Describe the mechanism by which tRNA molecules are charged and then briefly discuss how the same tRNA can be used to translate more than one codon. The diagram to the right may be helpful.



20. (8 pts) Please do **one** of the following choices.

**Choice A:** Discuss the mechanism by which some DNA polymerases remove incorrectly incorporated bases. What is the consequence of lack of this function in a polymerase and how does it affect the treatment of HIV?

**Choice B:** Discuss how editing occurs during the charging of tRNAs by certain amino acids, such as Ile and Val.



21. (12 pts) The diagram to the right shows an expression vector. The numbers refer to essential features that are contained in most expression vectors. Please do both parts of this question.

i) Complete the following table. The start and stop codons have been done for you as an example (6 pts)

Number	Name	Involved in mRNA synthesis (Yes/No)	Involved in protein synthesis (Yes/No)
1			
2			
3			
4			
5			
6	Start codon	No	Yes
7	Stop codon	No	Yes
8			

ii) Pick any **three** of the above features, except 5, 6, and 7, and discuss their role in either maintenance of the plasmid in the cell, or the expression of protein (6 pts)

22. (10 pts) The following DNA contains a protein coding sequence that you would like to express in bacteria. The sequence, along with the protein translation, is given in bold:

|-----500 bases-----|

AGCTGCTC**ATGCTCCCCACA**. . . . .**GTGAGGGGAAATTA**ACCGCCGGCG  
 TCGACGAG**TACGAGGGGTGT**. . . . .**CACTCCCCTTTAAT**TGGCGGCCGC  
**MetLeuProThr. . . . .ValArgGlyLysStop**

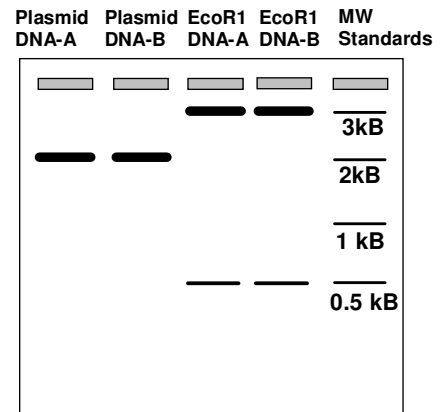
The expression vector (complete diagram shown in the previous question) contains a single EcoRI site between the start and stop codons that are contained in the vector, i.e. the vector sequence is:

--TTAGTAGGGCACCTCA**ATGGAATTC**TTAA-- ,

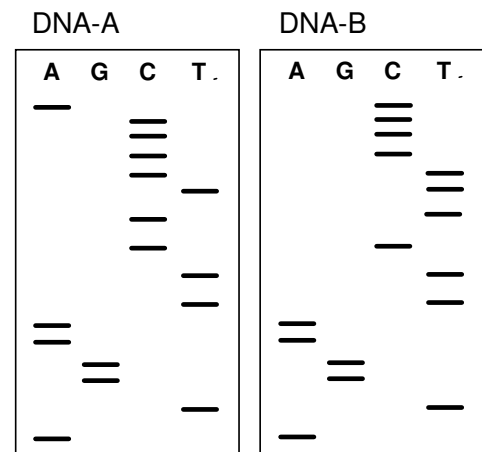
consequently it is not necessary to amplify the start and stop codons from the DNA, just the sequence that codes for the remaining amino acids (LeuPro----GlyLys).

Give the sequence of the left and right primers that would generate the desired PCR product. Make the total length of your primers 12 bases, i.e. don't worry about adjusting the length to generate the correct  $T_M$ . Write the sequence of the final PCR product.

23. (10 pts) After cutting the PCR product and the vector, and ligating the mixture, you isolate two different plasmids. The undigested and digested DNAs are shown on the gel on the right, along with molecular weight standards. Plasmid A and plasmid B are identical based on this gel. The lower portion of the DNA sequencing gel of both of these plasmids, using the primer GGGCACCTCA, is also shown on the right.



i) Briefly explain why the migration distance of the uncut plasmid is different (faster) than the fragment generated after cutting the plasmid (4 pts).



ii) Plasmid A generates the required protein after induction with IPTG, but plasmid B does not. Why? Use the back of the previous page to answer this question (6 pts).

24. (6 pts) Briefly explain how the different DNA molecules in the "A" lane in the above sequencing gels were generated.

25. (8 pts) Please do **one** of the following choices.

**Choice A:** How would you modify the protein produced in the previous question to cause it to be exported out of the cell? How does this modification lead to export of the protein?

**Choice B:** How would you modify the protein produced in the previous question to facilitate purification by affinity chromatography? What type of chromatography resin would you use?

26. (12 pts) Discuss the roles of the ribosome binding site, the start codon, and the stop codon on the overall process of protein synthesis.

Bonus questions (2 pts each).

1. In what way is the ribosome like an apple?
2. In what way is a peppermint patty like IPTG?