This exam consists of **17** pages. Be sure that you have all of the pages. There are a total of 152 points on the exam, budget about 1 min/point. There are two bonus questions scattered in the test as well.

A1:	_____/15
B1:	_____/ 5
B2:	_____/ 3
B3:	_____/20
B4:	_____/ 6
B5:	_____/ 3
B6:	_____/10
B7:	_____/20
B8:	_____/ 5
B9:	_____/ 8
B10:	_____/18
B11:	_____/ 5
B12:	_____/ 4
B13:	_____/ 6
B14:	_____/ 6
B15:	_____/ 8
B16:	_____/10
Bonus	_____/ 6
TOTAL	_____/152

Part A: Multiple Choice 1.5 pts each - total of 15

1. DNA absorbs UV light at ____ nm and proteins absorb at ____ nm.
   a) 260, 260
   b) 260, 280
   c) 280, 260
   d) 280, 280

2. Which amino acid has a sidechain that often stabilizes extracellular proteins by the formation of crosslinks in the protein chains?
   a) Alanine
   b) Cysteine
   c) Methionine
   d) Tyrosine

3. A ligand that binds to a protein more strongly than another ligand will
   a) Have a smaller dissociation constant (K_D).
   b) Have a larger dissociation constant (K_D).
   c) Have a smaller association constant (K_A).
   d) have a slower kinetic on-rate.

4. In both hemoglobin and myoglobin the oxygen is bound to.
   a) the iron atom in the heme group.
   b) the nitrogen atoms on the heme.
   c) a hydrophobic pocket in the protein.
   d) the surface of the protein.

5. During any successful purification scheme, you would expect
   a) the number of different proteins in the sample to decrease.
   b) the specific activity to decrease.
   c) the specific activity to increase.
   d) both a and c are correct.

6. In gel electrophoresis both proteins and DNA are separated on the basis of their:
   a) charge-to-mass ratio.
   b) molecular weight.
   c) positively charged sidechains.
   d) different isoelectric points.

7. Which of the following fatty acids would have the lowest critical micelle concentration (CMC)? (The CMC is the highest concentration of monomeric fatty acid that can be present in solution before micelles appear.)
   a) C_4-COOH
   b) C_5-COOH
   c) C_6-COOH
   d) C_7-COOH
   e) C_8-COOH

8. If the Gibbs free energy, ΔG, of a system is positive then:
   a) More reactants will spontaneously form.
   b) More products will spontaneously form.
   c) It is not possible to extract any energy from the system.
   d) both a and c are correct.

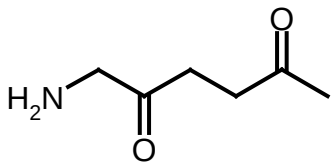
9. T_M refers to:
   a) the temperature at which 50% of a DNA molecule is denatured
   b) the temperature at which 50% of a protein molecule is denatured
   c) the temperature at which membranes are 50% fluid.
   d) all of the above.

10. During replication, overwinding or overtightening of DNA is caused by ____ and removed by ____:
   a) DNA ligase, Gyrase
   b) Dna B (Helicase), DNA polymerase
   c) DnaB (Helicase), Gyrase
   e) Single Stranded Binding Protein, DnaB (Helicase)

Part B:

1. Protein Structure (5 pts)

i) Complete the chemical structure of the dipeptide shown to the right by adding the correct atoms to the mainchain. (i.e. don't draw any sidechains). (1 pt).



ii) Draw the sidechain for a non-polar residue and a positively charged reside on your peptide, indicate which residue is of which type (2 pts).

iii) Name your dipeptide (1 pt):

iv) Circle the bond that is planer and trans (1 pt).

2. Complete the following 'fill in the blanks', the first example has been done for you as an example (3 pts).

i) Amino acid side chains are to proteins as <u>nucleotide bases</u> are to DNA.

ii) The mainchain atoms in protein are represented by _____ sugars in DNA.

iii) The peptide bond in proteins is analogous to the _____bond in DNA.

iv) The amino acid phenylalanine has the same number rings as _____ bases in DNA.

v) The bond between the C_α and C_β atoms in a protein serves the same role as the _____ bond in DNA.

vi) The amino terminus of a protein is analogous to the _____ end of a DNA strand.

vii) A dimeric protein has the same number of chains as _____ stranded DNA.

3. Please do **four** of the following five choices. *Read each choice carefully, some ask you to discuss two items while others give you a choice between one item or another*. (20 pts) (Two choices are on the next page)

Choice A: Discuss the role of the hydrophobic effect in the formation of the folded state of globular water soluble proteins ***and*** biological membranes. You answer should provide a clear molecular description of the hydrophobic effect and the relative importance of the hydrophobic effect in each process (5 pts).

Choice B: Describe, or give an example of a hydrogen bond, and discuss the role of hydrogen bonds in the formation of folded proteins *or* double stranded DNA. Your answer should discuss the relative importance of this interaction to the stability of the folded form. (5 pts)

Choice C: What is 'configurational entropy' and how does it affect the thermodynamics of protein *or* DNA folding. (5 pts).

Question 3 continued:

Choice D: Explain why the salt concentration can have a large effect of the stability of double stranded DNA, but has little effect on the stability of proteins.(5 pts)

Choice E: A nucleotide base, such as adenosine, will bind *non-covalently* to the blunt end of a double stranded piece of DNA , assuming a position similar to as it would if were covalently linked to the DNA. The reaction can be described as the following equilibrium: (5 pts)

```
A-G-G-C-G-G        +        A        →    A-G-G-C-G-G A
T-C-C-G-C-G                                T-C-C-G-C-C
```

What molecular interaction stabilizes this complex?

4. Please do **one** of the following two choices. Please indicate the choice that you are answering. (6 pts)

Choice A: Describe, or draw, the structure of a tRNA. Your answer should also include a discussion of the location of the attached amino acid in a charged tRNAs and the general nature of the interaction of the tRNA with its anticodon.

OR

Choice B: A number of amino acids are associated with more than one codon. For example, the amino acid Phe can be incorporated into a peptide chain whether the codon is UUU or UUC, yet there is only one tRNA molecule that is charged with Phe. Briefly explain how this occurs.

5. Please do one of the following two questions. Please indicate the choice you are answering (3 pts):

Choice A: Briefly explain why RNA is more easily hydrolyzed by base (e.g. NaOH) than DNA. Your answer should include a discussion of the mechanism of hydrolysis.

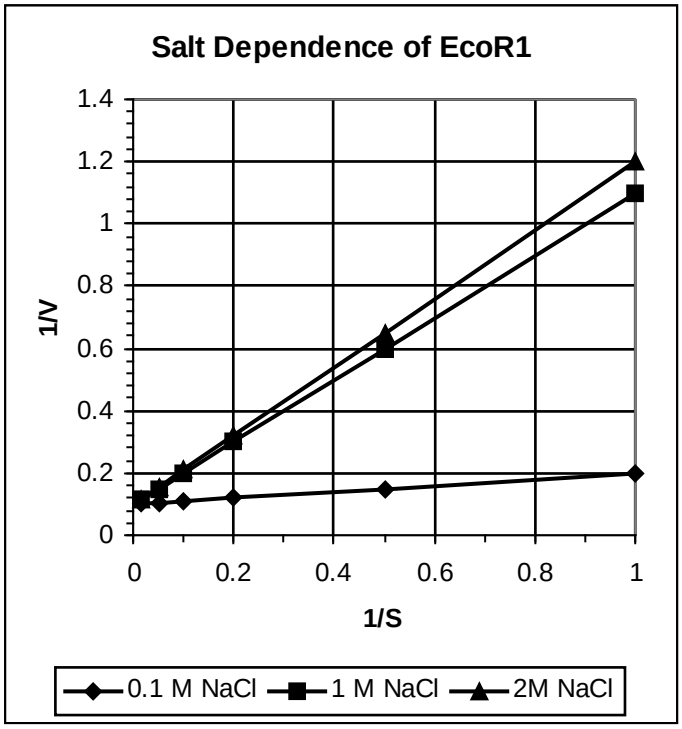
OR

Choice B: How does the chemical structure of RNA differ from DNA?

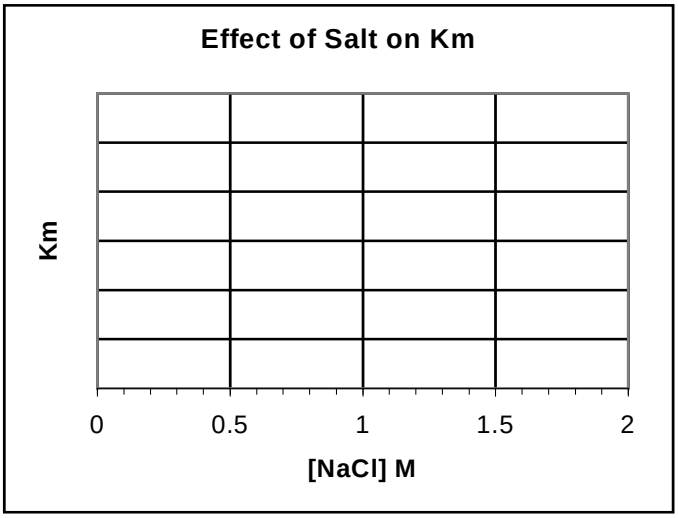
6. Describe the step associated with *either* lagging strand synthesis in DNA replication *or* the process of elongation during synthesis of proteins on the ribosome. Your answer should include a brief description of the molecules involved, both proteins and nucleic acids, as well as a clear indication of the order of events during the process. A well labeled sketch would be an acceptable answer (10 pts).

7. (20 pts) The kinetics of EcoR1 cleavage of DNA was measured at three different salt concentrations (0.1 M, 1M and 2M) and the velocity (products formed/unit time) are shown in the following table. The substrate was GAATTC (double stranded, of course). In this enzyme, the rate of hydrolysis of the DNA (k_2 or k_{CAT}) is very slow, such that $K_M \approx K_D$. V_{MAX} = 10.

[s] nM	v [NaCl]=0.1M	v [NaCl]=1M	v [NaCl]=2M
1	5.00	0.91	0.83
2	6.67	1.67	1.54
5	8.33	3.33	3.12
10	9.09	5.00	4.76
20	9.52	6.67	6.45
70	9.86	8.75	8.64



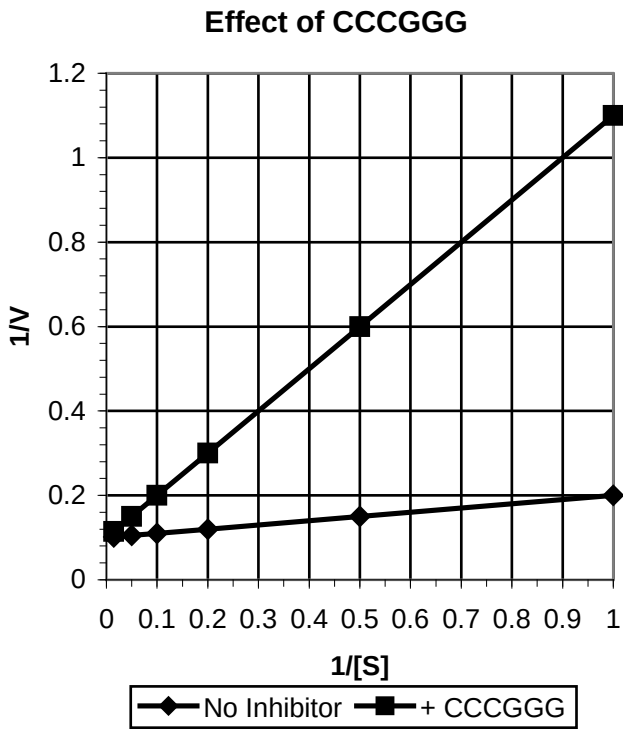
i) Determine the K_M at *each* of these salt concentrations by whichever means you like. Sketch a graph of K_M versus [NaCl] on graph below. (5 pts)



ii) Explain the dependence of the K_M on the salt concentration. Your answer should include a discussion of the general nature of the interaction of the restriction enzymes with the DNA and why this interaction may be affected by salt. (3 pts).

7, continued.....

The first experiment (lowest salt concentration) was repeated in the presence of the following DNA molecule: CCCGGG (double stranded), at a concentration of 9 nM. The resultant enzyme kinetic data is plotted on a double reciprocal plot shown to the right.



iii) Is CCCGGG a competitive or non-competitive inhibitor of the enzyme? Justify your answer *either* by reference to the structure of CCCGGG *or* by analysis of the enzyme kinetic data. (4 pts)

iv) Determine the dissociation constant (K_D) of CCCGGG to Eco R1 from the enzyme inhibition data. (4 pts)

v) Using your value from part I, sketch as accurately as possible, *either* the binding curve *or* the Hill plot for the binding of CCCGGG to EcoR1. Be sure to label the axis, including units, and include a numerical as well.

You should comment on whether you think the binding of CCCGGG to EcoR1 is cooperative or not *and* how the cooperativity (or lack of) is reflected in your plot. If you were unable to obtain a binding constant from *part iv*, state an assumed value and proceed with the problem. (4 pts)

8. Please do **one** of the following two questions. Please indicate your choice. (5 pts).

Choice A: Suggest a method to purify EcoR1 from a complex mixture of proteins by affinity chromatography. Discuss how would you elute the enzyme off of the column.

OR

Choice B: Explain how you would prove that restriction enzymes are homodimeric using SDS-PAGE gel electrophoresis and gel filtration chromatography.

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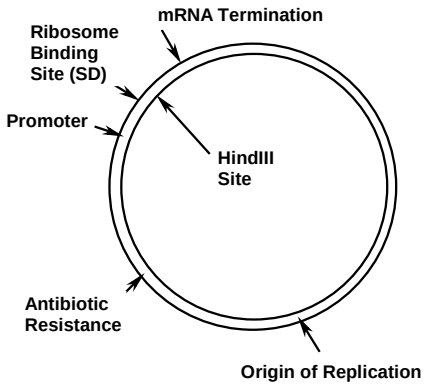
9. Allosteric effects play an important role in the regulation of biochemical processes. Briefly describe the nature of allosteric effects and then select *one* example from the following list and describe how allosteric effects control its function. Your answer should include a description or structure of the allosteric activator or inhibitor. (8 pts)

1. Hemoglobin
2. PFK
3. lac repressor

10. (18 pts) The following is a short segment of human DNA that contains the DNA sequence that encodes a human growth hormone. This hormone, if produce in large quantities in bacteria, can be used to treat a growth deficiency in people. The first three codons of the gene have been translated into the protein sequence for you. The *entire* gene for the growth hormone is in italics and consists of 11 codons, beginning with ATG and ending with TGA.

```
AGGCGTAGTGCTTTGC ATG TTT TGT CAT CAC CGT AGT GCT GAT GGG TGA TGTAGTCTG
                 Met Phe Cys
```

The above gene needs to be inserted into an expression vector/plasmid such that human growth hormone can be produced effectively in bacteria. This vector has the structure shown to the right.



The actual sequence of this vector between the promoter (-35 & -10) and the HindIII restriction site (boxed) is:

```
   -35                  -10            SD
TTGACATTTATGCTTCCGGCTCGTATAATGTGTGGAACAGGAAAGAAGCTT
```

i) The sequence of the growth hormone shows two codons that are underlined, ATG and TGA. Explain the role of *either* of these two triplets of bases in protein synthesis. (2 pts)

ii) Explain the role of *either* the antibiotic resistance gene *or* the origin of replication in the *maintenance* of this plasmid in the bacteria cell (2 pts).

iii) Explain the role of *either* the -35 and -10 regions *or* the role of the SD sequence in the production of the human growth hormone. (2 pts)

iv) The recognition sequence for HindIII is A^AGCTT. Write the double stranded form of the six base sequence and show this DNA after treatment with HindIII. (1 pts)

Question 10, continued:

v) The following product was obtained after polymerase chain reaction using the growth hormone DNA as template.

AAGCTTATGTTTTGTCATCACCGTAGTGCTGATGGGTGA**AAGCTT**
TTCGAATACAAAACAGTAGTGGCATCACGACTACCCACT**TTCGAA**

Describe, or draw a suitable sketch, how this fragment is inserted into the expression vector. Include a brief description of how the covalent bonds are reformed after the gene is inserted into the plasmid (2 pts)

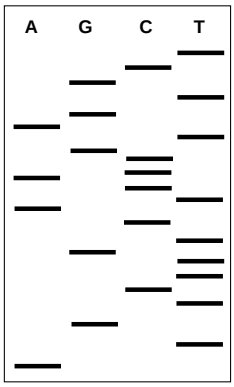
vi) (3 pts) After inserting the growth hormone gene into the vector you note that the bacterial cells that produce growth hormone die quickly such that very little growth hormone can be produced. The cell death is due to the toxic effects of the production of human growth hormone within the bacteria. Briefly describe how you would modify the expression vector to *either*:

- regulate (ie. turn on and off) the production of the human growth hormone, *or*
- export the hormone out of the bacterial cell.

Bonus! (3 pts). In fact, only 50% of the plasmids produced growth hormone (killing the cells), while the other 50% did not produce any growth hormone at all! Explain this observation.

Question 10, continued

vii) Please do **one** of the following **two** choices (6 pts).



Choice A: A DNA sequencing reaction was performed using the primer:
5'-AGGCGTAGTGCTTTGC-3', with the human growth hormone DNA as the template. The sequencing gel is shown to the right.

a) The DNA sequence for the hormone gene shown on the previous page contains an error. Using the sequencing gel, identify the error and indicate its location on the DNA sequence. The sequence is repeated here (2 pts):

AGGCGTAGTGCTTTGCATGTTTTGTCATCACCGTAGTGCTGATGGGTGATGTAGTCTG

b) Does this error change the protein sequence? Briefly, justify your answer. (1 pt)

c) Briefly describe the reaction components that were used to generate the DNA bands in the 'A' lane.(3 pts)

Choice B:

a) Show the PCR primers that would be required to generate the PCR product shown at the top of the previous page. You should give the sequence of the first 10 bases of each primer. (3 pts)

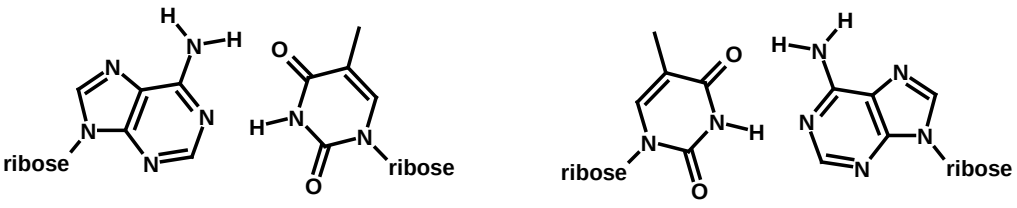
b) Briefly describe the components that would be required for PCR and indicate how the process works. Feel free to use a diagram to illustrate your answer.(3 pts)

11. (5 pts) The following shows an AT base pair and a TA base pair. The first would be found in the sequence:

XAX while the second would be found in the sequence: XTX
XTX XAX

Restriction endonucleases can easily distinguish between these two sequences due to the formation of hydrogen bonds in the major groove. If restriction endonucleases bound in the minor groove would they be able to differentiate between an AT and a TA basepair?

Begin by marking *all* non-watson crick hydrogen bond donors (D) and acceptors (A) in the major *and* minor groove of the following AT and TA base pairs. (3 pts). And then justify your answer by discussing how an enzyme would interact with each basepair via the minor groove. (2 pts)



12. List the major metabolic pathways (e.g. TCA cycle, fatty acid oxidation (FAO), glycolysis, oxidative phosphorylation (OxPhos), in the correct order, that the following compounds would be processed by while being converted to energy (ATP) .

You need only do ***two*** of the four. (4 pts).

Sucrose (table sugar):

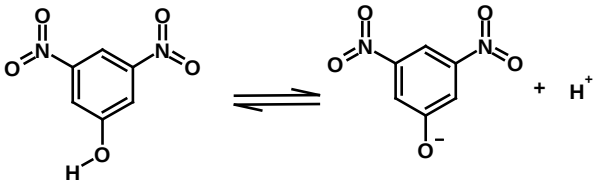
Olive oil (triglyceride):

NADH:

Amino acids:

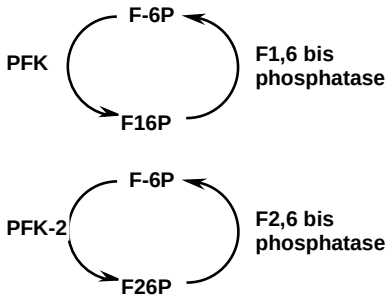
13. Provide a *general* description of a biological membrane. You should comment on the nature of the lipids found in the membrane and their important physical properties. Indicate the location of the various classes of membrane proteins with respect to the membrane and provide a *brief* discussion relating the location of each class to its function. You can answer this question with a well labeled diagram. (6 pts).

Bonus! Dinitrophenyl (DNP) is a weak somewhat *non-polar* acid, as indicated in the diagram to the right. At one time it was widely used as a diet drug, before the FDA pulled it off the drug store shelf. Its mode of action was to reduce the efficiency of ATP synthesis during oxidative phosphorylation. Suggest how this might occur. (3 pts).



14. Please do *one* of the following two questions. (6 pts).

Choice A: In the liver, the hormone epinephrine causes the release of glucose from glycogen as well as the net synthesis of glucose from pyruvate via gluconeogenesis. Explain how both of these processes are coordinately regulated by protein phosphorylation. You answer should include a brief description of the G-protein coupled receptors and the signal transduction pathway.



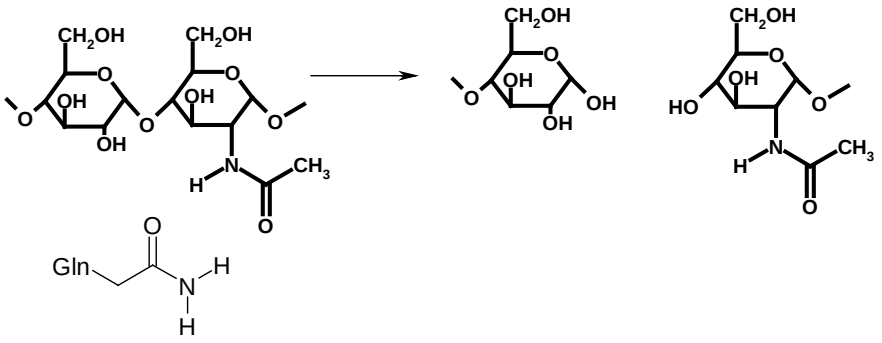
Choice B: In the muscle tissue, the hormone epinephrine also causes the release of glucose from glycogen. In contrast, glucolysis is activated, instead of gluconeogenesis. Given that the regulation of PFK and 1,6 bisphosphatase are the same in both tissues, explain the most likely difference in effect of protein phosphorylation on enzyme activation in the liver versus the muscle tissue.

15. (8 pts) Please do *both* sections of this question.
Peptide bond cleavage can be accomplished by any of the following enzymes: trypsin, chymotrypsin or HIV protease. Using one of these enzymes as examples, or any other enzyme that you think appropriate, discuss **both** of the following general attributes of enzyme catalysis.

i) Substrate specificity. Why do enzymes recognize specific substrates. Provide an example. (4 pts).

ii) Why do enzymes enhance the rate of chemical reactions? Your answer should include a discussion of the transition state and the role that amino acids play in the catalytic process. Provide an example.

16. (10 pts) A virus utilizes an enzyme to cleave polysaccharides off the surface of cells as a necessary step prior to infection of cells. This enzyme catalyzes the reaction shown to the right.



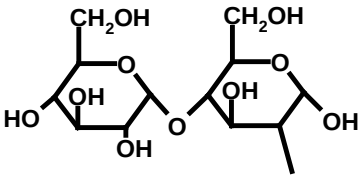
The diagram shows part of the polysaccharide substrate (bold) as well as one of the amino acid sidechains, from a Gln residue, within the active site of the enzyme. This residue is distant from the cleaved bond and therefore *not* involved in catalysis.

Please answer the following questions:

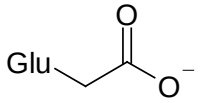
i) What is the general name for this type of reaction? Briefly justify your answer (2 pts).

ii) Describe the type of linkage between the saccharides (e.g. β(2-6)). (1 pt)

The disaccharide of glucose and N-acetylglucose (shown to the right) can be an effective inhibitor against infection by the virus. As with many other viruses, there is a high rate of mutation in the viral proteins and enzymes. One such mutant enzyme was isolated and the Gln was found to be replaced by a Glutamic acid residue.



iii) Indicate, on the diagram to the right, how you would modify the disaccharide drug such that it would bind effectively to the mutant enzyme. The mutant sidechain (Glu) is shown. Briefly explain why your modification of the disaccharide will lead to an drug that is effective at binding to and inhibiting the mutant enzyme (4 pts)



iv) Assuming that this is an RNA virus similar to HIV, explain why high levels of mutations are found in the viral genetic material? Your answer should compare the properties of various DNA polymerases (3 pts).