

There are a total of 170 pts on this exam in 12 pages. 1 min/pt is a very comfortable pace.

1. (7 pts) Hydrogen bonds play an important role in many biochemical interactions. Please answer the following questions:

- i) Define, or give a general description, of a hydrogen bond (4 pts)
- ii) Give an example that illustrates the biochemical and thermodynamic importance of hydrogen bonding in **either** a) protein structure, or b) DNA structure.(3 pts)

**A hydrogen bond forms between the following atoms: X-H Y, where X and Y are electronegative atoms (3 pts).**

**The proton becomes electropositive and interacts with the electronegative Y, creating the hydrogen bond (1 pt)**

- **Protein secondary structure - stabilized by hydrogen bonds.**
- **DNA structure - hydrogen bonds stabilize the correct base pair.**

**(3 pts for example)**

2. (8 pts) In addition to hydrogen bonding, the following thermodynamic factors:

i) van der Waals, ii) electrostatics, iii) hydrophobic effect, iv) conformational entropy, play a role in the stability of proteins, biological membranes, and DNA.

i) For each of four interactions, state whether the interaction is primarily an enthalpic or entropic effect and briefly describe the molecular nature of the factor (4 pts).

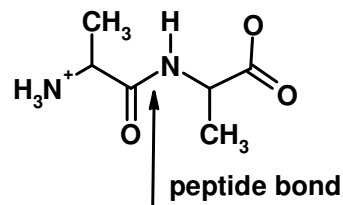
ii) Select **one** of the following **three** structures and discuss the relative importance of all of the above four factors in stabilizing the native state (4 pts).

- a) the tertiary structure of globular proteins
- b) the double stranded structure of DNA
- c) phospholipids bilayers.

	van der Waals	Electrostatics	Hydrophobic effect	Conformational entropy
	Enthalpic - interaction between dipoles/induced dipoles <b>(+1 pt)</b>	Enthalpic + ion entropy: Interaction between charged groups. <b>(+1 pt)</b>	Entropic - reduction in entropy of the system by ordering of water around non-polar groups. <b>(+1 pt)</b>	Entropic - large number of configurations in unfolded form increase entropy. <b>(+1 pt)</b>
<b>Globular Proteins (+1 pt each)</b>	Tight packing of core optimizes hydrophobic effect	Little role	Burial of non-polar groups in core drives folding.	Unstable for folded state.
<b>dsDNA (+1 pt each)</b>	Interaction between faces of the basepairs is energetically favorable	Phosphate-phosphate repulsion destabilizes double stranded.	Little role - bases are polar and solvent exposed in both cases.	Unstable for folded state.
<b>Phospholipid membranes (+1 pt each)</b>	Stabilizing force, responsible for controlling solid-fluid transitions.	Little role	Burial of non-polar acyl chain in the interior of the membrane drives assembly.	Little role

3. (3 pts) Draw any dipeptide and label the peptide bond. Name one feature of the peptide bond.

Diagram should be correct with correct sidechains - correct ionization state of termini not required. (2 pts)



Peptide bond is planar and usually trans (+1 pt)

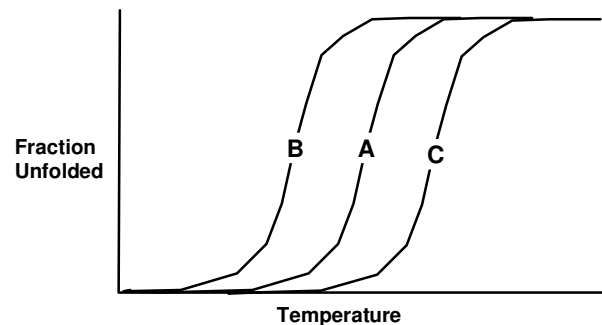
4. (8 pts) Compare and contrast the **primary** structure of DNA and proteins. Your answer should include a brief description of the monomeric units (including mainchain and backbone), how they are linked together, and the standard convention for naming these polymers.

	Protein	DNA
Mainchain	Amino-C $\alpha$ -CO-	Phosphate + ribose
Sidechain	One of 20 functional groups, eg. Methyl (alanine)	Purine or pyrimidine base
Linkage	Carboxy group of one residue is linked to the nitrogen of the next via peptide bond.	Phosphate group joins the 3'OH to the 5'OH carbons on the ribose or deoxy ribose
Naming	Amino to carboxy terminus	5' to 3' end.

5. (6 pts) Please do one of the following two choices.

**Choice A:** A 40 residue amino acid sequence spontaneously forms a helical hairpin (two helices packed against each other, joining by a short turn) in a lipid bilayer. Discuss the general features of the amino acid sequence of this protein.

**Choice B:** A protein contains an isoleucine residue in its hydrophobic core. The temperature dependence of unfolding of two mutants and the wild type were measured, giving the curves to the right. One mutant contained an isoleucine to valine change, the other an isoleucine to tryptophan change. Which unfolding curves (A, B, C) corresponds to which proteins (Ile, Val, Trp)? Briefly justify your answer.



**Choice A:**

Residues in the helix would show a non-polar residue

every 3.4 residues - presenting a hydrophobic surface to the acyl-chains (+4 pts) Non-polar residue generally larger than alanine for the insertion to be energetically favorable. (+2 pts).

**Choice B:**

Ile - Curve C, most stable (+2 pts).

Val - Curve A, less stable due to loss of methyl group, small decrease in vdw. (+2 pts).

Trp - Curve B, least stable due to large bulky sidechain interfering with vdw packing of many residues in the core. (+2 pts).

6. (1 pt) Give **one** important use of heme-iron ( $\text{Fe}^{2+/3+}$ ) in biochemical processes.

Oxygen transport in hemoglobin/myoglobin

Electron transport, specifically cytochrome C.

7. (5 pts) Compare and contrast the properties of HIV reverse transcriptase to polymerase III (Pol III is used to replicate the DNA found in *E. coli*).

	Pol III	RT
Template (+1 pt)	DNA	RNA or DNA
Direction of synthesis (+2pt)	5' → 3'	Same
Nucleotides used (+1 pt)	dNTPs	Same
Error correction (+1 pt)	Yes, 3' → 5' <i>exo</i>	NO

8. (8 pts) When a polymerase encounters an “A” in the template, two possible basepairs that could form are shown on the right.

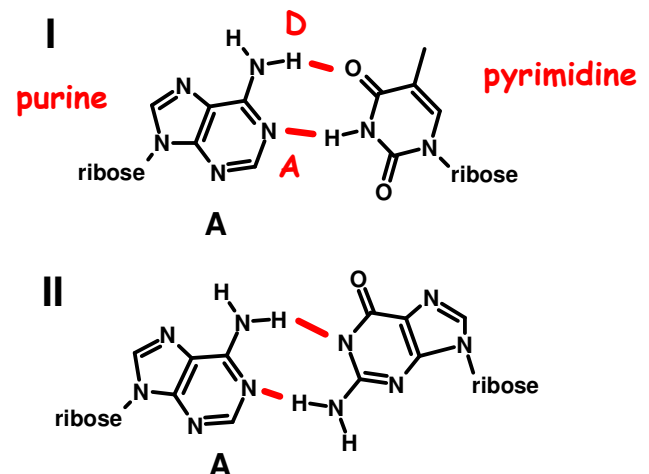
i) In the top pair (I) which base is a purine? Which is a pyrimidine? (+ 2pts)

ii) Identify “Watson-Crick” hydrogen bond donors and acceptors on the A base, and indicate which hydrogen bonds could form during basepairing in the active site of the polymerase for both basepairs I and II.

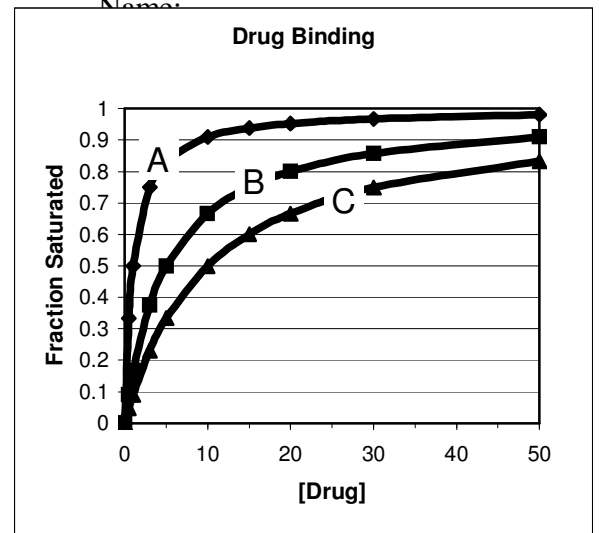
**Two W-C hbonds in each case. (+ 2pts)**

iii) Which of the two basepairs indicates the correct base to pair with the “A”, the top one (I) or the bottom one (II)? Briefly justify your answer.

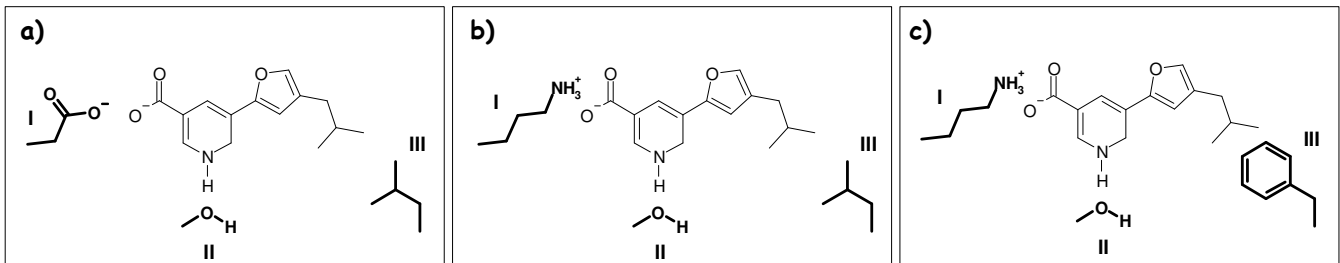
**I, since it is a purine-pyrimidine pairing which is the correct size. II is too large. (+ 4 pts)**



9. (12 pts) The binding of a drug to two mutant and wild-type reverse transcriptases (RT) is being studied. The fractional saturation was measured for all three drug-protein complexes. This drug binds to the wild-type RT with high affinity. Which binding curve (A,B,C) corresponds to which drug-protein pair (a, b, c) in the figure below (I, II, and III are sidechains from RT.) Which pair do you think is the wild-type protein (6 pts).



**Curve A high affinity = protein c) - many interactions.**  
**Curve B lower affinity = protein b) - loss of Phe interaction.**  
**Curve C lowest affinity = protein a) - unfavorable charge-charge interaction.**  
 (+3 for correct order, +3 for some description.)

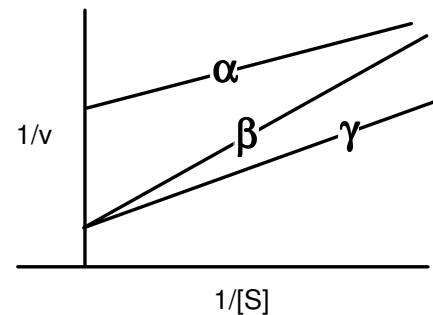


ii) Based on the binding curve, is the binding cooperative or not? What type of data analysis/plot would you do to verify your conclusion? How would you interpret this plot? (2 pts)

**The curves look to be non-cooperative, positively cooperative binding would show an S-shaped curve (+1 pt)**  
**A Hill plot would be used, the slope at the point where the curve crosses the x-axis is the Hill coefficient.**  
**The binding is cooperative if that is not equal to 1. (+1 pt)**

iii) If steady-state enzyme kinetics were measured in the presence of the drug and the wild-type enzyme, which of the lines ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) in the double reciprocal plot shown to the right would correspond to:

- a) the data acquired with the drug present? ( $\alpha$ )
  - b) the data acquired with no drug? ( $\gamma$ )
- Briefly justify your answer (4 pts).



**(+1 pt) for getting the correct curves**

**The enzyme is a polymerase, so the drug is clearly a mixed-type inhibitor. Therefore V-max will change and the y-intercept will be different.**

**Since this is an inhibitor, the y-intercept must be higher (lower Vmax), so  $\alpha$  is the inhibited data and  $\gamma$  is the data without inhibitor.  $\beta$  represents a competitive inhibitor.**

**(+3 pts for explanation)**

10. (8 pts) Provide a brief description of allosteric effects. Your answer should discuss the fundamental basis of allosteric effects and the general nature of homotropic and heterotropic allosteric effectors. Illustrate your answer with any example you like.

**Allosteric effects occur when the binding of one ligand (or phosphorylation) causes a change in the conformation of a protein. This change in shape can affect:**

- the binding of other ligands.
- the enzymatic activity.

**Homotropic compounds affect their own binding (e.g. oxygen to Hb)**

**Heterotropic compounds affect the binding of other compounds. (+ 4pts)**

**Examples:**

Hemoglobin:

- $O_2$  affects its own binding, causing positive cooperativity that is essential of optimal oxygen transportation.
- BPG affect the binding of  $O_2$ , allowing altitude adjustment.

Glycogen Metabolism:

- Phosphorylation of proteins causes activation of glycogen phosphorylase and inactivation of glycogen synthase.

Lac Repressor

- Binding of lactose causes it to release from DNA, allowing mRNA to be made. Normally this allows the bacteria to turn on genes required for lactose metabolism.

Glycolysis

- PFK and F16bisphosphatase are allosterically regulated by AMP, ADP and ATP to insure that the correct pathway is operated according to the needs of the cell.

**(+ 4 pts for example)**

11. (8 pts) Enzymes serve to catalyze the chemical transition of specific substrates to products. Please answer **one** of the following two choices:

**Choice A:** Using the framework of transition state theory, discuss the method by which enzymes increase the rate of chemical reactions. Provide one example of a reaction mechanism to illustrate your answer.

**Choice B:** Enzymes are specific for their substrates. How is substrate specificity achieved by enzymes? Illustrate your answer by discussing **two** enzymes with **different** substrate specificities.

**Choice A:**

Rate proportional to  $[X]$  (+2 pts)

$[X]$  increases when the energy required to reach the transition state is decreased. (+2 pts)

Energy decreased by pre-ordering of active site residues, therefore reduced entropy cost

Energy also decreases in some enzymes by direct and unique interactions with the transition state. (+2 pts)

Examples: Serine proteases, HIV protease (+ 2pts).

**Choice B:**

Substrates that are preferred have slow off-rates due to a number of interactions between the enzyme and the substrate (+4 pts). e.g. the negatively charged Asp residue in trypsin, the non-polar residues in chymotrypsin or HIV protease. (4pts for example)

12. (1 pt) Most enzymatic data is analyzed using steady-state kinetics. During the measurement of product formation, which of the following are assumed to be constant? [Circle correct choice]

- a) the substrate, [S]
- b) the enzyme-substrate complex [ES] (+ 1pt)**
- c) the product, [P]
- d) the enzyme-product complex [EP]
- e) all of the above.

13. (2 pts) Please do **one** of the following two choices:

**Choice A:** You wish to separate hemoglobin from ribosomes. What separation technique would you use?

**Choice B:** In a protein purification scheme the specific activity increases. Define the specific activity and discuss why it increases during purification.

**Choice A:**

Gel filtration - separation by size **(1 pt)**. Hb is much smaller than a ribosome. **(1 pt)**

**Choice B:**

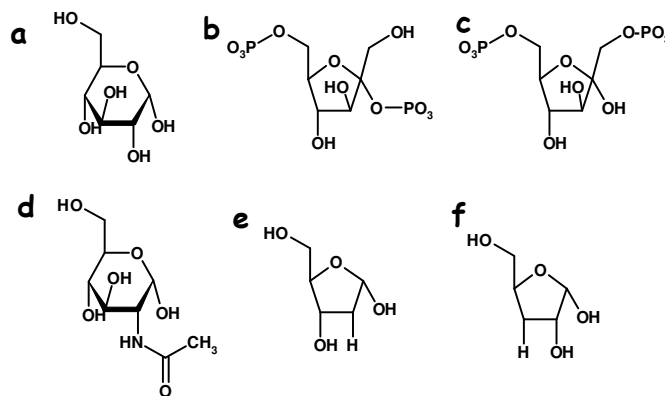
The specific activity is: (the amount of target protein)/(total protein) after a step in the purification scheme. **(1 pt)**

Since a useful step should decrease the amount of contaminating proteins, the specific activity should increase after a purification step. **(1 pt)**

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

**Choice A:** The diagram on the right shows a collection of monosaccharides. Indicate which monosaccharide (a – f) best characterizes the following descriptions:

- i) Is found in DNA, but not RNA. **(e)**
- ii) Is found in cellulose **(a)**
- iii) Is found in glycogen **(a)**
- iv) Is found in bacterial cell walls **(d)**
- v) Is the substrate of a key regulatory step in glycolysis. **(c)**
- vi) Is a compound that activates a key regulatory step in glycolysis **(b)**



**Choice B:** How does a triglyceride differ from a phospholipid (a labeled drawing is sufficient). What is the normal biological function of each of these compounds?

A triglyceride has three fatty acids esterified to glycerol.

A phospholipid has two fatty acids, plus a phosphate, esterified to glycerol.

**(+ 3pts)**

Triglycerides are used for storage of fats, phospholipids form membranes **(+ 3pts)**

15. (3 pts) Briefly discuss how the permeability properties of biological membranes are important for **one** of the two choices:

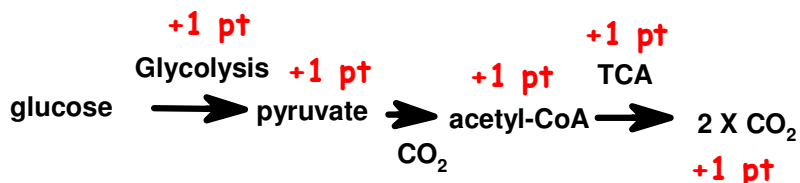
**Choice A:** oxygen transport.

**Choice B:** production of ATP in the mitochondria.

**Choice A:** The membrane is freely permeable to non-polar compounds, such as oxygen. Allowing oxygen to freely enter the red blood cell and bind to hemoglobin.

**Choice B:** The membrane is impermeable to ions, such as hydrogen ions. This allows a different concentration of hydrogen ions to exist across the inner mitochondrial membrane. The energy stored in this gradient can be used to make ATP by ATP synthase.

16. (10 pts) Briefly discuss the steps (pathways & major intermediates) that occur in the complete oxidation of glucose to produce ATP when oxygen is present. Your answer should also discuss how the energy that is released by oxidations during this process is captured for subsequent conversion to ATP.



- Oxidation of glucose to pyruvate, and finally to  $\text{CO}_2$  produces electrons which are used to produce NADH and  $\text{FADH}_2$ , both storing some of the energy from the oxidation (+2 pts)
- NADH and  $\text{FADH}_2$  are oxidized in electron transport, this energy is stored in a proton gradient. (+1 pt)
- The proton gradient is used by ATP synthase to make ATP. (+1 pt)

17. (8 pts) Protein kinases and phosphatases play an important role in the regulation of carbohydrate metabolism. Please answer both i) and ii).

i) Compare and contrast the reactions catalyzed by these two enzymes (6 pts).

ii) Selecting conditions of **either** low or high blood glucose levels, discuss how kinase/phosphatase activity controls **either** glycogen synthesis/degradation **or** glucose synthesis/degradation. (+2 pts)

i)

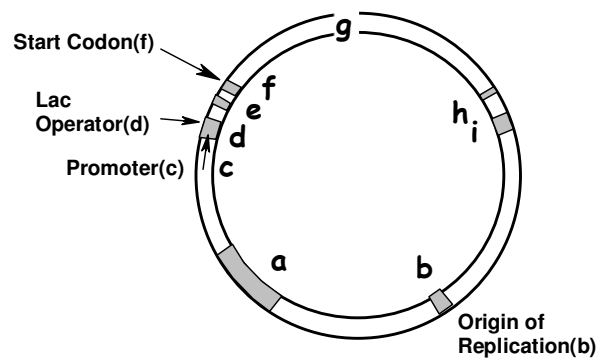
**Kinases** transfer a phosphate from ATP to another compound. In the reverse direction they would transfer a phosphate from a compound to ADP to form ATP. (+3 pts)

**Phosphatases** remove a phosphate by hydrolysis, or in the reverse direction add inorganic phosphate. (+3 pts)

ii)

Low Blood Sugar		High Blood Sugar	
Kinases are activated, causing protein phosphorylation.		Phosphatases are active, causing protein dephosphorylation.	
<b>Glycogen degradation</b>	<b>Glucose synthesis (gluconeogenesis)</b>	<b>Glycogen Synthesis</b>	<b>Glucose oxidation (Glycolysis)</b>
Glycogen phosphorylase is active when phosphorylated. Glycogen synthase is inactive when phosphorylated.	F26P levels are low because F26P bisphosphatase is active. F26P inhibits F16bisphosphatase in gluconeogenesis.	Glycogen phosphorylase is in-active when dephosphorylated. Glycogen synthase is active when dephosphorylated.	F26P levels are high because F6P kinase-2, which makes F26P is active. F26P activates PFK in glycolysis.

18. (10 pts) The following is diagram of an expression vector. Several of the labels are missing from this diagram, however all of the elements are in the correct position.



i) Provide the missing labels for a, e, g, h, and i.

a) antibiotic resistance gene

e) ribosome binding site.

g) codons

h) stop codon

i) mRNA termination

ii) Discuss the role of any **4 of the 9** labels in the expression of recombinant protein. Be sure to indicate your choices.

- a) Antibiotic resistance gene: Provides a method to insure the plasmid remains in the bacteria. Only those bacteria with the plasmid will grow in the presence of the antibiotic.
- b) Origin of replication: Insures that the plasmid will be replicated in the bacteria.
- c) Promotor: Site where RNA polymerase binds to initiate the synthesis of mRNA.
- d) Lac operator: On/off switch for mRNA production. The lac repressor binds at this site, turning off transcription. Addition of IPTG turns transcription on.
- e) Ribosome binding site - positions mRNA on the ribosome.
- f) Start codon - required to initiate protein synthesis on the ribosome.
- g) Codons - give order of amino acids for the protein.
- h) Stop codon - end of protein synthesis
- i) mRNA termination - signals RNAP to stop making mRNA

iii) What would you add to the DNA sequence of the vector to cause the protein to be exported out of the cell?

Add codons that would specify the sequence of the leader (or signal) peptide at the amino terminus of the protein.



19. (5 pts) Restriction endonucleases always recognize sequences that are the same on the top and the bottom strand and cut in the same location on each strand. With reference to the structure of the protein, briefly describe why this type of sequence specificity and cutting site occurs.

Restriction endonucleases are homodimeric proteins. One of the subunits recognizes the top strand, the other the bottom strand. Since the subunits are identical, the DNA sequences on both strands must be equal.

Similar argument for the cut site.

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

**Choice A:** Briefly explain how dideoxy nucleotide triphosphates are used to determine the sequence of DNA. Your answer should include a description of the reagents/compounds that are needed for this reaction.

**Choice B:** Briefly explain how PCR (polymerase chain reaction) leads to the amplification of DNA segments. Your answer should include a description of the reagents/compounds that are needed for this reaction.

**Choice A:**

- In DNA sequencing, it is necessary to generate molecules that begin at the same location and end with a known base.
- Using a primer, a polymerase produces molecules that begin at the same location.
- The use of a dideoxy nucleotide causes chain termination, giving molecules that end in known bases.

**Choice B:**

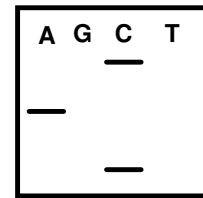
- In PCR two primers are selected that point toward each other. The left primer has the same sequence as the top strand. The right primer has the same sequence as the bottom strand.
- Denaturation of the template, followed by annealing of the primers.
- DNA polymerase activity will extend from each primer, copying the template between the two primers.
- After several cycles the DNA between the two primers becomes selectively amplified.

21. (6 pts) Please do one of the following two choices.

**Choice A.** The following two DNA molecules were mixed together and used for a DNA sequencing reaction. Draw the resultant sequencing gel.

5'-G-T-C-T-G-3'

3'-A-T-G-C-A-G-A-C-G-T-G-5'



The primer will anneal to the template, forming the following primer-template complex:

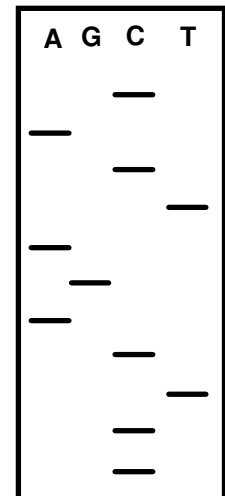
5'-G-T-C-T-G-3'  
3'-A-T-G-C-A-G-A-C-G-T-G-5'

Fragments formed by DNA polymerase activity would be the primer plus C, CA, CAC, giving the gel shown on the right.

**Choice B:** Read the sequence from the sequence gel shown on the right. Be sure to indicate the 5' and 3' ends of your answer.

The bottom of the gel are the nucleotides closest to the 5' end of the primer and would be added first by the polymerase. The sequence is

5'-CCTCAGATCAC



22. (7 pts) The HIV protease gene from a mutant (inactive) HIV virus was sequenced and part of the sequence that was obtained from the sequencing gel is shown below. The complete nucleotide and protein sequence of HIV protease is also given. Identify the nucleotide and amino acid change (6 pts) and briefly explain why this virus is inactive (1 pt).

DNA sequence from gel: 5'-AAAGGAAGCTCTATTAACAACAGGA

**HIV protease.**

5'-CCTCAGATCACTCTTTGGCAA  
ProGlnIleThrLeuTrpGln<sub>7</sub>

CGACCCCTCGTCACAATAAGGATAGGGGGGCAACT**AAAGGAAGCTCTATTAGATACAGGA**  
ArgProLeuValThrIleArgIleGlyGlyGlnLeuLysGluAlaLeuLeuAspThrGly<sub>27</sub>  
GCAGATGATACAGTATTAGAAGAAA**TGA**ATTTGCCAGGAAAATGGAAACCAAAAATGATA  
AlaAspAspThrValLeuGluGluMetAsnLeuProGlyLysTrpLysProLysMetIle<sub>47</sub>  
GGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTACGATCAGATACCTGTAGAAATCTGT  
GlyGlyIleGlyGlyPheIleLysValArgGlnTyrAspGlnIleProValGluIleCys<sub>67</sub>  
GGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCT**GTC**AACATAATTGGAAGA  
GlyHisLysAlaIleGlyThrValLeuValGlyProThrProValAsnIleIleGlyArg<sub>87</sub>  
AATCTGTTGACTCAGATTGGTTGTACTTTAAATTT**C**ccattagtcctattgaaact-3'  
AsnLeuLeuThrGlnIleGlyCysThrLeuAsnPhe

uLysGluAlaLeuLeuAspThrGly

**Wild-type sequence:** AAAGGAAGCTCTATTAGATACAGGA

**Mutant sequence:** AAAGGAAGCTCTATTAACAACAGGA

The asp codon has been changed to Thr. This would inactivate the enzyme since asp-25 is required for catalytic function.

23. (12 pts) The following shows the complete nucleotide sequence of one of the surface proteins from swine flu virus (the HA protein). The entire length of this gene is 1700 bases, beginning with ATG and ending with TAA. Note that there is a BamHI restriction site (GGATCC) at position 230. You wish to produce this protein in bacteria for the purposes of making a vaccine to save the world from a potential pandemic.

HA gene sequence

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1  atgaaggcaa tactagtagt tctgctatat acatttgcaa cgcgcaaatgc agacacatta
61 tgtatagggtt atcatgcgaa caattcaaca gacactgtag acacagtact agaaaagaat
121 gtaacagtaa cacactctgt taaccttcta gaagacaagc ataacgggaa actatgcaaa
181 ctaagagggg tagccccatt gcatttgggt aaatgtaaca ttgctggctg gatcctggga
241 aatccagagt gtgaatcact ctccacagca agctcatggt cctacattgt ggaaacatct
...
1441 tgctttgaat tttaccacaa atgcgataac acgtgcatgg aaagtgtcaa aaatgggact
1501 tatgactacc caaaatactc agaggaagca aaattaaaca gagaagaaat agatggggta
1561 aagctggaat caacaaggat ttaccagatt ttggcgatct attcaactgt cgccagttca
1621 ttggtactgg tagtctccct gggggcaatc agtttctgga tgtgctctaa tgggtctcta
1681 cagtgtagaa tatgtattta a

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- i) (4 pts) Design primers to amplify the coding region for this protein, placing EcoRI sites (GAATTC) sites at both ends of the PCR product. After PCR, your final product should be (EcoRI sites underlined):

GAATTCATGAAGG...~1700 bases....TATTTAAGAATTC.

Ignore the melting temperature requirement and make your primers a total of 12 bases in length, including the six bases from the EcoRI sites. Note that only the first and last 300 bases, and only the top strand, is shown above; you may need to generate part of the complementary strand in your primer design.

Left primer: *GAATTCATGAAG*

Right primer: *GAATTCTTAAAT*

(+1 pt)

(+ 3 pts)

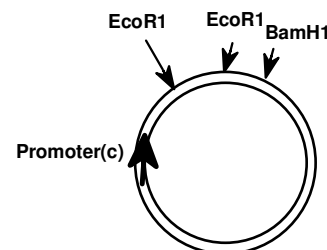
- ii) (4 pts) You have the following expression vector at your disposal. Briefly describe how you would insert the HA gene (PCR product) into the expression vector.

Cut vector with Eco R1 (+ 2pt)

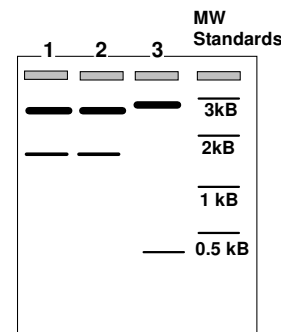
Cut PCR product with Eco R1

Mix DNA

Add DNA ligase. (+ 2pt)



- iii) (4 pts) After inserting your PCR product into the expression vector you transform the DNA into bacteria and check for production of the HA protein. You find that some bacterial produce the HA protein while others do not. You isolate the DNA from a number of bacteria, digest it with BamHI, and separate the DNA fragments by gel electrophoresis. Note that the HA gene contains an internal site for BamHI, at position 230. On this gel samples 1 and 2 produce the protein, while sample 3 does not. Explain why a sample 3 does not produce protein. [use the back of the previous page if you need more space.]



In the case of 3 the fragment has gone in backwards, placing the BamHI site that was within the gene close to the BamHI site on the vector, giving the small <0.5 kB fragment. (+4 pts)

/12

24. (8 pts) Briefly describe the structure of the ribosome (4 pts) and then answer **one** of the following three choices (4 pts):

**Choice A:** Describe the events that occur during elongation of the growing polypeptide chain.

**Choice B:** Describe the events that occur during termination of protein synthesis.

**Choice C:** Although both AAA and AAG code for lysine (Lys) there is only one tRNA<sup>LYS</sup>. Briefly explain how a single tRNA can recognize both codons.

### Ribosome structure

Contains both rRNA and proteins. (+1 pt)

30 S subunit - responsible for binding mRNA (+2 pts)

50 S subunit - responsible for peptide bond formation. (+1 pt)

### Choice A: Elongation

1. The ribosome has 3 tRNA binding sites, one for the exiting uncharged tRNA (E), one that holds the tRNA-peptide (P) and one that hold the incoming charged tRNA (A)
2. tRNA-met is in the P-site.
3. next tRNA-AA binds to the A-site
4. amino group of AA in A-site attacks peptide-tRNA ester, transferring growing chain to tRNA in the A-site.
5. Ribosome shifts, the uncharged tRNA in the P-site goes to the E(exit) site and leaves. The elongated protein is in the P-site.

### Choice B: Termination.

Stop codon in A-site causes protein release factor to bind.

Protein release factor catalyzes hydrolysis of peptide from the last tRNA in the P-site.

### Choice C: Wobble base pair

The anti-codon loop for tRNA<sup>LYS</sup> must be 3'-UUU.

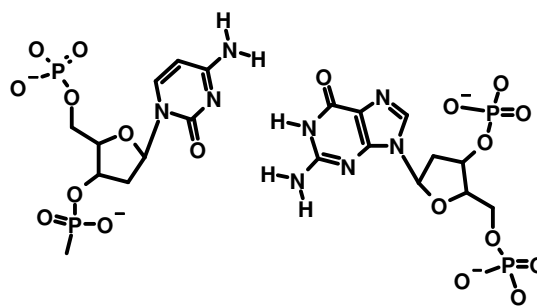
The first two codon-anticodon interactions are typical W-C basepair (A=T).

The last codon-anticodon interactions are W-C for AAA, but a wobble GU basepair.

25 (10 pts) The following binding data was obtained for a protein that binds to double-stranded nucleic acid.

Discuss how this protein recognizes the nucleic acid, including which groove the protein binds to. Support your answer by reference to the experimental data. The following diagram of a basepair may be useful to illustrate your answer.

Nucleic Acid	Conditions	K <sub>D</sub>
AACTT	0.1 M NaCl	10 <sup>-9</sup> M
AAGTT	0.1 M NaCl	10 <sup>-9</sup> M
AATTT	0.1 M NaCl	10 <sup>-8</sup> M
AACTT	0.5 M NaCl	10 <sup>-7</sup> M



- The effect salt on the binding (row 1 versus row 4, same DNA - different salt) indicates involvement of the phosphate group in the protein DNA interaction (+ 5 pts).
- The bases must also be recognized because AATTT has lower affinity than AAGTT or AACTT (+2 pts)
- The fact that AACTT and AAGTT both bind with equal affinity suggests minor groove binding because a C-G basepair has identical H-bonding to G-C in the minor groove. (+ 3 pts)

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