

This exam has 14 pages and contains 220 points. Allot 1 min/2 pts.

Part A: Multiple Choice. Please circle the best answer. 1.5 pts/question 18 pts total.

1. 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.**
2. Which of the following statements is incorrect about *most* DNA polymerases
 - a) require a primer.
 - b) synthesize in the 5' to 3' direction.
 - c) require a template.
 - d) synthesize in the 3' to 5' direction.**
3. DNA Gel Electrophoresis is similar to SDS-PAGE of proteins because
 - a) DNA and proteins are separated according to their molecular weight **(+1 pt)**
 - b) Both techniques rely on a constant charge to mass ratio.
 - c) Both techniques utilize the sieving properties of gels.
 - d) All of the above are correct.**
4. A PCR reaction contains
 - a) mRNA.
 - b) tRNA.
 - c) DNA polymerase.**
 - d) dideoxy-NTPs.
5. In the chain-terminator method of DNA sequencing, an "A" will be read on a sequencing gel when
 - a) ddATP is included in the DNA synthesis reaction.**
 - b) A is present on the template stand.
 - c) ddTTP is included in the DNA synthesis reaction.
 - d) T is present on the primer stand.
6. The RNA polymerases that transcribe bacterial DNA are
 - a) multisubunit enzymes.**
 - b) monomeric and very large.
 - c) interchangeable with DNA polymerases.
 - d) only active inside the cell.
7. Removal of the signal peptide from a protein that is translocated across a membrane is accomplished by
 - a) A serine protease, such as Trypsin.
 - b) Signal Peptidase.**
 - c) HIV protease.
 - d) fMet aminopeptidase.
8. During replication, overwinding or overtightening of DNA is caused by ____ and removed by ____:
 - a) DNA ligase, Gyrase.
 - b) Helicase, DNA polymerase.
 - c) Helicase, Gyrase.**
 - d) DNA polymerase, Gyrase.

Part A: _____ / 18

1: _____ / 10

2: _____ / 7

3: _____ / 8

4: _____ / 18

5: _____ / 12

6: _____ / 16

7: _____ / 6

8: _____ / 6

9: _____ / 4

10: _____ / 16

11: _____ / 18

12: _____ / 8

13: _____ / 12

14: _____ / 14

15: _____ / 4

16: _____ / 10

17: _____ / 6

18: _____ / 8

19: _____ / 19

Total: _____ / 220

9. Which of the following chromatography methods would be appropriate to use if the pH of the solution was lower than the isoelectric pH (pI) of a protein?
- anion exchange.
 - cation exchange.**
 - Gel filtration.
 - Affinity chromatography.
10. Which of the following 'forces' is the most unfavorable for protein folding and the formation of double stranded DNA?
- Configurational Entropy.**
 - Hydrophobic Interactions.
 - Van der Waals Interactions.
 - Hydrogen Bonds.
11. Energy released by the *total* oxidation of glucose is ultimately stored as:
- a concentration gradient across a membrane.
 - on NADH and FADH₂.
 - as ATP.**
 - as ADP.
12. Direct coupling of metabolic reactions requires that
- the product of one is the substrate of the next step.
 - ATP be involved in the reaction **(+1 pt)**
 - the favorable and unfavorable reactions occur on different enzymes.
 - the favorable and unfavorable reactions occur on the same enzyme.**

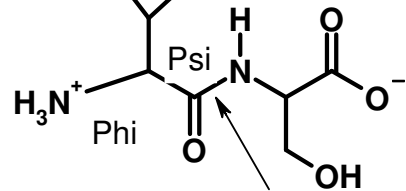
Part B:

1. (10 pts)

- Draw the structure of a dipeptide in the space to the right.
The first amino acid can be any non-polar amino acid and the second can be any polar or charged amino acid (4 pts).
- Name the peptide that you have drawn (1 pt).

Val-Ser

- Indicate the peptide bond with an arrow and list two important characteristics of the peptide bond (3 pts).



The peptide bond is planer and usually trans, as shown in the drawing.

- Indicate the phi and psi angles associated with one residue (2 pts).

As shown on the drawing.

2. (7 pts)

a) Provide a brief, one or two sentence description or definition of the following (6 pts):

i) primary structure: **The linear sequence of amino acids**

ii) tertiary structure: **The folded form of a single chain - the positions of all atoms are known.**

iii) quaternary structure: **A description of a protein with multiple chains.**

b) Give *one* example of a protein that has a quaternary structure (1 pt).

Hemoglobin, antibodies, RNA polymerase, reverse transcriptase, HIV protease.

3. (8 pts) Two important forms of secondary structure are the α -helix and the β -sheet (formed from β -strands). Please answer the following questions.

a) What interaction(s) stabilizes these secondary structures (2 pts).

Hydrogen bonding (1 $\frac{1}{2}$) between mainchain atoms (1/2 pt).

b) Compare and contrast the structure of the mainchain (backbone) and sidechain groups in these two structures (a labeled drawing is fine) (5 pts).

In an α -helix the mainchain follow a helical pattern with the sidechains pointing out. (2 $\frac{1}{2}$ pts)

In a β -sheet the strands are arranged in a parallel (or anti-parallel) manner, with the side chains pointing above or below the sheet. (2 $\frac{1}{2}$ pts)

c) What is a Ramachandran plot? What is its relationship to a α -helix and β -strand (1 pt).

It is a two dimensional plot where each point is defined by the phi and psi angles of a single residue.

Residues in α -helix or β -strand are found in different regions of the Ramachandran plot since they have different phi and psi angles.

4. (18 pts)

a) Provide a molecular description of the hydrophobic effect. Is it an enthalpic or entropic term (6 pts).

- **It is the ordering of water molecules around exposed non-polar groups.**
- **The ordered water molecules are released with the non-polar group is buried.**
- **The entropy gain due to release of the water is favourable.**

b) Briefly discuss the role of the hydrophobic effect in *four* of the following. If the hydrophobic effect has no *significant* role, briefly explain why and describe the major energetic term that is involved. (12 pts)

i) Stability of double stranded DNA.

- **No effect (bases are polar and not completely buried in dsDNA)**
- **Most important effect that stabilizes double stranded DNA is base stacking (pi-pi stacking, van der Waals)**

ii) Well-packed core of globular proteins.

- **No contribution from the hydrophobic effect.**
- **The well packed core occurs due to van der Waals interactions.**

iii) Formation of lipid bilayers.

- **Hydrophobic effect is most significant, causing the non-polar acyl chains to form a bilayer structure.**

iv) Melting of lipid bilayers.

- **No contribution from the hydrophobic effect.**
- **The melting is determined by van der Waals interactions between acyl chains.**

v) Non-polar interior of globular proteins.

- **Hydrophobic effect is most significant, causing the non-polar amino acid side chains to form the non-polar core structure.**

vi) Phe-Ala as a substrate for Chymotrypsin *or* HIV protease.

- **Hydrophobic effect is most significant, causing the non-polar amino acid side chains on the substrate to interact with non-polar residues in the active site.**
- **(+ 2pts if van der Waals was stated)**

vi) Lys-Ala as a substrate for Trypsin.

- **No contribution from the hydrophobic effect.**
- **The binding of the substrate is due to an electrostatic interaction between the + charge on the lysine and the neg charge on an Asp residue in Trypsin.**

5. (12 pts) Please do **one** of the following two choices.

Choice A: Define or describe allosteric effects and discuss the importance of these effects in biochemical systems. Illustrate your answer with an example.

Allosteric effects either increase or decrease the activity of a protein or enzyme by the binding of a ligand (or by protein phosphorylation). The binding cause some sort of change in shape of the protein (+ 4 pt)

The enzyme exists in two states relaxed (R) or tense (T) with the relaxed being the active form.

- **Allosteric activators bind and stabilize the R form.**
- **Allosteric inhibitors bind and stabilize the T form.**

(+4 pts)

Examples (+4pts)

- Oxygen binding to hemoglobin increases its affinity, which is important for efficient delivery of oxygen to the tissues.**
- PFK is activated by fructose 2,6 P, allowing glycolysis to be regulated (indirectly) by hormones.**
- The lac repressor binds lactose (or IPTG) such that genes encoding lactose metabolism (or recombinant protein) can be turned on when needed.**

Choice B: Explain, in general terms, why enzymes enhance the rate of reactions. Illustrate your answer with an example of a specific enzyme.

- **Enzymes lower the energy of the transition state relative to the reactants (+4 pts)**
- **The rate of reaction is proportional to the transition state.**
- **Since the energy of the transition state is lower, there will be more transition state, and the rate will be higher. (+4 pts)**

The transition state is stabilized by two methods:

- a decrease in entropy due to the pre-arrangement of catalytic residues in the active site. For example the catalytic triad in serine proteases (Ser, His, Asp) or the two Asp residues in HIV protease.**
- By forming direct interactions between the transition state and the enzyme, e.g. the oxyanion hole in serine proteases forms a hydrogen bond with just the transition state.**

+2 for each general point, +2 for an example (only one specific example was required)

6. (16 pts) I was working in my garden on Saturday using my arms to lift a very heavy tool.

a) (2 pts) After about 10 min of *very* vigorous lifting my arms became quite weak. What compound(s) did I deplete in my muscles after 10 min?

I have converted all of my ATP to ADP and P_i. Plus I have oxidized most of my glucose and reduced the levels of glycogen in my muscle tissue.

b) (10 pts) After resting for about 2-5 min, my arm strength was pretty much back to normal. Describe the events that transpired to restore energy to my arms. You should *briefly* discuss (i.e. name) *all* of the metabolic pathways that would be involved, how they might be regulated, and the involvement of any hormones.

1. **The use of glucose by my muscles has lowered the glucose levels in my blood.**
2. **Glucagon is secreted (by the pancreas) (+2 pts)**
3. **Glucagon causes enzyme phosphorylation in the liver, activating glycogen phosphorylase, producing glucose from glycogen. (+2 pts)**
4. **F26P levels will be low in the liver, indirectly due to enzyme phosphorylation. (+2 pts)**
5. **Glycolysis is off since PFK is activated by F26P. Gluconeogenesis is on since fructose 1,6 bis phosphatase is inhibited by high levels of F26P, this produces glucose. (+2 pts)**
6. **Glucose goes from liver into blood.**
7. **Glucose in muscle is either stored as glycogen, or oxidized to produce ATP via the following pathway (+ 2pts)**
glycolysis --> TCA --> electron transport ----> ATP synthase.

c) (4 pts) After about 2 hours of this activity I noticed that it became increasingly more difficult to restore the energy levels in my arms and maintain this activity. How did my metabolism change, and why?

I have used up all of glycogen in the liver (and muscles) I am starting to oxidize fats.

7. (6 pts) Compare and contrast feedback versus product inhibition of metabolic pathways. Provide *one* example of a feedback inhibitor.

A product inhibitor inhibits the enzyme that created it (+2 ½ pts)

A feedback inhibitor is a compound from a subsequent step in the pathway inhibiting an earlier step, i.e. it is not a direct product of the reactions (+ 2 ½ pts)

Example:

PFK is inhibited by ATP (1 pt).

8. (6 pts) The Hill plot for the binding of the restriction endonuclease EcoR1 to GAATTC (double stranded) is shown to the right. The slope of the line is equal to 1.

a) What does this result tell you about the cooperativity of binding? (4 pts)

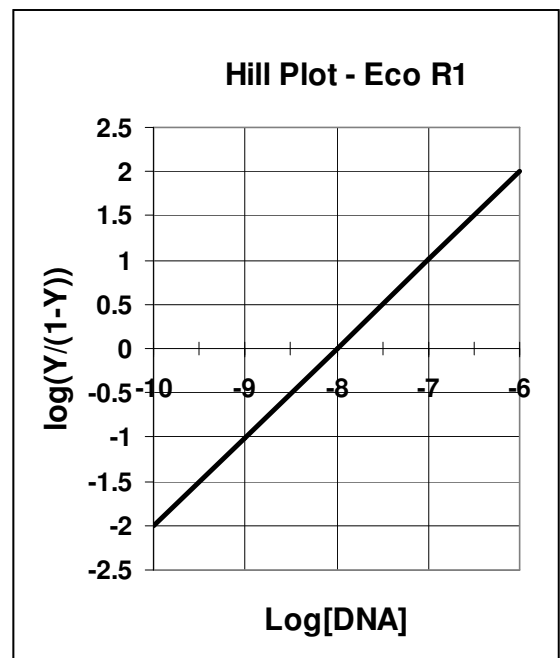
It is non-cooperative since the Hill coefficient is one.

(+4 pts)

b) Is this conclusion consistent with the structure of restriction endonucleases and how they interact with DNA? Why? (2 pts)

Yes, the enzyme only binds one GAATTC sequence, therefore it can't be cooperative.

(+2 pts)



9. (4 pts) Define specific activity and briefly describe its usefulness in protein purification.

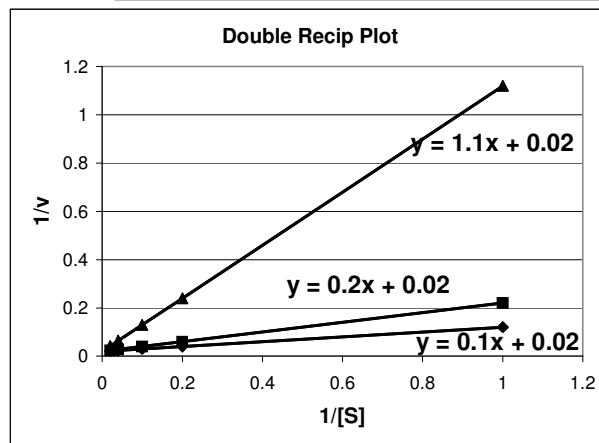
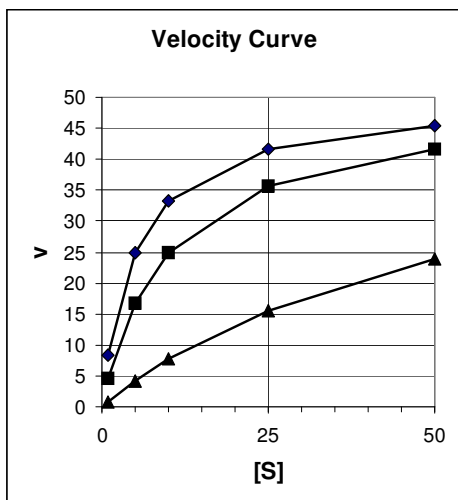
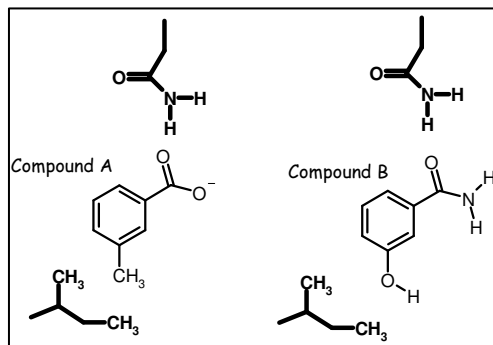
The specific activity is the activity of the protein that is being purified divided by the total amount of protein:

$$\text{Specific Activity} = \frac{\text{Enzyme Activity}}{\text{Total Protein}}$$

(+2 pts)

Since the total amount of protein should decrease during a purification, the specific activity increase can be used to determine if a step in the purification scheme actually increased the purity. (+2 pts)

10. (16 pts) Two compounds that bind to the same site on an enzyme are shown to the right, along with some amino acid sidechains from the enzyme (shown in bold). The initial rate of product formation was measured in the absence of either inhibitor, and in the presence of equal amounts (1 μM) of either inhibitor. The data are plotted on both velocity curves and on a double reciprocal plot, however none of the curves are labeled. The equation of each line in the double reciprocal plot is given.



a) On the basis of the kinetic data, are these competitive or non-competitive inhibitors? Why? (4 pts).

They all have the same y-intercept, or V_{MAX} therefore they are competitive.

b) Based on your answer to part a), where do you suppose these compounds are binding – to the active site or elsewhere? (2 pts).

Competitive inhibitors bind to the active site.

c) Determine the K_I for each inhibitor from the double reciprocal plots (4 pts).

The lowest straight line corresponds to the uninhibited reaction, since $1/v$ is plotted.

The K_I is obtained from α : $K_I = [I]/(\alpha-1)$.

α is obtained from the ratio of the slopes.

For the upper curve: $1.1/0.1 = 11$, $K_I = 1 \mu\text{M} / (11-1) = 0.1 \mu\text{M}$.

For the lower curve: $0.2/0.1 = 2$, $K_I = 1 \mu\text{M} / (2 -1) = 1.0 \mu\text{M}$

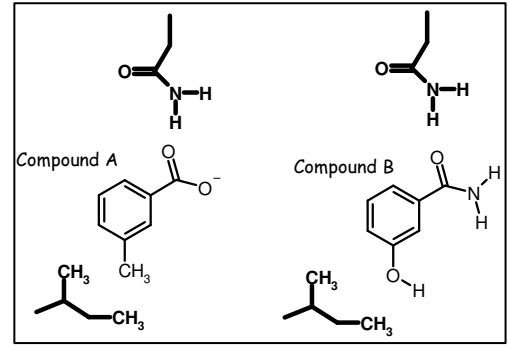
Question 10, continues...

d) Which K_I is associated with which molecule? Justify your answer by reference to the interaction between the molecules and the enzyme. The structure of the enzyme inhibitor complexes have been repeated to the right. (4 pts).

The K_I values are $0.1 \mu\text{M}$ and $1 \mu\text{M}$.

The lower the K_I the better the binding.

Compound A forms a nice non-polar interaction with the enzymem while compound B has a polar group. Therefore compound A binds with the K_I of $0.1 \mu\text{M}$.



e) How would you change the *enzyme* to enhance the interaction to the compound that binds less tightly? (2 pts)

Replace the non-polar isoleucine group with something that can form H-bonds with the -OH group on compound B, e.g. Ser, Thr, Asn, Gln.

11. (18 pts) The following shows the structure of a single stranded nucleic acid.

a) (6 pts) Label the following items on the structure using the Roman numeral and an arrow that clearly points to the item.

- i) 5' end.
- ii) 3' end.
- iii) A ribose.
- iv) A phosphodiester bond
- v) A purine.
- vi) A glycosidic bond.

b) (2 pt) Add *all* missing charges: **One/phosphate**

c) (2 pts) Write the sequence of this nucleic acid.

5'-UGAT

d) (2 pts) Write the sequence of the complementary strand.

5'-ATCA

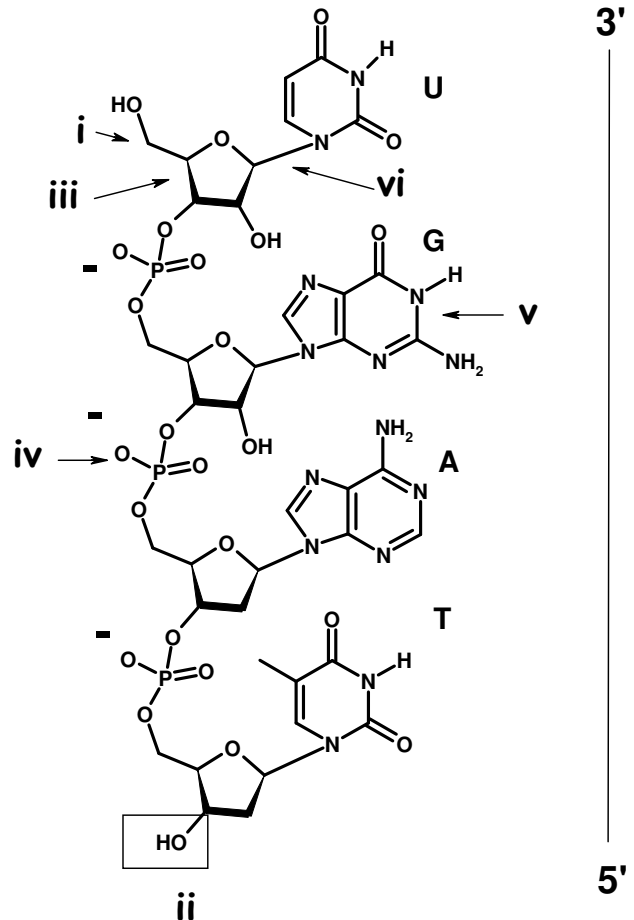
e) (2 pts) If this were a primer for DNA synthesis, indicate where the next nucleoside would be added with a **box**.

3'-OH group

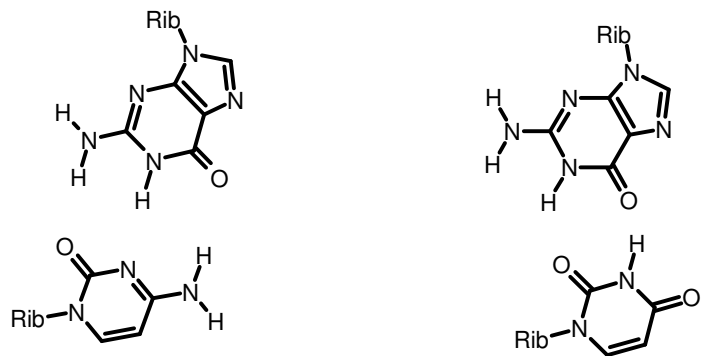
f) (2 pts) Could this nucleic acid be found in Okazaki fragments during DNA replication? Justify your answer.

Yes - the 5' is RNA, the 3' end is DNA. It would represent an RNA primer that has had two bases added.

g) (2 pts) Indicate, by means of a sketch, where the complementary strand would be located. Be sure to indicate its direction (e.g. 5'-3'). **As drawn - antiparallel.**



12. (8 pts) Two codons, CAU and CAC, code for the amino acid Histidine. However, in most organisms there is only one tRNA that can be charged with Histidine and recognize these codons. Explain how this occurs. Your answer should include a discussion of tRNA–mRNA interactions and provide specific details about base-base interactions. You may find the diagrams shown to the right useful in illustrating your answer.



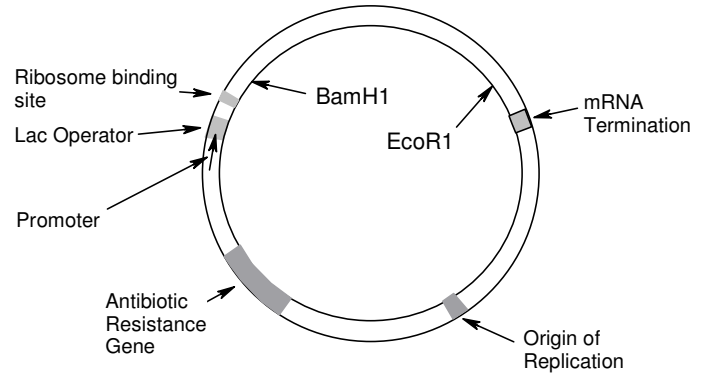
The first two bases of the anticodon loop will form “Watson-Crick” -H bonds with the mRNA. The 5' base of the anticodon can recognize either a U or a C. If it is a G it can form a G-U “wobble” basepair the U, as shown in the upper right. The G can form a normal W-C basepair with the C, as shown in the upper right. As a diagram:

tRNA anticodon: 3'-GTG-5' 3'-GTG-5'
 mRNA Codons: 5'-CAU-3' 5'-CAC-3'

13. (12 pts) Select any **one** of the three polymer synthesis reactions that were discussed in the course (DNA replication, RNA synthesis, protein synthesis) and describe the process of synthesis. Your answer should state the template, monomeric units, and the final product. You should also describe the initiation events, polymerization, and termination (briefly).

| | DNA Replication | mRNA Production | Protein Synthesis |
|--------------------|--|--|--|
| Template | DNA | DNA | mRNA |
| Monomers | dNTP | NTP | Amino Acids |
| Product | DNA | mRNA | Protein |
| Initiation | <ol style="list-style-type: none"> dsDNA is opened. ssDNA coated with SSB Helicase and Gyrase added. RNA primers made by primase. | <ol style="list-style-type: none"> RNA pol binds to promoter. Open complex forms, exposing single stranded DNA template. No primer is required. | <ul style="list-style-type: none"> 30s, 50s ribosomal subunits, mRNA and tRNA^{MET} form initiation complex. Start codon is in the P site along with the tRNA^{MET}. |
| Elongation | <ul style="list-style-type: none"> PolIII does the leading strand in a contineous manner. Lagging strand Synthesis: <ol style="list-style-type: none"> PolIII PolI exo removes RNA primer. PolI 5'-3' pol fills gap caused by removal of RNA bases. DNA ligase joins phosphodiester backbone. | NTPs are added to 3' end, reading template strand. | <ol style="list-style-type: none"> Next tRNA, with its amino acid bind to the A site. Peptide bond formation occurs, moving peptide from P-site to A-site. Ribosome translocates: tRNA in P-site goes to E-site. tRNA-peptide in A site-goes to P-site. |
| Termination | It happens | mRNA termination signals cause termination. | Stop codon causes termination factor to bind. Hydrolysis of peptide from tRNA occurs. |

14. (14 pts) The following show a diagram of an expression vector (plasmid). The nucleotide sequence of a portion of the expression vector, from the promoter to the mRNA termination site is shown below.



The recognition sequence for BamH1 is G[^]GATCC while that for EcoR1 is G[^]AATTC.

mRNA Start RBS
TTGACATTTATGCTTCCGGCTCG**TATAAT**GTGTG**GAAT****TGTGAGCGGATAACAATTT****CACACA**AGGACGGATCC---GAATTC-----mRNA Term.
Promoter **Lac operator** **BamH1** **EcoRI**

a) Explain the role of *four* of the following in the production of recombinant protein in bacteria (12 pts).

i) Origin of replication:

Insures that plasmid will be replicated inside bacterial cell.

ii) Antibiotic resistance gene:

Bacteria containing the plasmid are resistant to killing by the antibiotic. Allows a mechanism to identify bacteria with plasmid inside.

iii) Promoter:

Site where RNA polymerase binds and begins to make mRNA.

iv) Lac operator:

Site where lac repressor binds. When its bound, no mRNA can be made, therefore no protein can be made. Addition of IPTG causes lac repressor to leave DNA, turning on mRNA production.

v) Ribosome binding site:

Responsible for binding of mRNA to ribosome.

b) What two important features that are important for *mRNA translation* are missing from this expression vector? Where should they be located with respect to the other features listed above? (2 pts)

There is no start codon or stop codon. The start codon should be adjacent to the ribosome binding site and the stop codon should be adjacent to the mRNA termination site:

Promoter ---- RBS --- Start ----- Stop ----- mRNA termination.

15. (4 pts) If the following DNA was treated with BamHI, what are the products?

GGGGATCCCC
CCCCTAGGGG

Bam HI cuts between the Gs in the sequence GGATCC, on both strands. The products will be:

GGG GATCCCC
CCCCTAG GGG

16. (10 pts) You want to produce human growth hormone in bacteria. The gene & associated peptide sequence for growth hormone is as listed below.

MetProArgSerArgAspValAlaSerTyrCys
GCCCTTAAATGCCCGCTCGCGGGACGTCGCCAGTTATTGTTAATTTACTG
CGGGAATTTACGGGGCGAGCGCCCTGCAGCGGTCAATAACAATTAATGAC

- a) Give the sequence of the PCR primers that would be required to amplify the gene for human growth hormone such that it would be correctly inserted into the expression vector, as it is shown on the previous page. Don't worry about the T_M, simply make both primers 12 bases long. Be sure to write both primers in the 5'-3' direction (5 pts).

The Bam HI site should be at the start codon and the EcoRI site needs to be at the other end. We also need the start and stop codons because they are not in our vector.

The left primer is the sequence from the upper left, with the BamHI sequence on its 5' end: GGATCCATGCCC

-1 pt if they had the right region, but didn't start at the Met codon, as this moves the SD sequence too far from the Met codon, i.e. GGATCCGCCCTTT will result in the loss of 1 pt.

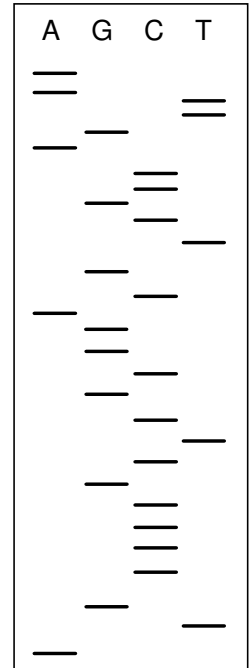
The right primer is the sequence from the lower left, with the EcoRI sequence on its 5' end: GAATTCTTAACA

Anything is fine here, as long as they have the stop codon (eg. GAATCCCAGTAAATT.. is ok)

- b) Briefly describe how you would insert the PCR product into the expression vector (5 pts).

1. Digest the PCR product with Bam HI and Eco R1
2. Digest the vector with Bam HI and Eco R1
3. Mix, the sticky ends will cause the two fragments to join.
4. Treat with DNA ligase to reform phosphodiester bonds.

17. (6 pts) You produce recombinant growth hormone in bacteria, but find that it is inactive in humans. The DNA sequencing gel is shown to the right. Does this gel explain why the protein is non-functional? Justify your answer.



The expected sequence is:

Met Pro Arg Ser Arg Asp Val Ala Ser Tyr Cys
 ATG CCC CGC TCG CGG GAC GTC GCC AGT TAT TGT TAATTTACTG

The sequence off of the gel is:

ATG CCC CGC TCG **CGG** ACGTCGCCAGTTAA

is missing the first **G** of the **Asp** residue. This will shift the reading from, causing the remaining amino acids to be incorrect. e.g. the next amino acid is **Thr**, not **Asp**.

18. (8 pts) After correcting the mistake in the DNA for the growth hormone (see previous problem), you find that it is impossible to purify the human growth hormone from the complex mixture of proteins in the bacterial cells. Briefly describe how you would fix this problem by **one** of the following two approaches. Please indicate your choice.

Choice A: Describe what changes you would make to the expression vector such that the growth factor was exported out of the cell.

Add the appropriate codons for the leader peptide to the amino terminus of the protein. This could be done by PCR, i.e. having a very long 5' end on the left primer. In practice it would be easier to get an expression vector that already has the leader peptide included.

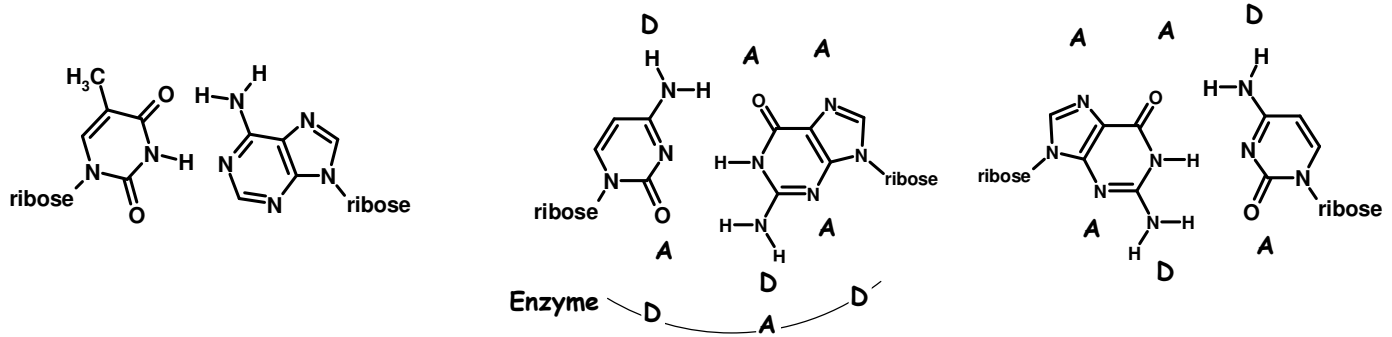
Choice B: It is possible to purify proteins using affinity chromatography by including a segment of 6 His residues at either the amino-terminus or the carboxy terminus of the protein. The codon for His is CAC. Describe how you would modify the PCR primers to add these His residues.

The sequence CAC CAC CAC CAC CAC CAC would have to be added to one end. For example, to add this to the amino terminus, the PCR primer would be:

GGATCC ATG CAC CAC CAC CAC CAC CAC CCC CGC, where the spaces have been added for clarity. Note that the Met codon has to be placed before the 6 His codons, -1 pt if it is not.

19. (19 pts) A T-A and a C-G basepair are shown below.

a) (2 pts) Identify the minor and major groove in both basepairs.



The major groove is at the top, the minor on the bottom. To identify the grooves, a circle drawn through the riboses and the smaller arc is the minor groove.

b) (5 pts) A hypothetical protein will bind to *either* GC or CG basepairs, but only weakly to AT or TA basepairs. Is the protein binding in the major or minor groove via hydrogen bonds? Justify your answer.

Since the protein can't tell the difference between GC or CG it must be binding in the minor groove. The pattern of hydrogen bond acceptors and donors for a CG basepair (shown above) is A-D-A. The GC base pair has the same pattern A-D-A. If it were binding in the major groove, the pattern of H-bond donors and acceptors is different and so you would have an different interaction between the enzyme and each basepair. The pattern of acceptors and donors on the enzyme must be DAD, which and interact with either CG or GC via the minor groove.

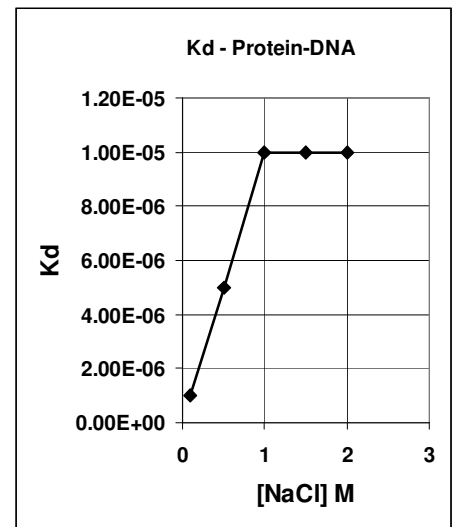
c) (10 pts) The K_D for the binding of the protein to double stranded DNA composed entirely of GC bases was measured as a function of salt concentration and the data is plotted in the graph to the right.

i) Does the affinity, or binding strength, to the DNA increase or decrease as the [NaCl] increases? Justify your answer. (2 pts).

The K_D is increasing as the salt conc increases, so the affinity is decreasing, small K_D values indicate tight binding, larger K_D values, weaker binding.

ii) Based on your answer to part i, what additional interactions occur between the protein and the DNA besides hydrogen bonding? Justify your answer. (4 pts)

Since salt is reducing the affinity it must be an electrostatic interaction between positively charged groups on the protein (e.g. Lys) and the negatively charged group on the phosphate of the DNA.



iii) How much do *each* of these interactions (Hydrogen bonds and the interaction stated in part ii) contribute to the standard free energy of binding (Assume $RT=2.5$ kJ/M). (4 pts).

The K_D at low salt is 10^{-6} M and at high salt 10^{-5} M.

The relationship between ΔG° and K_{EQ} is $\Delta G^\circ = -RT \ln K_{EQ} = -RT \ln (1/K_D)$

At low salt: $\Delta G^\circ = -2.5 \ln (10^6) = -2.5 (2.3) (6) = -34.5$ kJ/M

At high salt: $\Delta G^\circ = -2.5 \ln (10^5) = -2.5 (2.3) (5) = -28.75$ kJ/Mol.

At low salt both H-bonding and electrostatics contribute to the standard energy, but at high salt only hydrogen bonds. Therefore the H-bonds contribute -28.75 kJ/M and the electrostatic -5.75 kJ/M (=28.75 - 34.5)