This exam contains 100 points in 6 pages. Use the space provided. On questions with choices, all of your answers will be graded and you will receive the highest grade.

Part 1 – Multiple Choice 12 pts.

1. A prokaryotic mRNA molecule can contain multiple start and stop codons.
   a) True b) False (operons produce a single mRNA that codes for 2 or more proteins)

2. All hormones require a membrane bound receptor for signaling.
   a) True b) False (e.g. estrogen can cross the membrane)

3. Cellulose is a polysaccharide consisting of _glucose_ units linked with _beta(1-4)___ glycosidic linkages (fill in the blanks).

4. Genes are generally regulated as operons in
   a) eukaryotic cells
   b) prokaryotic cells.

5. If an apple is used to represent a ribosome, then the stem is
   a) the emerging polypeptide chain.
   b) a tRNA molecule carrying an amino acid.
   c) the mRNA

6. Okazaki fragments are
   a) an intermediate in the synthesis of the lagging strand.
   b) short fragments composed entirely of DNA or entirely of RNA.
   c) an intermediate in the synthesis of the leading strand.
   d) only observed in cells with defective polymerases.

7. The aminoacyl tRNA synthetase that attaches the amino acid valine to tRNAs can attach valine to any tRNA.
   a) True b) False (if this were true, codons would no longer be read properly, valine would be incorporated at locations that should have other amino acids).

8. The spontaneous formation of micelles, membranes and soluble proteins is due to this effect or force:
   a) hydrophobic effect
   b) van der Waals
   c) hydrogen bonding
   d) favorable electrostatic interactions.

9. Which of the following are disaccharides (select all that apply).
   a) Glycogen
   b) Glucose
   c) Lactose
   d) Sucrose

10. Which of the following can add a phosphate to an -OH group on a protein or a small molecule?
   a) Phosphatase
   b) Kinase
   c) Polymerase
   d) DNA ligase

11. Which of the following is responsible for removing phosphate groups from phosphorylated molecules?
   a) Phosphatase
   b) Kinase
   c) Ligase
   d) Protease

12. mRNA splicing occurs in both eukaryotic cells and prokaryotic cells.
   a) True b) False

Part 2 – Short Answer:

1. (6 pts) You want to produce human growth hormone in bacteria so that you can administer the growth hormone to individuals who cannot make their own. Assume that you are starting with the mature (sliced) mRNA for the growth hormone; list (do **not** describe) the steps that you have to perform to insert the codons for the growth hormone into a plasmid that would express the hormone in bacteria.

a) PCR to convert the mRNA to DNA and to amplify the sequence and add restriction sites to the end of the PCR product. You have to convert the mRNA to DNA for two reasons, to add the restriction sites, restriction enzymes only work on double stranded DNA.
b) digest both PCR product and plasmid with restriction enzymes
c) mix and ligate, codons are now inserted into the plasmid.

2. (11 pts) The following DNA sequences are present on an expression plasmid.

A) Start codon
B) Ribosome binding site
C) Promoter
D) mRNA termination
E) Lac operator sequence
F) Stop codon
G) Antibiotic resistance gene

i) Give the correct order of these sequences on the expression plasmid. You can draw the entire plasmid or a linear representation of it (3 pts).

Promoter-lac operator-Ribosome binding site-start codon-stop codon-mRNA termination
the antibiotic resistance gene can be anywhere on the plasmid.

ii) Please do **one** of the following choices (6 pts)

Choice A: Indicate which sequences are directly involved in the production of mRNA and briefly describe (**one** sentence) their role in that process.

Choice B: Indicate which sequences are directly involved in the synthesis of proteins and briefly describe (one sentence) their role in that process.

Choice A:
Promoter – RNA polymerase binds and starts production of mRNA
lac operator sequence – lac repressor binds and prevents production of mRNA
mRNA termination – signals end of mRNA production to RNA polymerase.

Choice B:
ribosome binding site – binds mRNA to ribosome
Start codon – required for formation of the initiation complex (50s+30s+tRNA-fmet)
Stop codon – required for release of protein from last tRNA on the ribosome

iii) To initiate the production of mRNA for the growth hormone you would add either lactose or IPTG to the culture media. Why does the addition of this compound start the production of mRNA (2 pts)?

When IPTG (or lactose) bind to the lac repressor, it causes a change in the shape of the repressor so that it no longer binds to the operator sequence – this allows RNA polymerase to make mRNA because the lac repressor protein is no longer blocking its access to the DNA.

3. (2 pts) You have isolated a new compound from fungi that inhibits RNA polymerase from bacteria but not the RNA polymerase in the fungi. What additional property of this compound should you check before considering developing it for use as an antibiotic to treat people?

Although fungi are eukaryotic (like humans) their RNA polymerase may be sufficiently different from our RNA polymerase so the compound may inhibit human RNA polymerase. You should test to see if the antibiotic will inhibit the human polymerase.

4. (10 pts) You have isolated several new antibiotics from fungi. Please do **one** of the following choices.

Choice A: The antibiotic causes all new proteins to remain attached to the ribosome. What step in the process is this antibiotic inhibiting? Provide a brief description of the normal process that is being inhibited.

Choice B: The ribosomes begin to make proteins but only short, or premature, proteins are produced by the ribosome. Provide a brief description of the normal process that is being inhibited.

Choice A:

Since the antibiotic is interfering with the release of the protein, the stop codon is not functioning.

The possibilities include – the release factor can't bind, or if it can it cannot hydrolyze the bond between the protein and the last tRNA..

Choice B:

This suggest that the antibiotic is blocking the exit tunnel, so initiation can occur, followed by the addition of a few amino acids (so the A site and the peptide bond forming active site are also functional). However, once a certain length is reached no additional amino acids can be added.

5. (6 pts) Mutations in mRNA splicing can cause the production of non-functional proteins. State one of the possible mutation sites in the **intron** and briefly describe why the resultant protein might be non-functional.

Changes in the 5' splice site, the branch point (A) or the 3' splice site, would prevent splicing. The intron will be left in the mRNA, this will either:

- add additional amino acids to the protein, likely preventing it to function properly
- introduce a stop codon which would cause the premature termination of the protein.

6. (8 pts) Please do **one** of the following choices.

Choice A: What are the roles of helicase and gyrase in DNA replication?

Helicase uses ATP energy to unwind the DNA at the replication fork so that polymerases have access to the template. The unwinding by helicase overtwists the DNA in front of fork, the overtwists are removed by gyrase.

Choice B: What is the difference between leading and lagging strand synthesis?

In leading strand synthesis, the polymerase can copy the entire strand as on continuous piece. In lagging strand synthesis, synthesis is done in pieces because the polymerase has to wait until the template is open before it can begin. The short pieces are called okazaki fragments.

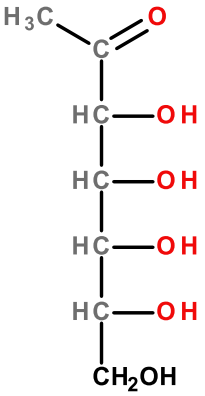
Choice C: Why are two DNA polymerases used during replication? What role do they play?

Pol III replicates most of the DNA on both the leading and lagging strand. It cannot remove RNA primers so a second polymerase, Pol I is required for that. PolI replaces the RNA primer with DNA.

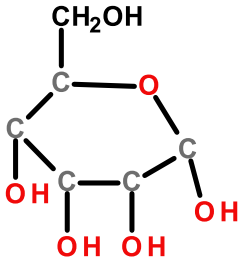
7. (8 pts) Please do **one** of the following choices:

Choice A: The monosaccharide on the right is missing some atoms.

i) Modify the diagram such that it would be a monosaccharide, either an aldose or a ketose. Indicate whether you have drawn an aldose or a ketose. Add a C=O to the first carbon to make an aldose or the second carbon to make a ketose. Remaining carbons should have an –OH group.



ii) Draw the cyclic structure of the monosaccharide and indicate the chirality (α or β) of the new chiral center. See diagram, the a-form is shown (OH on carbon 1 is down). Hydrogens have not been drawn



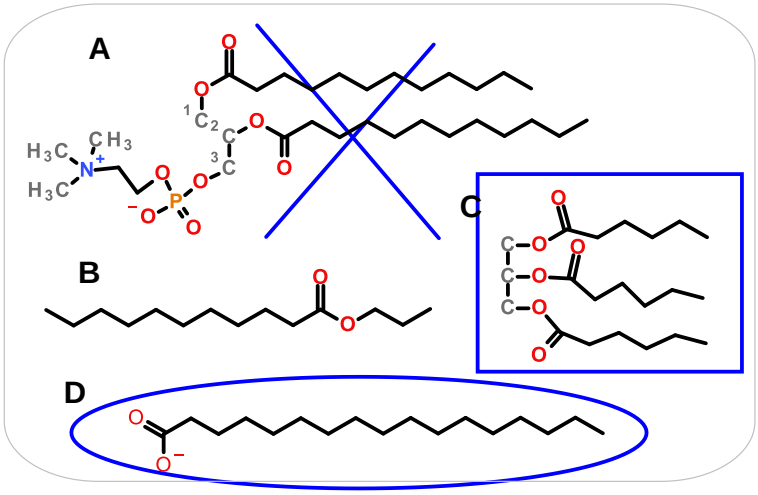
Choice B: The diagram shows the structure of a number of lipids.

i) Circle the compound that could be found in soaps or detergents.

ii) Put a box around the compound that is used for energy storage.

iii) Put an X through the compound that is found in cell membranes.

iv) What important lipid is missing from these structures and what is the role of that lipid in defining the properties of mammalian membranes (5 pts).



Cholesterol, important for keeping membranes fluid.

8. (6 pts) Please do **one** of the following choices:

Choice A: Briefly describe the structure of the bacterial cell wall.

Choice B: How does the antibiotic penicillin function to prevent the growth of bacteria?

Choice A:

Linear polymers of NAM and NAG joined by beta (1-4) linkage.

Crosslinked by proteins between each NAM unit.

Choice B:

Penicillin is a covalent inhibitor of the enzyme that forms the protein crosslink in the cell wall.

The bacterial cell wall is weakened by the absence of the crosslink and the bacteria are now susceptible to osmotic lysis, in particular the influx of water into the cell will cause the cell to burst.

9. (9 pts) Please answer **both** questions regarding **integral** membrane proteins.
   i) Describe the location of polar, charged, and non-polar amino acids on the membrane protein (3 pts).
   ii) Briefly describe the function of one type of integral membrane protein (6 pts).

Note: This question asked about integral membrane proteins, NOT membrane anchored proteins. In the case of integral membrane proteins most of the protein is embedded in the membrane.

i)

polar and charged – are found on the part of the protein that is NOT in contact with the non-polar lipid tails.

non-polar residues – are found on the surface of the protein that contacts the non-polar lipid tails

ii)

Transporters – have a channel through the protein that allows things to enter and exit the cell, can be passive or active.

Signal transduction – bind something outside the cell (e.g. hormone) an transmit, via allosteric change, the signal to inside the cell, e.g. glucagon receptor.

Cargo receptor – brings something across the cell membrane, e.g. the LDL receptor.

10. (2 pts) The concentration of salt in a red blood cell is 0.15 M NaCl. A red blood cell is placed in pure water. Will it shrink, swell, or remain the same size? Why?

- Water can cross the membrane, but salt cannot.
- Water will spontaneously flow from high to low concentration.
- The water concentration outside the cell is higher (it's pure water)
- So water will enter the cell, it will swell (get larger)

11. (10 pts) Please do **one** of the following choices:
   Choice A: Explain how cholesterol production is regulated in the body.
   Choice B: What is the consequence of the loss of the LDL receptor by genetic mutation and how is this disease treated by the use of statins?

Choice A:

- The liver releases cholesterol that it has made as VLDL particles, as cholesterol leaves the VLDL particles they become LDL particles.
- LDL particles, which contain unused cholesterol, are brought into the liver cell by the LDL receptor, a process called endocytosis.
- The cholesterol leaves the internal membrane compartment (endosome).
- Cholesterol inhibits its own synthesis (feedback inhibition), so the more cholesterol that is taken up, the less that is synthesized.

Choice B:

- Without an LDL receptor the liver cell does not know if it is making too much cholesterol.
- The liver will then make excess cholesterol, increasing the levels in the body, usually a bad thing.
- Statins are competitive inhibitors of one of the enzymes in the cholesterol biosynthesis pathway, which would reduce the amount of cholesterol produced by the liver.

12. (10 pts) Please do **one** of the following choices:

Choice A: A protein can either be released as a soluble protein by the cell or anchored in its cell membrane. What are the common features of these two proteins and how do they differ?

Choice B: How is the release of glucose from glycogen controlled by the hormone glucagon under conditions of low blood sugar?

Choice C: A change in receptor signaling is responsible for a large number of breast cancer cases. What is this change and what is one of newer methods used to treat this disease that involves a protein as a drug.

Choice A:

- Since both are exported by the cell they would both have an amino-terminal leader sequence that would cause the ribosome to interact with the rough ER and the protein would be exported into the inside of the rough ER.
- The protein that is to be anchored in the membrane has an additional non-polar segment that will hold the protein in the membrane.

Choice B:

1. Glucagon binds to its receptor.
2. The receptor activates a G-protein by causing the exchange of GTP for GDP
3. The activated G-protein activates adenyl cyclase, producing cAMP
4. cAMP activates protein kinases
5. Glycogen phosphorylase becomes activated when it is phosphorylated
6. Glucose is released from glycogen to increase blood glucose levels.

Choice C:

- The receptor for a growth hormone is over-expressed, this makes the cells more responsive to the growth hormone and they grow uncontrollably.
- An antibody (the protein drug) binds to the receptor and prevents it from being activated by the growth hormone.
- The cancer cell no longer gets a signal to divide.