This exam consists of **100 points** on 5 pages. **Please allot 1 min/2 pts**. On questions with choices, all of your attempts will be graded and you will receive the highest scoring attempt.

1. (10 pts) A plasmid, or expression vector, contains the following DNA sequences that result in the production of a protein in E.coli.

   -Promotor-----Lac Operator-----Start codon-----Codons-----Stop codon-----mRNA termination-

   i) One important element is missing, what is it, and where would you place it on the above diagram? *Briefly justify your answer (2 pts).*

   ii) Which of the above DNA sequence is involved in *initiating* the synthesis of mRNA? (2 pts)

   iii) Which of the above DNA sequences is involved in *regulating or controlling* the production of mRNA. Briefly describe how it works (5 pts).

   iv) Name one other DNA sequence, which is not shown here, that is present on all plasmids. What is its function? (1 pt)

i) The missing element is the ribosome binding site. It should be located between the Lac operator and the start codon.

ii) The **promotor** initiates the synthesis of mRNA

iii) The lac operator is required to regulate or control the production of mRNA.

- It binds the lac repressor protein.
- When the repressor is bound mRNA is not made.
- If an inducer is added, lactose or IPTG, the inducer binds to the lac repressor,
- The lac repressor is released from the DNA, allowing mRNA to be made.

iv) Origin of replication – so that the plasmid is replicated along with the chromosomal DNA. Antibiotic resistance gene. Allows growth of plasmid containing bacteria in the presence of antibiotic, cell that have lost the plasmid will be killed.

2. (6 pts) Compare and contrast the synthesis of DNA by a DNA polymerase to the synthesis of RNA by a RNA polymerase. Give **one** similarity and **two** differences.

Similarities: Template required, both generate polymers in the 5' to 3' direction.

Differences: dNTP versus NTPs, DNA polymerases require a primer, DNA polymerases have proofreading.

3. (5 pts) DNA replication requires the following proteins: a) Helicase, b) Gyrase (a topoisomerase), c) Single stranded binding protein. Pick any **one** of these proteins and briefly discuss its role in DNA replication.

Helicase – uses ATP to separate the double stranded DNA during replication.

Gyrase – unwinds the DNA in front of the fork, releasing overtwisting due to helicase activity.

Single Stranded Binding Protein – binds to the single stranded DNA and prevents it from reforming double strands.

4. (8 pts) Please do **one** of the following choices:

Choice A: Why is DNA synthesis on the lagging strand discontinuous?

Choice B: What are Okazaki fragments and what happens to them during DNA replication?

Choice A: During replication the two templates run in opposite directions because DNA is anti-parallel.

On one strand (leading) the polymerase is moving in the same direction as the replication fork and can polymerize DNA in a continuous manner.

On the other strand (lagging) the polymerase is moving in the opposite direction as the replication fork. Therefore it has to wait until the fork has exposed the template before it can begin. This means the DNA is copied in short, discontinuous fragments (Okazaki fragments).

Choice B:

Okazaki fragments are generated during lagging strand synthesis. They contain the RNA primer and a segment of replicated DNA. The RNA is removed by PolI, and the resultant single stranded DNA is duplicated by polI. After this the fragments are joined by DNA ligase.

5. (8 pts) Please do **one** of the following choices:

Choice A: A number of antibiotics inhibit protein synthesis in bacteria. Assume that an antibiotic binds in the *A-site* of the ribosome. What steps in protein synthesis would still be possible?

Choice B: A new antibiotic has been developed that binds to stop codons on the ribosome, how would this interfere with protein synthesis?

Choice A:

Initiation could still occur. So the mRNA, 30s, 50s, and $tRNA^{fmet}$ would assemble

The tRNA would be in the P-site.

The antibiotic would block the A-site, so no new tRNA can come in and the protein will not be elongated.

Choice B:

This antibiotic would block the binding of the release factor, thus the newly synthesized protein would remain bound to its tRNA (in the P site) and not be released from the ribosome.

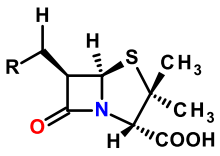
~~6. (5 pts) X-ray crystallography is used to determine the atomic structure of biological molecules. Briefly describe why the scattering of X-rays can be used to determine the position of atoms.~~

~~The electrons around the atoms scatter the X-rays.~~

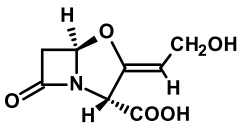
~~The relative position of the atoms affects the intensity of the scattered X-rays~~

~~Since the intensity (and phase) are affected by the positions, it is possible to work backwards from the scattering to the positions.~~

7. (8 pts) The antibiotic penicillin is used to treat bacterial infections. The structures of penicillin and clavulanic acid are shown on the right. Do both parts i) and ii) of this question, you have choices for part ii).



penicillin



clavulanic acid

i) Briefly describe/draw the structure of bacterial cell walls.

ii) Complete **one** of the following choices:

Choice A: How does penicillin inhibit the synthesis of the bacterial cell wall.

Choice B: How does clavulanic acid enhance penicillin's effectiveness towards resistant bacteria?

i) The bacterial cell wall consists of parallel linear polysaccharides that are alternating NAM and NAG which are joined by β(1-4) linkages.
The NAM units are crosslinked by polypeptide chains.

ii) **Choice A:** penicillin binds to penicillin binding proteins, which are the enzymes that catalyze the protein crosslinking of the cell walls. The penicillin forms a covalent bond with the enzyme, inhibiting it. The penicillin is slowly released from the enzyme.

Choice B: Clavulanic acid binds to β-lactamase, the enzyme that degrades penicillin. By inhibiting the enzyme, the penicillin is not destroyed and is thus effective at inhibiting the enzyme the synthesizes the cell wall.

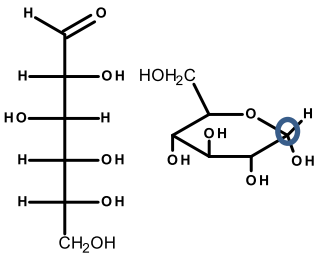
8. (8 pts) The following diagram shows the linear and cyclic form of three monosaccharides.

i) Label each pair of structures with its correct name (one of ribose, glucose, or fructose).

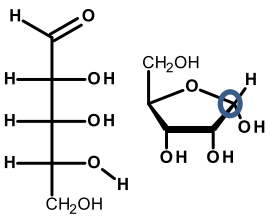
ii) Pick any **one** of the pairs of structures and (do both a and b):

a) indicate whether it is an aldose or a ketose. Justify your answer.

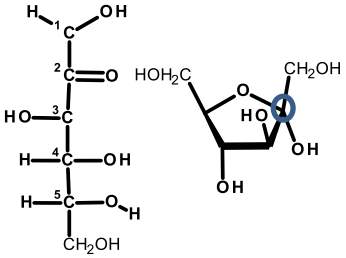
b) indicate the anomeric carbon and state whether it is α or β in its configuration.



Glucose
aldose



Ribose
aldose



Fructose
ketose

Aldoses have an aldehyde group at carbon 1, which is found on carbon 2 for ketoses.
The anomeric carbon is 1 on aldoses, and 2 on ketoses (circled). All of the above are α, since the -OH on the anomeric carbon points down.

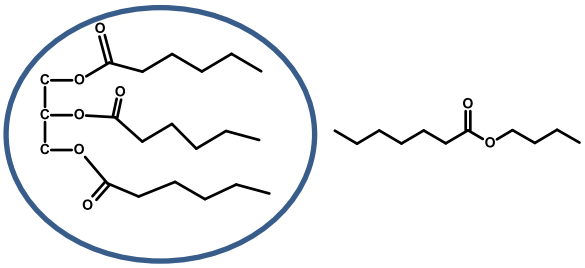
9. (2 pts) Name **one** common dissacharide and give the name of the new bond that is formed when the two monosaccharides are joined.

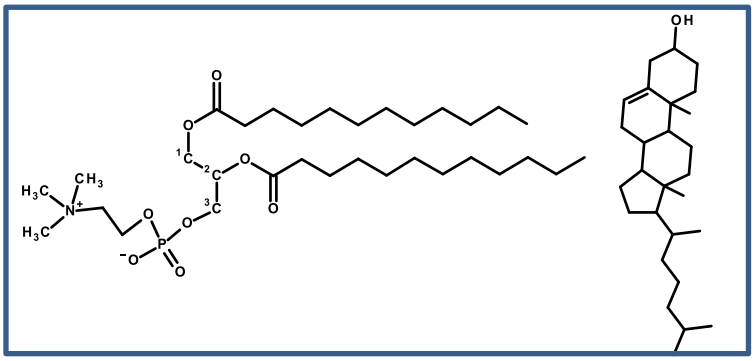
Lactose or sucrose. Glycosidic bond.

10. (6 pts) Briefly compare and contrast glycogen (or starch) to cellulose by completing the following table:

	Glycogen (or starch)	**Cellulose**
Monomeric unit	glucose	glucose
Type of linkages between the units, e.g. β(1-4)	α(1-4) and α(1-6) branches	β(1-4)
Biological function (energy storage or structural)	Energy storage	structural

11. (6 pts) A number of lipids are shown below.
i) Label each lipid with its correct name (e.g., wax, fatty acid, cholesterol, triglyceride, phospholipid)
ii) Circle the lipid(s) that is/are used for energy storage.
iii) Put a box around the lipid(s) that you would find in a biological membrane.





Triglyceride Wax Phospholipid Cholesterol
Energy storage found in membrane found in mem.

~~12. (8 pts) Olive oil is a triglyceride that melts at 10°C and is therefore a liquid at room temperature. Coconut oil is a triglyceride that melts at 25°C and is therefore a solid at room temperature. What is the most likely difference between the fatty acids in these two oils and how does this difference affect their melting temperatures?~~

~~The Olive oil either has shorter fatty acids or has fatty acids with more cis double bonds. This reduces the van der Waals interactions between the molecules, lowering the melting temperature. The shorter chains have fewer atoms that can interact. The cis double bond makes the chain crooked, or kinked, reducing van der Waals.~~

13. (8 pts) Please do **one** of the following choices. Please answer **all** of the questions within a choice.

Choice A: mRNA processing. Please answer **all** of the following:

i) In what type of organism does mRNA splicing occur (circle correct): prokaryotic or eukaryotic?

ii) Briefly describe what occurs during mRNA splicing, in what way is the mRNA modified?

iii) What is alternative splicing and what is its importance in the production of antibodies by B-cells and plasma cells.

Choice B: The immunoglobin heavy chain has both a leader sequence and a stop-transfer (membrane anchor) sequence.

i) Where would you find the leader sequence and what would happen if it were absent?

ii) Where in the protein sequence would you find the stop-transfer sequence and what would happen if it were absent?

Choice A:

i) eukaryotic.

ii) Introns are removed and exons are joined. The order of the exons is always the same.

iii) Alternative splicing leads to a variation in the number of exons that are found in the mature mRNA, i.e. some exons are also spliced out – the order of the remaining exons is the same.

In the case of the heavy chain on antibodies, there are two exons at the end of the gene. Only one of these exons is found in the final mRNA. One exon will cause the protein to be secreted, the second will cause the protein to be retained in the membrane.

Choice B:

i) The leader sequence is found at the amino terminus. If it was missing, the protein would not be imported into the rough ER because the amino terminus would not be recognized by the signal recognition particle.

ii) The stop transfer sequence would be near the C-terminus of the protein. If it were missing, then the heavy chain would be completely exported into the lumen of the rough ER and thus the antibody would be secreted out of the cell and not be bound in the membrane.

14. (2 pts) What "force" or interaction drives the spontaneous assembly of fatty acids into micelles and phospholipids into membranes? Please circle the correct answer:

Hydrogen bonding van der Waals Electrostatics Hydrophobic effect

~~15. (6 pts) Please do **one** of the following choices.~~

~~**Choice A:** Briefly describe the structure and function of the K^+ channel.~~

~~**Choice B:** How might you modify the K^+ channel so that it would let larger ions (Rb^+) through, but not allow small ions through?~~

~~**Choice A:**~~

~~It is a transmembrane protein that carries K^+ through the membrane as a passive transporter. It has a non polar outside and a polar inside. The channel itself is just the right size to bind desolvated (dehydrated) K^+ ions via mainchain C=O interactions.~~

~~**Choice B:**~~

~~You would have to make the selectivity channel (described above) wider. This would actually be hard to do since the channel is made up of the mainchain C=O atoms.~~

16. (4 pts) A eukaryotic cell is placed in distilled water. Will the cell shrink or expand? Why?

Water will flow inward. Since there is more salt inside the water concentration is lower, thus water flows spontaneously from the high concentration outside the cell to the lower concentration inside the cell.

Bonus (1 pt each)

1. What is lactose intolerance? Inability to produce the enzyme lactase, which breaks down lactose to galactose and glucose.

2. What enzyme in DNA replication is inhibited by anti-cancer drugs?

Topoisomerases.

3. Name a disease that is due to mRNA splicing defects.

Inability to produce growth hormone.

4. What protein, if incorporated into the membrane of the cell, would increase its rate of change in size under the conditions specified in question 16. Aquaporin.