In the spirit of the honor code, I pledge that I have neither given nor received help on this exam.

____________________________________
Signature

1_______
2_______
3_______
4_______
5_______
6_______
7_______
8_______
9_______
10_______
11_______
12_______
13._______
1. (16 points) Below is a diagram of a bacterium.

Is this a gram-negative or a gram-positive organism? (circle one)

Label the indicated parts.

What is the composition in terms of major macromolecules of

a._____________________________________________

b._____________________________________________

c._____________________________________________

d. ______________________________________________

Where are the enzymes of the glycolysis pathway located? __________

Where are the cytochromes located? __________

Where is the DNA located?__________________

If this bacterium were of the other type with respect to the Gram stain, what would be 3 major differences in the cell structure?

1. 

2. 

3. 
2. (7 points) Construct a functioning transcriptional unit for the highly transcribed gene *ara* out of the following DNA fragments so that transcription can occur from left to right.

A) ATG  B) TTGACA N<sub>25</sub> TATAA N<sub>10</sub>  
C) AAAAAA  D) ORF-Ara  E) AGGAGGA  
F)  

Explain the roles of A, B, C, E, and F as they relate to transcription and translation.

A) _____________________________________________  
B) _____________________________________________  
C) _____________________________________________  
E) _____________________________________________  
F) _____________________________________________  

3. (6 points) Complete the following table indicating the phenotype (+ or -) of the various \textit{lac} operon mutant \textit{E. coli} strains. The dotted line in the fourth and fifth rows indicates operons on different plasmids.

\begin{itemize}
  \item i: repressor
  \item y: permease
  \item p: promoter
  \item a: transacetylase
  \item o: operator \( o^c \): operator constitutive
  \item \( P_{trp} \): \textit{trp} operon promoter
  \item z: \( \beta \)-galactosidase
\end{itemize}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textit{E. coli} Genotype & Is \( \beta \) galactosidase made on media containing as a carbon source & Ability to grow on media containing glucose, lactose and excess tryptophan \textbf{Ability to grow on media containing lactose and no glucose or tryptophan} \\
& maltose & lactose & \\
\hline
\textit{i}^{+} \textit{p}^{+} \textit{o}^{+} \textit{z}^{+} \textit{y}^{+} \textit{a}^{-} & & \\
\hline
\textit{i}^{-} \textit{p}^{+} \textit{o}^{+} \textit{z}^{+} \textit{y}^{+} \textit{a}^{+} & & \\
\hline
\textit{i}^{-} \textit{p}^{+} \textit{o}^{+} \textit{z}^{+} \textit{y}^{+} \textit{a}^{+} & & \\
\hline
\textit{i}^{+} \textit{p}^{+} \textit{o}^{+} \textit{z}^{+} \textit{y}^{+} \textit{a}^{-} & & \\
\hline
\textit{i}^{-} \textit{p}^{+} \textit{o}^{+} \textit{z}^{+} \textit{y}^{+} \textit{a}^{+} & & \\
\hline
\textit{i}^{-} \textit{p}^{+} \textit{o}^{+} \textit{z}^{+} \textit{y}^{+} \textit{a}^{+} & & \\
\hline
\textit{i}^{+} \textit{P}_{trp} \textit{o}^{+} \textit{z}^{+} \textit{y}^{+} \textit{a}^{-} & & \\
\hline
\end{tabular}
\end{table}

4. (4 points) Tryptophan is an amino acid. The genes for tryptophan biosynthesis \textit{trpEDCBA} are found in an operon and are regulated by a repressor and by attenuation. For each \textit{E. coli} mutant shown in the table below, put a checkmark indicating the amount of gene mRNA produced if the bacteria were grown in the stated medium.

\begin{itemize}
  \item o: operator \( o^c \): operator constitutive
  \item \textit{P}: tryptophan promoter
  \textit{EDCBA}: genes for tryptophan biosynthetic enzymes
  \item \textit{R}: tryptophan repressor gene
\end{itemize}

\begin{itemize}
  \item 1 - mRNA leader sequence region 1
  \item 2 - mRNA leader sequence region 2
  \item 3 - mRNA leader sequence region 3
  \item 4 - mRNA leader sequence region 4
\end{itemize}

\begin{itemize}
  \item + means the region is present, - means the region is absent.
\end{itemize}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
\textit{E. coli} Genotype & Level of gene mRNA produced in minimal medium with glucose and excess tryptophan & Level of gene mRNA produced in minimal medium with glucose only \\
& & None & Low & High & None & Low & High \\
\hline
\textit{R}^{+} \textit{P}^{-} \textit{o}^{+} 1^{+} 2^{+} 3^{+} 4^{+} \textit{EDCBA} & & & & \\
\hline
\textit{R}^{-} \textit{P}^{-} \textit{o}^{+} 1^{+} 2^{+} 3^{+} 4^{+} \textit{EDCBA} & & & & \\
\hline
\textit{R}^{+} \textit{P}^{-} \textit{o}^{+} 1^{+} 2^{+} 3^{+} 4^{+} \textit{EDCBA} & & & & \\
\hline
\textit{R}^{-} \textit{P}^{-} \textit{o}^{+} 1^{+} 2^{+} 3^{+} 4^{+} \textit{EDCBA} & & & & \\
\hline
\end{tabular}
\end{table}
5. (16 points) Fill in the following table with respect to bacterial growth.

<table>
<thead>
<tr>
<th>Organism and medium</th>
<th>Conditions</th>
<th>Growth rate (fast, medium, slow, or none)</th>
<th>biochemical process producing energy</th>
<th>Electron donor</th>
<th>Final electron acceptor</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> with glucose and nutrient broth (rich medium)</td>
<td>aerobic 37°</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> with glucose and nutrient broth</td>
<td>anaerobic 37°</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> with CO₂, NO₃, H₂SO₄, and salts</td>
<td>aerobic 37°</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Thiobacillus</em> in medium with H₂SO₃, and salts</td>
<td>aerobic 25°</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
6. (7 pts.) Shown below are the components of the chemotaxis system in *E. coli*.

![Chemotaxis System Diagram]

Complete the table with the phenotype of each of the following mutants:

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>cheR</em>-</td>
<td></td>
</tr>
<tr>
<td><em>cheB</em>-</td>
<td></td>
</tr>
<tr>
<td>CheA where the histidine responsible for becoming phosphorylated is replaced with arginine</td>
<td></td>
</tr>
<tr>
<td>CheY lacking histidine</td>
<td></td>
</tr>
<tr>
<td>Increased binding affinity of MCP for attractant</td>
<td></td>
</tr>
</tbody>
</table>

For wild type *E. coli*:

In an environment that maintains constant levels of chemoattractant, *E. coli* will show ________________ methylation of MCP.

In an environment where the levels of chemoattractant increase, *E. coli* will show ________________ sensitivity to the attractant over time.

7. (6 pts.) In *E. coli*, porin regulation controls osmotic pressure. This is a two-component system in
which the sensor kinase, EnvZ detects the environmental signal (in this case, changes in osmolarity) and passes the signal on to the response regulator, OmpR. When OmpR receives the signal, it activates the transcription of the *ompC* gene (which makes OmpC, a porin with a smaller pore) and represses transcription of the *ompF* gene (which makes OmpF, a porin with a larger pore).

<table>
<thead>
<tr>
<th>Physical location in the cell in the presence of the environmental signal</th>
<th>Sensor kinase protein</th>
<th>Response regulator protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Will the protein be phosphorylated in the presence of the signal?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

What size pores (large or small) will the bacteria synthesize if they lack phosphatase, an enzyme that removes the phosphoryl group from the response regulator? __________

What size pores (large or small) will the bacteria synthesize if the sensor cannot be phosphorylated? __________

8. (3 pts.) Draw the location of an obligately aerobic bacterium in the tubes below which are filled with nutrient broth and glucose with the following conditions and mutations:

<table>
<thead>
<tr>
<th>Wild type</th>
<th>Mutation in ability to sense oxygen</th>
<th>Mutation so that oxygen is avoided</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

medium → air
9. (8 points) Answer the following questions indicating “clear, cloudy or none”.

What type of plaques does T7 make on wild type *E. coli* K12? _______________

What type of plaques does T7 make on *E. coli* K12 (λ)? _______________

If we replace the early promoter in T7 with the late promoter what type of plaques will it make on *E. coli* K12? _______________

If we replace the early T7 promoter with λ Pr what type of plaques will it make on *E. coli* K12? _______________

10. (10 points) Fill in the following table indicating by a + when each of the viral genes is expressed. **Mark only one space for each gene.**

<table>
<thead>
<tr>
<th>virus</th>
<th>early genes</th>
<th>delayed early or intermediate genes</th>
<th>late genes</th>
<th>not a viral gene product</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4 nuclease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4 head proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4 lysozyme</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4 DNA polymerase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4 sigma factor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T7 ribosomes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4 t-RNAs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>polio coat proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>polio protease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>polio lipid biosynthesis enzymes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

11. (3 points) Fill out the following table indicating the type of plaques (clear, cloudy, or none) which would be formed if λ of the indicated genotype infected the strains of *E. coli* shown. The genetic map of λ is given below.

\[
P_R \text{ cro t}_{R1} \text{ CII } \text{ t}_{R2} \text{ Q t}_{R3} \text{ P}_{R\text{late SR}}...A...
\]

<table>
<thead>
<tr>
<th>xis att int</th>
<th>CIII</th>
<th>t_{L1}</th>
<th>N</th>
<th>P_L</th>
<th>CI</th>
<th>P_{RM}</th>
<th>P_{RE}</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>λ genotype</th>
<th>bacterial host</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild type</td>
<td><em>E. coli</em> K12 (-)</td>
</tr>
<tr>
<td>P_R no longer binds CI</td>
<td><em>E. coli</em> K12 (λ)</td>
</tr>
<tr>
<td>P_{RM} replaced with P_{lac} and the cells are grown on minimal medium with glucose</td>
<td></td>
</tr>
</tbody>
</table>
12. (5 points) Fill in the following table with respect to λ phage by checking one column.

<table>
<thead>
<tr>
<th>λ genotype</th>
<th>plaques</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>clear</td>
</tr>
<tr>
<td>delete C₁</td>
<td></td>
</tr>
<tr>
<td>delete t₁ and tᵣ₁</td>
<td></td>
</tr>
<tr>
<td>reduce binding of Cᵢ and Cᵢᵢ to Pₑ</td>
<td></td>
</tr>
<tr>
<td>delete Pₑ</td>
<td></td>
</tr>
<tr>
<td>delete N</td>
<td></td>
</tr>
</tbody>
</table>

13. (9 points) Chromobacteria are colored bacteria; colonies usually appear pale purple or pink. In looking at a plate of Chromobacterium you observe a colony which contains excess pigment and is bright purple. List 3 possible different regulatory mutations acting at different sites in the DNA which could account for this phenotype. Note the pigment is a product of enzymatic reactions and is not a protein itself.

1. 
2. 
3. 

Pick one of your suggestions and state (briefly) how you could test to determine whether it is correct. State which of your mechanisms you are testing 1, 2, or 3.

Proposed mechanism to be tested:
Test: