Name ______________________________________________________
First      Last
(Please Print)
PID Number __________-__________

HOUR EXAM I
BIOLOGY 422
FALL, 2014

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_______
1. (13 points) Below is a diagram of a bacterium.

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

Label the indicated parts on the drawing.

![Diagram of a bacterium with labeled parts A, B, C, D, and F.]

What is the composition in terms of **major macromolecules** of each of the parts you labeled on the drawing above (be sure to indicate more than one type of macromolecule if more than one type plays a major role)? The letters below should correspond to the letters above.

A. _____________________________________________
B. _____________________________________________
C. _____________________________________________
D. _____________________________________________
E. _____________________________________________
F. _____________________________________________

If this bacterium were of the other type with respect to the Gram stain, which of the above structures would be different? In what way?

[Blank space for answer]
2. (10 points) Where in a bacterial cell would you expect to find each of the following? (Note: not present is a possible answer).

Mitochondria ________________________

ATP synthesis complex of proteins________________

Proteins for synthesizing small molecules involved in quorum sensing (acylhomoserine lactones) __________________________

Response sensor________________________________________

DNA_____________________________________________

A protein which interacts with streptomycin or erythromycin _____________________________

RNA polymerase   ________________________________

Flagellar motor __________________________

True          or               false  (circle one)
We can easily grow all of the bacteria found in the human gut in the laboratory.

3. Viable cell counts (4 points)
You make 10-fold dilutions of a 1 ml culture and plate 0.1 ml of the following dilutions on agar plates. Calculate the viable cell counts.

<table>
<thead>
<tr>
<th>dilution</th>
<th># of colonies</th>
<th>viable cell count</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-5}$</td>
<td>489</td>
<td></td>
</tr>
<tr>
<td>$10^{-6}$</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>$10^{-7}$</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Which number would you report in a scientific journal article?   _____________________
4. (9 points) Bacterial Growth. Use curves shown below to answer the following 4 questions.

1) An *E. coli* pro- strain is growing at time 0. All of the proline in the culture medium is depleted at time A. __________

2) Wild-type *E. coli* is growing in the presence of glucose and lactose at time 0. Glucose is depleted at time A. __________

3) Different doses of a small molecule are being tested as an antibiotic against a newly identified pathogen. The pathogen is growing at time 0, and the small molecule is added at time A.
   a. A low dose is tested, which enables the pathogen to more efficiently utilize glucose. (Glucose is plentiful in the culture medium.) __________
   b. A higher dose is tested, which is bacteriostatic. __________
   c. The highest dose tested is bactericidal. __________

4) A culture of a mesophile was placed at 25°C at time 0, then at 37°C at time A. __________
5. (16 points) Gene regulation – the lac operon.

Fill in the blank with the name of each item (from the word bank above) indicated in the schematic. (Note: not all required parts are shown.) (12 points)

1) ____________________________ 7) ____________________________
2) ____________________________ 8) ____________________________
3) ____________________________ 9) ____________________________
4) ____________________________ 10) ____________________________
5) ____________________________ 11) ____________________________
6) ____________________________ 12) ____________________________

In which of the above locations could you make a transposon insertion that would result in a bacterium that can grow on glucose but not lactose? (Consider only the numbered locations shown in the diagram) (4 points)
6. (8 points) Given the indicated genotypes and levels of glucose and lactose in the table below, determine the status of lacZ transcription and the ability of the cells to grow on lactose. Fill in the table with either ++ (maximum transcription), + (moderate transcription), or – (no transcription).

<table>
<thead>
<tr>
<th>Bacterial genotyp</th>
<th>lacZ transcription (++, +, or -) in medium containing</th>
<th>Growth on lactose (Yes or no)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose, little lactose</td>
<td>No glucose, high lactose</td>
</tr>
<tr>
<td>lacI&lt;sup&gt;−&lt;/sup&gt;, lacA&lt;sup&gt;−&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>crp&lt;sup&gt;−&lt;/sup&gt;, lacY&lt;sup&gt;−&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Promoter lac&lt;sup&gt;−&lt;/sup&gt; (&lt;sup&gt;φ&lt;/sup&gt;lac&lt;sup&gt;−&lt;/sup&gt;)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lacI&lt;sup&gt;−&lt;/sup&gt;,lacY&lt;sup&gt;−&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

7. (8 points) Gene Regulation – attenuator.

Write in the blank whether each condition described below will result in either A or B situation above and whether the trpE gene would be transcribed (yes or no). (This is a trp<sup>R</sup>- bacterium, i.e. no repressor is present.)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Situation (A or B)</th>
<th>trpE transcription (Y or N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal medium, glucose, no tryptophan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal medium, glucose, high tryptophan levels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal medium, glucose, and tryptophan, no other amino acids, trp t-RNA synthase is mutated so that it adds arg to the trp-t-RNA.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal medium, glucose, no tryptophan. A mutation in region 3 causes 3-4 pairing to be more energetically favorable than 2-3 pairing</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
8. (6 points) Indicate which of the following are suitable targets for antibiotics and state your reasons briefly for considering them to be a target or to be unsuitable as a target.

<table>
<thead>
<tr>
<th>Bacterial substance or process</th>
<th>Is this a potential target? yes or no</th>
<th>Why? Briefly.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell wall</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell membrane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycolysis (glucose metabolism)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytochromes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

9. (8 points) Bacterial genetics. For all parts of this question use the materials on the reagent shelf on the last page of the exam. What substances from the reagent shelf must I add to a minimal salts mixture to obtain growth of each of the following?

Wild type *E. coli* ____________________________________________

*E. coli lacZ⁺Y⁺A⁺ trpE⁻bio⁻* ________________________________

*E. coli lacZ⁻Y⁺A⁺ trpE⁺bio⁻argR⁻malB⁻* ________________________________

If I carry out the following cross between an Hfr and an F- strain of *E. coli* what should I put in the medium to obtain each of the following types of recombinants (in the F- strain)?

*E. coli Hfr lacZ⁺Y⁺A⁺ trpE⁺bio⁻malB⁺ade⁺SmS⁵*  
X  
*E. coli F- lacZ⁻Y⁺A⁺ trpE⁻bio⁻malB⁻ade⁻SmR⁶*

1. *trp⁺* recombinants __________________________________________
2. *mal⁺* recombinants __________________________________________
3. *ade⁺* recombinants __________________________________________

The cross was carried out and recombinants were obtained after varying time intervals shown in the table.

<table>
<thead>
<tr>
<th>gene</th>
<th>Number of recombinants after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min.</td>
</tr>
<tr>
<td>trp⁺</td>
<td>0</td>
</tr>
<tr>
<td>mal⁺</td>
<td>0</td>
</tr>
<tr>
<td>ade⁺</td>
<td>0</td>
</tr>
</tbody>
</table>

Which gene is closest to the origin of transfer? _______

Which gene is farthest from the origin of transfer? _______
You are studying a newly isolated bacterial species, *X. amarillo*, which degrades cellulose when grown at 25°C but not at 37°C. Use transposon mutagenesis to determine which genes contribute to this ability. You have already determined that *X. amarillo* is transformable. Fill in the blanks below with reagents from your reagent shelf (listed at the end of the question).

1. Purify ________ DNA to introduce the transposon into *X. amarillo*.
2. Transform ________ with the DNA you obtained in #1.
3. Identify the mutants you want by plating the transformants on ____________________________ at ___ °C, and screening for ________________________________.

One of your isolated mutants degrades cellulose at both temperatures. What hypothesis would explain this phenotype?

Using the reagent shelf, how would you test your hypothesis?
11. (7 points) Chemotaxis
Draw an *E. coli* cell, including the flagellae, which is swimming.

Draw an *E. coli* cell, including the flagellae, which is tumbling.

In the following drawing, 4 different bacterial cells move from point A to point B. The paths they took are indicated. A schematic of the chemotaxis system from *E. coli* is shown on the right.

Which bacterium could have a deletion in *cheA*? _______________
Which bacterium could have a deletion in *cheZ*? _______________
Which bacterium could have a deletion in the flagellar motor? _____________
Which bacterium could contain a mutation where CheA is always phosphorylated? _________________

What would be the phenotype of a *cheB* mutant?

______________________________
Information page

<table>
<thead>
<tr>
<th>amino acids</th>
<th>vitamins</th>
<th>sugars</th>
<th>antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>threonine (thr)</td>
<td>biotin (bio)</td>
<td>glucose (glu)</td>
<td>streptomycin (sm)</td>
</tr>
<tr>
<td>leucine (leu)</td>
<td>thiamine (thi)</td>
<td>lactose (lac)</td>
<td>rifampicin (rif)</td>
</tr>
<tr>
<td>histidine (his)</td>
<td></td>
<td>maltose (mal)</td>
<td>ampicillin (amp)</td>
</tr>
<tr>
<td>tryptophan (trp)</td>
<td>nucleic acid bases</td>
<td>arabinose (ara)</td>
<td>tetracycline (tet)</td>
</tr>
<tr>
<td>arginine (arg)</td>
<td>adenine (ade)</td>
<td></td>
<td>neomycin (neo)</td>
</tr>
<tr>
<td>proline (pro)</td>
<td>cytosine (cyt)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

polysaccharides

cellulose

Reagent shelf for question 9

Minimal salts medium
Nutrient agar
Glucose, maltose, lactose
Tryptophan, arginine, biotin, adenine
Penicillin, streptomycin, kanamycin, tetracycline

Reagent shelf for question 10

E. coli
X. amarillo
Competent E. coli
Competent X. Amarillo
Plasmid pUNC1 containing a transposon which encodes a transposase, ampR gene, and a broad-host range origin of replication
Plasmid pUNC2 containing a transposon which encodes a transposase, tetR gene, and an origin of replication which works only in E. coli
Plasmid pUNC3 pUNC1 containing a transposon which encodes a rifR gene, and a broad-host range origin of replication. The transposase is located elsewhere on the plasmid.
Plasmid pUNC4 pUNC1 containing a transposon which encodes an kanR gene, and an origin of replication which works only in E. coli. The transposase is located elsewhere on the plasmid.
Plasmid pUNC5 containing a transposon which encodes a smR gene, and an origin of replication which works only in E. coli.
Minimal medium plates and any necessary additions
Nutrient agar plates
A kit to purify DNA from bacteria
Cellulose
A dye which stains cellulose red (Congo red)
All necessary antibiotics and chemicals