General information for all questions on this exam.
complex medium - medium containing nutrient broth or agar
minimal medium - medium containing salts but no carbon source,

<table>
<thead>
<tr>
<th>amino acids</th>
<th>sugars</th>
<th>vitamins</th>
<th>antibiotics</th>
<th>nucleic acid bases</th>
</tr>
</thead>
<tbody>
<tr>
<td>tryptophan</td>
<td>glucose</td>
<td>biotin</td>
<td>ampicillin</td>
<td>adenine</td>
</tr>
<tr>
<td>arginine</td>
<td>maltose</td>
<td></td>
<td>tetracycline</td>
<td>cytosine</td>
</tr>
<tr>
<td>methionine</td>
<td>lactose</td>
<td></td>
<td>kanamycin</td>
<td></td>
</tr>
<tr>
<td>leucine</td>
<td>arabinose</td>
<td></td>
<td>gentamycin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>streptomycin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rifampicin</td>
<td></td>
</tr>
</tbody>
</table>

I. (10 points) In a cross between 2 strains of E. coli with the following genotypes
E. coli met^+^ade^+^lac^+^mal^+^bio^+^rif^+^ Hfr
E. coli met^+^ade^+^lac^+^mal^+^bio^+^rif^+^ F^-

what medium would you use to select for recombinants for each of the following genes:

met

ade

lac

II. (13 points) You preform an interrupted mating between two E. coli strains with the following genotypes:
leu^+^trp^+^mal^+^Sm^+^ Hfr
leu^+^trp^+^mal^+^Sm^+^F^-
The transconjugants are plated on the following media at the times indicated.
Medium 1: minimal medium, glucose, leucine, streptomycin
Medium 2: minimal medium, maltose, leucine, tryptophan, streptomycin
Medium 3: minimal medium, glucose, tryptophan, streptomycin

The number of transconjugants recovered are as follows:

<table>
<thead>
<tr>
<th>Time</th>
<th>0</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>20 min</th>
<th>25 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium 1</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>16</td>
<td>26</td>
<td>36</td>
<td>46</td>
</tr>
<tr>
<td>Medium 2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>11</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>Medium 3</td>
<td>0</td>
<td>3</td>
<td>18</td>
<td>33</td>
<td>48</td>
<td>63</td>
<td>78</td>
</tr>
</tbody>
</table>

Graph these data on the following page. Be careful to label the axes and to indicate which line represents which data.
What are the map positions (in minutes) of the genes and what is their order of transfer?


If you replica plated colonies from medium 1 at 16 min to medium 3 would most of them grow? yes or no (circle one)

If you replica plated colonies from medium 3 at 16 min to medium 2 would most of them grow? yes or no (circle one)

III. (10 points) You wish to determine the gene order and relative distances of three genes from *E. coli*. You prepare DNA from *E. coli* which is *leu*"*pro"*lac*" and use it to transform *E. coli* which is *leu*"pro*"*lac*". You select for cells which are *pro*" or *leu*" and test them for the ability to make proline or leucine or to grow on lactose. You obtain the following results:

<table>
<thead>
<tr>
<th>Selected marker</th>
<th><em>leu</em>&quot;</th>
<th><em>pro</em>&quot;</th>
<th><em>lac</em>&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>leu</em>&quot;</td>
<td>100</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td><em>pro</em>&quot;</td>
<td>40</td>
<td>100</td>
<td>80</td>
</tr>
</tbody>
</table>

Draw a diagram to show the gene order and relative distances.

Why didn’t you plate the original transformation on medium to select for *lac*" cells?
IV. (8 points) RNA viruses that grow in mammalian cells have to solve the problem that eukaryotic cells generally only make one protein using one piece of mRNA. Different viruses have used different strategies to circumvent this problem. Describe the strategy used by brome mosaic virus (a bromo virus).
V. (12 points) Name two benefits a **prokaryote** can get from association with a eukaryote (You may not use an example more than once).

<table>
<thead>
<tr>
<th>Benefit to prokaryote</th>
<th>prokaryote involved</th>
<th>eukaryote involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Name two benefits a **eukaryote** can get from association with a prokaryote (You may not use the above examples)

<table>
<thead>
<tr>
<th>Benefit to eukaryote</th>
<th>prokaryote involved</th>
<th>eukaryote involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

VI. (7 points) An outbreak of a new disease has struck a number of children who went to a party on Halloween. The disease presents with a mild fever and the appearance of a black and orange spotted rash. All the children ate candy at the party. One of the children fed some of the candy to her pet hamster who also developed a black and orange spotted rash. None of the candy remains. A bacterial agent is suspected. How do you isolate and identify the bacterium responsible?
For questions VII and VIII you have available the following reagents to use in your experiments: competent *E. coli*
competent *P. halloweenii*
mice
hamsters
rams

*E. coli met*"tra" carrying the plasmid pABC shown on the right.
*E. coli Hfr*
E. coli pro"lac" carrying the plasmid pUNC shown on the right.
restriction enzymes and other enzymes which interact with DNA
All the media ingredients given at the beginning of the exam and any other ingredients
commonly used in gene cloning.
A clone of erythrogenic toxin obtained from a neighboring lab.

VII. (11 points) You are successful in isolating the bacterium which caused the black and orange spots which you name *Pseudomonas halloweenii*. You decide to use transposon mutagenesis to identify the major genes involved in the pathogenesis of this bacterium.

Fill in the missing blanks in the protocol shown below:

1. Grow ___________________ and ___________________.

2. Mix the two cultures and allow them to ___________________.

3. Select the resulting bacteria you desire by plating on ______________ medium
containing______________.

4. Test the surviving bacteria to determine which ones have mutations in genes required for virulence by ___________________.

This procedure selects/ screens (circle one) for bacteria which carry the transposon and selects/ screens (circle one) for bacteria which are mutant in genes required for virulence.
VIII. (17 points) You find that the spots appear at sites far from the sites of bacterial infection. This causes you to suspect that the spots are caused by a toxin. You wish to clone the gene which encodes the toxin. A lab down the hall from you is working on Streptococcus and has clones of erythrogenic toxin from this organism. You suspect that your toxin may be related to erythrogenic toxin and ask them for a clone which they give to you. Fill in the blanks in the protocol given below for cloning the toxin gene.

1. Isolate ________ from ________________ and digest it with ________________.

2. Isolate ________ from ________________ and digest it with ________________.

3. Mix the products of #s 1 and 2 and add ________________ (enzyme).

4. __________ the product of # 3 into ________________ (cells).

5. Plate the resulting cells on ________________ medium containing ________________.

   Keep the ________________ colonies.

6. Identify the colony which contains the clone of the toxin gene by ________________

   ________________.
IX. (12 points) What is the biochemical mode of action of diphtheria toxin?

Is diphtheria toxin an exotoxin endotoxin enterotoxin? (circle as many as apply)

Diphtheria toxin is encoded by a lysogenic phage. The means that (circle as many as apply) there are C. diphtheriae which don’t make toxin the cells lyse and release phage when they produce toxin the phage always lyse the cells the phage never lyse the cells the phage can infect mammalian cells the toxin gene can be transferred in nature from one strain to another by transformation

True or false (circle one) A piece of nucleic acid (DNA or RNA) must encode a protein in order to produce disease in a eukaryote.