HOUR EXAM II

BIOLOGY 108

FALL, 2005

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

______________________________  Signature
1. (7 points)

If Curve B above is *E. coli* grown on glucose and lactose, what is the doubling time of the bacteria when they are using lactose as a carbon source? 

Curve A above is a depiction of *E. coli* grown in minimal medium with 0.02% glucose. Give two ways in which the growth curve would differ if *E. coli* were grown in nutrient broth (complex medium).

A ________________________________

B ________________________________

Using a few words, describe what is happening during the following growth phases of B (on the graph above).

1 ________________________________

2 ________________________________

3 ________________________________

5 ________________________________
2. (4 points) Above is a list of ingredients that you have available to prepare bacterial growth media for an experiment. You wish to transform a bacterium which is ara- leu- arg- ade- mal+ tetR with DNA from a bacterium which is ara+ leu+ arg- ade+ mal- tetS.

What medium would you use to select bacteria of each of the following genotypes?

leu+ _______________________________________

mal+ _____________________________________

ade+ ______________________________________

tetR _______________________________________

3. (9 points) You perform an interrupted mating between two E. coli strains with the following genotypes:

F-: SmR trp− bio− lac− met+
Hfr: SmS trp+ bio+ lac+ met−

The transconjugates are plated on the following minimal medium at the times indicated:

Medium 1: glucose, bio, sm
Medium 2: lactose, trp, bio, sm
Medium 3: glucose, trp, sm

The number of bacteria which grow on each medium are counted as follows:

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>18</td>
<td>38</td>
</tr>
<tr>
<td>Medium 2</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>45</td>
<td>70</td>
<td>120</td>
</tr>
<tr>
<td>Medium 3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>7</td>
<td>17</td>
</tr>
</tbody>
</table>

Graph these data on the following page. Label the axes and indicate which line represents which medium and mutant.

Draw a diagram below to show the gene order and relative positions in minutes.

Transferred last                         Transferred first

them
grow?
Yes or no (circle one)

If you replica plated colonies from the medium 3 30 min plate to medium 2 would most of them grow?
Yes or no (circle one)

4. (7 points) You wish to determine the gene order and relative distances of three genes from E. coli. You grow the generalized transducing phage P1 on E. coli which is leu$^+$ thi$^+$ cyt$^-$ and use it to transform E. coli which is leu$^-$ thi$^-$ cyt$^+$. You select for cells which are leu$^+$ or thi$^+$ and test them for the ability to make leu or thi or cyt. You obtain the following results:

<table>
<thead>
<tr>
<th>Selected marker</th>
<th>Percentage of selected cells which are</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>leu$^+$</td>
</tr>
<tr>
<td>leu$^+$</td>
<td>100</td>
</tr>
<tr>
<td>thi$^+$</td>
<td>70</td>
</tr>
</tbody>
</table>

Draw a diagram to show the gene order and relative positions

What medium did you use to test whether the thi$^+$ bacteria are cyt$^+$?
Reagent shelf
Fully stocked supply room with reagents and restriction enzymes

*E. coli*
*C. perfringens* NI
*C. perfringens* CS
Compentent *E. coli*
Compentent *C. perfringens* NI
Compentent *C. perfringens* CS

*E. coli* strain UNC-24 *tra*<sup>+</sup> *arg*<sup>-</sup> (carries plasmid pQL5 which encodes tet<sup>R</sup> and also contains Tn5 which encodes neo<sup>R</sup>. This plasmid can only replicate in enteric bacteria)

*E. coli* strain UNC-18 (carries plasmid pBLT, Amp<sup>R</sup>, Multiple Cloning Site (MCS), can only replicate in enteric bacteria)

Phage P1
Minimal medium, complex medium
X-gal
Glucose, Lactose
Collagen
Stain for collagen
Mice, rabbits, rats, canaries, parrots, gold fish, trout

5. (7 points) A new strain of *Clostridium perfringens* causing gas gangrene was isolated from a wound infection of a civilian in Iraq. The bacteria seem to be more virulent than the common strain CS both in humans and in rats. You believe that this may be due to the presence of a stronger or additional collagenase (enzyme which digests collagen) in the new isolate called NI. Since the new gene may not be closely related to the collagenase gene in the sequenced strain you decide to try to clone the gene(s) without using its sequence. Fill in the details in the following protocol designed to clone the collagenase gene(s) from NI. You have available the materials on the reagent shelf given above and all of the compounds listed in the chart before question 2. In addition you have invented a procedure to make and transform competent *C. perfringens* which appears to work on the NI as well as the CS strains.

1. Isolate DNA from ______________ and ____________ (process) it with ________.

2. Isolate DNA from ______________ and ____________ (process) it with ________.

3. Mix the products of 1 and 2 above and add ________ and ________.

4. Introduce the product into ______________ using ________________.

5. Plate the resultant bacteria on ________________________.

6. Identify the colonies which carry the collagenase gene by ______________.
6. (9 points) You are successful and obtain a gene for collagenase. All of your collagenase positive clones contain identical genes; when you sequence them you find them to be identical to the gene in the CS strain. Thus you can not account for the increased virulence of the NI strain by a change in the collagenase gene. Therefore you decide to look for genes which may be involved in the increased virulence using transposon mutagenesis. Fill in the details of the following protocol designed to isolate mutants in the genes involved in the increased virulence of NI. You have available the materials on the reagent shelf given above and all of the compounds listed in the chart before question 2.

1. **Grow** _______________ and introduce _______________ into it by _______________.

2. **Plate the resulting bacteria on** _______________________________ and keep _______________ for further testing.

3. **Identify the mutants which are reduced in virulence by** ___________________________________________ (be specific about how to do this).

4. In step 2 you are selecting / screening for the bacteria you want (circle one).

5. In step 3 you are selecting / screening for the bacteria you want (circle one).

7. (10 points) Eukaryotic cells tend to only make one protein per mRNA. Describe the strategy that the Polio virus employs to overcome this difficulty when growing in eukaryotic cells.

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Describe the strategy used by the tobacco mosaic virus (TMV).
8. (9 points) Different types of viruses use various enzymes to replicate their genomes. Some are incorporated into the virus and introduced into the host cell upon infection; some are encoded on the viral genome and some are borrowed from the host cell the virus infects. Complete the table below by naming the enzyme or enzyme package (for example, eukaryotic protein synthesis machinery) **required for replication** of the viral genome. If the type of virus does not utilize any enzymes of that origin, fill the box with “none.”

<table>
<thead>
<tr>
<th>Virus type</th>
<th>Enzyme(s) included in the virus</th>
<th>Enzyme(s) encoded on viral genome</th>
<th>Enzyme(s) or enzyme package from host cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>ssRNA + Strand such as polio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ssRNA – Strand such as influenza</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dsDNA such as T4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

9. (7 points) You are employed as a clinical microbiologist. You are treating a number of patients who have become ill, presenting symptoms associated with the respiratory system, e.g. coughing and having difficulty breathing. The patients all work in the same office area. A pet canary which they keep in the office has developed similar symptoms. You suspect a bacterial pathogen may be to blame, but you are still unsure as to the cause of the illness. Develop a protocol for determining if the patients are infected with a bacterial pathogen and if so, how to isolate the responsible bacterium. (You may use the contents of the reagent shelf listed in question 5.)
10. (6 points) Complete the following table:

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Bacterial agent (genus and species)</th>
<th>Site of action (tissue or cell type)</th>
<th>Damage caused (provide ONE major effect)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholera toxin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Botulism toxin</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

11. (9 points) Describe the biochemical mechanism of action of diptheria toxin on a human cell.

Why does diptheria toxin kill eukaryotic cells?

Why doesn’t diptheria toxin kill Corynebacterium diptheriae?
13. (8 pts) Contrast innate and adaptive immunity in the following table:

<table>
<thead>
<tr>
<th>type of immunity</th>
<th>specificity</th>
<th>effect of prior exposure</th>
<th>molecules involved</th>
<th>cells involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>innate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adaptive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>