Name ______________________________________________________

First       Last
(Please Print)

PID Number __________ - __________

HOUR EXAM II

BIOLOGY 422

FALL, 2007

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_______
1. Selection (7 pts)

A student in your lab performed a conjugation between two *E. coli* strains with the following genotypes:

F-: \( \text{his}^+ \text{ trp}^- \text{ leu}^- \text{ arg}^+ \text{ thr}^- \text{ lac}^- \text{ mal}^+ \text{ ara}^- \text{ neo}^S \text{ amp}^R \)

Hfr: \( \text{his}^- \text{ trp}^- \text{ leu}^+ \text{ arg}^- \text{ thr}^+ \text{ lac}^+ \text{ mal}^- \text{ ara}^+ \text{ neo}^R \text{ amp}^S \)

They had written the reagents used to make their selection plates, but not which transconjugant genotypes they were selecting for.

Using your deduction skills, please label the following plates with the genotype they were selecting for.

- minimal media       minimal media         complex media
  - glu         ara
  - trp         trp
  - thr         leu
  - amp

______________________________________________________________________________

You also want to select for a transconjugant with a *bio*^+^ genotype. What medium would you use to select for *bio*^+^?

______________________________________________________________________________

What medium would you use to select for *lac*^+^?

______________________________________________________________________________
2. (7 points) You wish to map the relative positions of three genes using transformation. You extract DNA from a bacterium which is \( cyt^- his^+ thr^+ \) and use it to transform a bacterium which is \( cyt^+ his^- thr^- \). You select for cells which are his\(^+\) or thr\(^+\) and determine their phenotype for the other 2 genes. You obtain the following results:

<table>
<thead>
<tr>
<th>Gene Selected</th>
<th>cyt(^+)</th>
<th>his(^+)</th>
<th>thr(^+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>his(^+)</td>
<td>30</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>thr(^+)</td>
<td>90</td>
<td>20</td>
<td>100</td>
</tr>
</tbody>
</table>

Make a diagram showing the gene order and relative positions.

Why didn’t you select for cells which were cyt\(^+\)?

3. (14 points) A. Single-stranded RNA viruses which infect eukaryotic cells face the problem that these cells generally only read one protein from a piece of mRNA. How has polio solved this problem? **Be specific and mention the steps and enzymes involved.**

B. TMV (a plant virus) has used a different strategy to solve the problem. Describe the strategy used by this virus. **Again be specific and mention the steps and enzymes involved.**
NOTE. FOR THE PROTOCOLS FOR QUESTIONS 4, 5, AND 6 YOU MAY ONLY USE REAGENTS LISTED ON THE LAST PAGE OF THE EXAM.

4. A. (7 points) A disease is spreading rapidly through New Jersey hospitals. Doctors there have been unable to isolate and identify the causative agent. Therefore, they have called Dr. House, and as the microbiologist on his team, he turns to you. Patients are suffering from sore throat. The patients throat then turns blue and they develop a fever and cough. One of the sick patients has a pet parrot that also has a blue throat and cough. You suspect a bacterial agent is to blame, but need to do some more research before you’ll know for sure. Using what is given in the reagent shelf, describe a protocol for identifying and isolating the causative agent. Be specific.

B. (2 points) Name two possible types of virulence factors that would allow this pathogen to invade the respiratory tract.

1. _______________________________________________________

2. _______________________________________________________

C. (3 points) Name three built-in resistance mechanisms not involving an immune response that reduce infection by this organism or prevent it from spreading to other systems.

1. _______________________________________________________

2. _______________________________________________________

3. _______________________________________________________
5. (10 points) You successfully isolate the organism causing the epidemic in New Jersey which you name *Pneumophilia azura*. You are curious as to its major virulence factors and decide to do a transposon mutagenesis. It is a gram negative bacterium which can grow on minimal medium. You have available the materials shown on the reagent shelf. Fill in the missing details in the protocol you will use to obtain your mutants.

1. Grow ___________ and introduce ________________ by ________________ (technique).

2. Plate on medium containing _________________ and ______________ to obtain transposon mutants.
3. Screen for mutants which are no longer virulent by ______________________
   ________________________________________________________________________
   __________________________________________ (be specific).

You carry out this protocol and fail to obtain any completely avirulent mutants although you are able to obtain non-motile mutants. Suggest a possible reason for your failure.

___________________________________________________________________________
6. (15 points) You are curious about the blue pigment produced by this bacterium and decide to attempt to clone the gene(s) responsible for its production. Fill in the blanks in the cloning protocol below. Reference the list of reagents.

1. Isolate DNA from  
   A. ___________________ and _______________ (process) it with ___________ to obtain fragments of DNA, and from  
   B. ___________________ and _______________ (process) it with ___________ to obtain fragments of DNA

2. Mix the fragments and add ___________________ and ___________.

3. Introduce DNA from step 2 by ___________ into ____________________ and grow on ________________.

4. Identify the clone which carries the genes for the blue pigment production by _________________.

You are having a bad week and your cloning procedure is unsuccessful although you are able to clone a leucine biosynthesis gene using the same procedure and organisms. Suggest two possible reasons for your lack of success. Chose one of your reasons and tell what you could do to fix the problem if this was the cause.

Reasons 1. __________________________________________________________

2. ______________________________________________________________

Method to fix one of these possible problems: ____________________________

________________________________________________________________________.
7. (9 points) Name three different diseases (bacterial or viral) which are of major importance in the tropics and have **different modes of transmission**. Fill in the following table for these diseases.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mode of Transmission</th>
<th>One Method for Controlling Spread of Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8. (8 points) Name two benefits a prokaryote can get from interaction with eukaryote. You must use **2 different** prokaryotes in your examples.

<table>
<thead>
<tr>
<th>Benefit to Prokaryote</th>
<th>example</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Name two benefits a eukaryote can get from interaction with prokaryote. You must use **2 different** eukaryotes in your examples. You can not use an example you used in the first part of this question.

<table>
<thead>
<tr>
<th>Benefit to Eukaryote</th>
<th>example</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
9. A. (12 points) Describe in detail the molecular mechanism of the action of cholera toxin. Be careful to be complete and clear about each step.

Some animals are not susceptible to cholera. However, their cells possess the same biochemical regulatory mechanisms as human cells. State a possible reason for their lack of susceptibility.

___________________________________________________________________________

The expression of the gene for diphtheria toxin is regulated by iron. If an activator is involved in this regulation then which regulatory mutations are likely to be most frequent (circle one)?
Mutants which always produce toxin       mutants which never produce toxin
both types of mutants will be equally frequent

10. Epidemiology  (6 points)
A) Every year, a number of Carolina students get infected with Fanatosium carolinia, otherwise known as “basketball fever.” The fever lasts for four months, causes giddiness and bouts of cheering, and confers immunity to other similar diseases. From December 2009 to February 2010, 6,000 new cases were reported to Student Health. Assuming a population of 20,000 students, what was the average incidence per month?

___________________________________________________________________________

B) Let’s suppose that there were 2,000 existing cases in November. Using the average incidence that you calculated above, what was the prevalence at the end of December?

___________________________________________________________________________

C) By the middle of March, 16,000 Carolina students were infected with Fanatosium carolinia. A Duke student tries to infect UNC students with the related bacterium F. dukia but only 2 students develop the disease. What is the phenomenon responsible for this result called?
Use the following list of reagents to answer questions for this exam.

<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></td>
<td>cytosine (cyt)</td>
<td></td>
<td>kanamycin (kan)</td>
</tr>
</tbody>
</table>

minimal medium without carbon source

Biochemicals
X-gal
IPTG

Supplementary Reagent shelf for use in questions 4, 5, and 6

dogs, cats, alligators, mice, rabbits, monkeys, parrots, goldfish, maize plants
non-specific media for growing bacteria (plates)
P. azura
Competent P. azura
E. coli
competent E. coli
E. coli arg tra rif<sup>R</sup> carrying pUNC5
E. coli arg tra rif<sup>R</sup> carrying pAM22
plasmid pUNC5 mob<sup>+</sup> amp<sup>R</sup> which possess a Mini-Tn<sub>5</sub> transposon that encodes kan<sup>R</sup> and has one EcoR1 site and an origin of replication which is only recognized by E. coli
plasmid pAM22 that has one HindIII site, one BamH1 site, and two EcoRV and BglII sites and an origin of replication which is only recognized by E. coli and encodes amp<sup>R</sup>
all the biochemicals and media on the list above
all restriction enzymes
DNA polymerase, DNA ligase, ATP, topoisomerase
all enzyme buffers
PCR primers and a PCR machine
lactose, cellulose, albumin (a protein), pectin
stain for β-galactosidase, stain for pectin, stain for proteins