I. (8 points) Translation and transcription are two of the most important processes involved in maintaining the life of a bacterium. Look at the following steps involved in both translation and transcription. Fill in the gaps so that you have a complete process.

A. Fill in the blanks in the following process.

a. Begin with a piece of dsDNA
b. The _____ binds to the DNA
c. The component from step b reads one strand of the DNA and uses dNTP's to synthesize _____
d. Aminoacyl-t-RNA synthases then add _____to the tRNA using the _____ to determine which ones to pair up..
e. Initiation begins with the formation of the initiation complex, consisting of tRNA, initiation factors, and an active _____ consisting of a 30S and 50S subunit.
f. The A Site, _____, and E Site then help position the proper amino acyl-t-RNAs to create a protein sequence.

B. Answer the following questions regarding Part A.

a. Which steps from Part A correspond to Translation? _____

b. Which steps from Part A correspond to Transcription? _____

II. (5 points) Draw a diagram of a bacterial operon containing two genes, Gene A and Gene B. The expression of these genes is regulated by cAMP activation and a repressor. Choose items from the following list in constructing your diagram. (NOTE: You will not use all of the items, and you can use an item more than once if needed.)

| | | |
|---|---|---|
| Promoter | activator | repressor |
| ATG | RBS | TGA, TAA, TAG |
| ORF A | ORF B | attenuator |
| Ribosome | cAMP | stem-loop |
| AT Rich Region | GC Rich Region | CAP Binding Site |
| Operator | Protein A | Protein B |
| Initiation factor | intein | intron |

Use the following template for your answer:

5' 3'

III. (8 points)

A. Which of the following processes or systems produce energy for the cell? (circle all those that apply)

| | |
|---|---|
| glycolysis | photosystem I |
| fermentation | photosystem II |
| assimilation | nitrogen fixation |
| bacterial rhodopsin | $CO_2$ fixation |
| anaerobic respiration | chemotaxis |

B. Which of the following processes or systems require iron containing proteins or cofactors? (circle all those that apply)

| | |
|---|---|
| glycolysis | photosystem I |
| fermentation | photosystem II |
| assimilation of sulfate | nitrogen fixation |
| bacterial rhodopsin | $CO_2$ fixation |
| anaerobic respiration | chemotaxis |

IV. (6 points) There are several forms of gene regulation present in any given cell, and for any given gene. The genes that regulate the biosynthetic components of tryptophan are no exception. Besides being regulated by repression, attenuation plays an important role in regulating the expression of the *trp* operon. The amount of Trp available to the cell decides how attenuation will work to remedy either an abundance of Trp, or a lack of Trp. Fill in the following table to describe what happens during attenuation of the *trp* operon, in a repressor minus cell, under the specified conditions.

| Trp conditions | translation of leader peptide halted (yes/no) | mRNA region(s) forming loops | RNA polymerase terminates before *trpE* (yes/no) |
|---|---|---|---|
| Trp in abundance | | | |
| Trp starvation | | | |

V. (6 points) What is the expected level of β-galactosidase if each of the following constructs is introduced into a *lacZ* deleted *E. coli* on a plasmid. Except for the deletion of the *lacZ* gene you can assume the chromosomal genes are wild type unless otherwise indicated.

| genotype | conditions | level of β-galactosidase produced (none, small, or large) |
|---|---|---|
| lacZ cloned behind its own promoter | lactose and glucose present | |
| lacZ cloned behind the T7 late gene promoter | glucose present | |
| lacZ cloned behind λ $P_{RE}$ | glucose present | |
| lacZ cloned behind the λ $P_R$ | glucose present | |
| lacZ cloned behind λ $P_R$ in a cell lysogenic for λ | glucose present | |
| lacZ cloned behind λ $P_R$ in a cell lysogenic for λ | glucose present, high levels of UV radiation present | |

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

| **amino acids** | **sugars** | **vitamins** | **antibiotics** | **nucleic acid bases** |
|---|---|---|---|---|
| tryptophan | glucose | biotin | ampicillin | adenine |
| arginine | maltose | | streptomycin | cytosine |
| methionine | lactose | | rifampicin | |
| leucine | arabinose | | neomycin | |
| | | | tetracycline | |
| | | | gentamycin | |

VI. (9 points) You preform an interrupted mating between two *E. coli* strains with the following genotypes:
*leu*$^+$*ade*$^+$*ara*$^+$*rif*$^S$ Hfr
*leu*$^-$*ade*$^-$*ara*$^-$*rif*$^R$F$^-$
The transconjugants are plated on the following media at the times indicated.
Medium 1: minimal medium, glucose, adenine, rifampicin
Medium 2: minimal medium, arabinose, leucine, adenine, rifampicin
Medium 3: minimal medium, glucose, leucine, rifampicin

The number of transconjugants recovered are as follows:

| Time | 0 | 5 min | 10 min | 15 min | 20 min | 25 min | 30 min |
|---|---|---|---|---|---|---|---|
| Medium 1 | 0 | 0 | 4 | 14 | 24 | 34 | 44 |
| Medium 2 | 0 | 0 | 1 | 6 | 11 | 16 | 21 |
| Medium 3 | 0 | 6 | 21 | 36 | 51 | 66 | 81 |

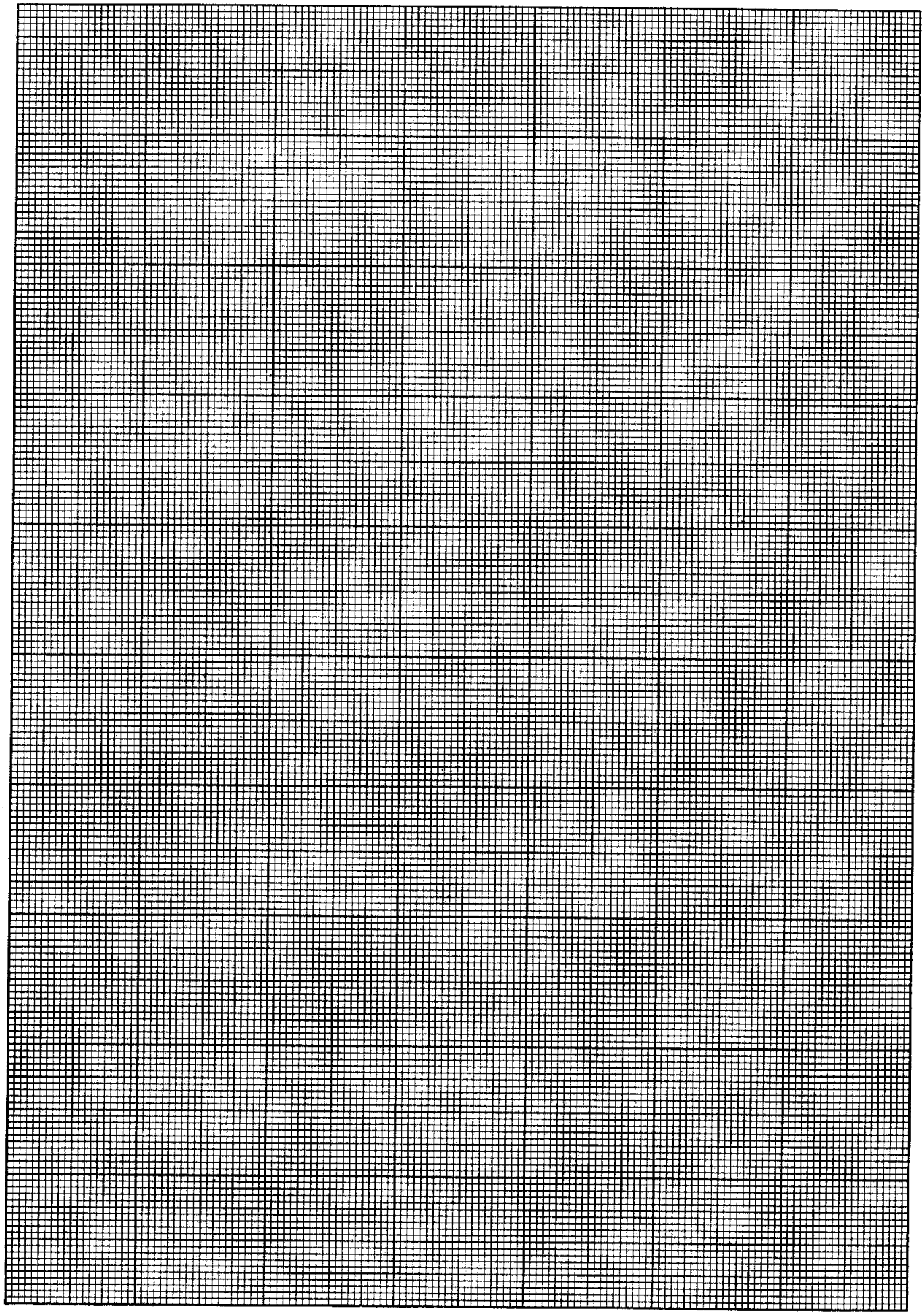
Graph these data on the following page. Be careful to label the axes and to indicate which line represents which data.

What are the map positions (in minutes) of the genes and what is their order of transfer?

first last

If you replica plated colonies from medium 1 at 15 min to medium 3 would most of them grow? yes or no (circle one)

If you replica plated colonies from medium 3 at 15 min to medium 2 would most of them grow? yes or no (circle one)



CITADEL® NO. 643 - CROSS SECTION - 20 SQUARES TO INCH

VII. (7 points)You wish to study the regulation of the expression of a protein adhesin made by pathogenic *E. coli*. For this purpose you want to identify all of the genes required for the expression of the adhesin. However, you don't want to include genes required for normal growth and metabolism. You have available the following:

wild type pathogenic *E. coli* (adhesin$^+$)
the laboratory nonpathogenic strain K12 of *E. coli* (adhesin$^-$)
defective λ phage carrying the transposon Tn*5* (encodes resistance to neomycin)
minimal medium
complex medium
all of the biochemicals listed at the beginning of question VI
mammalian cells to which adhesin$^+$ *E.coli* adhere
a plasmid pUNC encoding resistance to ampicillin and containing a multiple cloning site
the pUNC plasmid carrying the cloned adhesin behind a constitutive promoter pUNCcad

In order to identify genes required for the expression for the adhesin you decide to use transposon mutagenesis. Fill in the protocol you will use below.

1. Introduce _____ into _____ .
2. Select for bacteria which received the transposon by plating on _____.
3. Identify bacteria in which you have mutated a gene required for the expression of the adhesin by

| |
|---|
| |

In the preceding experiment you identify several different genes required for the expression of the adhesin. Name two different types of global regulators which you might expect to be included in the regulatory genes:

1._____

2._____

VIII. (12 points)

A. For the following if none is the correct answer write none in the space provided.

What enzymes, if any, must a single stranded negative strand RNA virus bring into the cell with it?

_____

What enzymes, if any, must a single stranded negative strand RNA virus encode?

_____

What enzymes, if any, must a double stranded DNA virus which multiplies in the nucleus bring into the cell with it? _____

What enzymes, if any, must a double stranded DNA virus which multiplies in the cytoplasm bring into the cell with it? _____

B. Draw the structure of a typical IgG molecule indicating the functional parts (use a stick drawing).

C. Fill in the following bits from a medical microbiology text.

When a wound in the skin is invaded by a foreign organism the mammalian host responds initially with an _____ which includes the following nonspecific defenses _____

_____.

Macrophage may phagocytise some bacteria to limit the spread of disease. What is one bacterial virulence factor which can reduce phagocytosis? _____

Some bacteria can survive in the phagosome. Name one _____

What are some virulence factors which allow bacteria to survive in the phagosome?_____

_____.

IX. ( 10 points) Which of the following diseases could potentially be eradicated and what are the **major reasons** for your answer?

| disease | Can this disease be eradicated? (yes or no) | List **different major reasons** for the ability or inability to eradicate the disease |
|---|---|---|
| rabies | | 1. |
| polio | | 1.<br>2.<br>3. |
| AIDS | | 1.<br>2. |
| mumps and measles | | 1.<br>2.<br>3. |

X. (5 points)The same immunization for polio has been used for the last 40 years. In contrast the immunization for influenza has to be changed frequently. Why is this so?

XI. (5 points) Complete the following table:

| reaction | reduction or oxidation | reason for reaction (that is, why does an organism do this?) |
|---|---|---|
| $NH_3 \rightarrow NO_2^-$ | | |
| $N_2 \rightarrow NH_3$ | | |
| $NO_3^- \rightarrow NO_2^-$ | | |
| $S^\circ \rightarrow S_2O_3^=$ | | |
| $S^\circ \rightarrow H_2S$ | | |

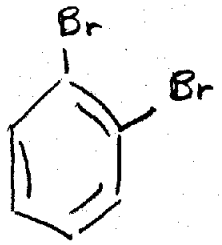
XII. (8 points) Microorganisms have been found in nearly every habitat researchers have been able to reach on earth. These include extremely diverse environments, such as deep sea vents and the interiors of animals, including humans. Even more interestingly, however, is the symbiotic relationships these microorganisms can have with other fauna in their environment. Fill in the following table for two classes of fauna that have been found to exist symbiotically with other microorganisms.

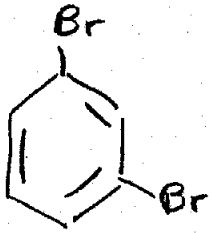
| type of eukaryote | area in the eukaryote where symbiosis occurs | benefit to host | which organisms are the primary producers in this ecosystem? |
|---|---|---|---|
| cow | | | XXXXXXXXXXX |
| tube worm | | | |
| Casuarina or alder root | | | |

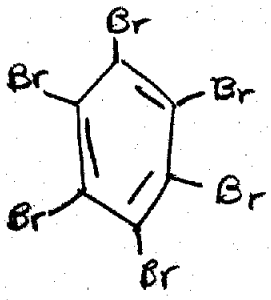
XIII. (8 points) There are many factors that determine the rate of biodegradation of compounds found in the soil, under **aerobic** conditions. Often the structure of the compound is very important.

A. Put the following compounds in order, from least degradable (1) to the most rapidly degraded (4)

A 


B 


C 


D 


1. slowest
2.
3.
4. fastest

B. If the compounds where found in **ANAEROBIC** conditions, which compound would be degraded first? _____

C. If you were to attempt to isolate the organisms which break down D by inoculating soil samples into medium with D as the sole carbon source is it likely that this strategy would allow you to culture the desired bacteria? Yes or no (circle one)

Why or why not?

XIV. (8 points) A colleague of yours tells you about a paper he is working on. He took some soil samples from several kinds of agricultural land and ran a series of assays on them. Now, he is going to publish data which quantitatively relates nitrogen fixation to amount of microbial diversity and total metabolic activity for these samples. On further questioning, however, you determine that:

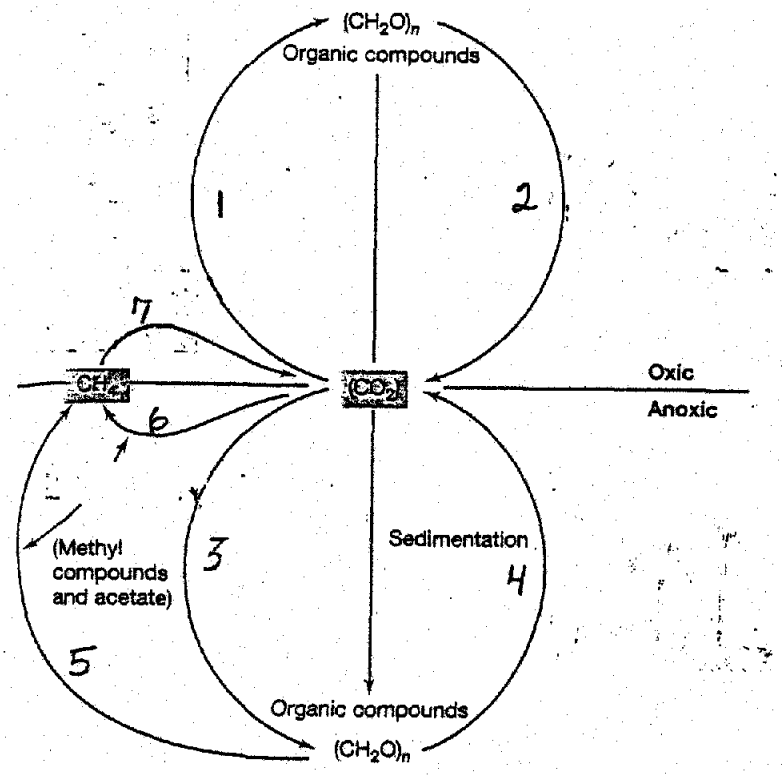
- He measured "nitrogen fixation" by assaying the rate at which acetylene was transformed to ethylene in his original samples
- He measured "microbial diversity" by counting the number of different kinds of microbes he could grow up on nutrient agar from each sample
- He measured "metabolic activity" by timing how quickly his cultures grew

Did your colleague measure "nitrogen fixation" appropriately? **yes no** (circle one)
If you answered "no," suggest an alternative assay. If "yes," write "OK."

Did your colleague measure "microbial diversity" appropriately? **yes no**
If you answered "no," suggest an alternative assay. If "yes," write "OK."

Did your colleague measure "metabolic activity" appropriately? **yes no**
If you answered "no," suggest an alternative assay. If "yes," write "OK."

XV. (6 points) Below is a diagram of the carbon cycle from your text:




Name a **type** of prokaryote which carries out reaction 1 _____

Name a **type** of prokaryote which carries out reaction 3 _____

Are there any eukaryotes which carry out reaction 3?_____

Which reactions can be carried out by archaebacteria?_____

If organisms which can carry out reaction 1 had never evolved, what would be the major consequence? _____

In a microbial mat, which reaction(s) of the above diagram is/are found primarily in the top layer?_____

XVI.. (9 points) Please fill out the following table by writing "yes" if the antibiotic indicated is effective against the referenced microbe, or "no" if there is no or minimal effect. Please do not use marks other than "yes" or "no." Assume that each microbe is representative of its domain.

| Substance | *Thermophilus aquaticus* (Archaea) | *Staphylococcus aureus* (Eubacteria) | *Candida albicans* (nucleus/cytoplasm only) (Eukaryote) |
|---|---|---|---|
| Penicillin | | | |
| Rifampicin (targets RNA polymerase subunit β) | | | |
| Experimental antibiotic: cleaves ether linkages between glycerol and fatty acids | | | |