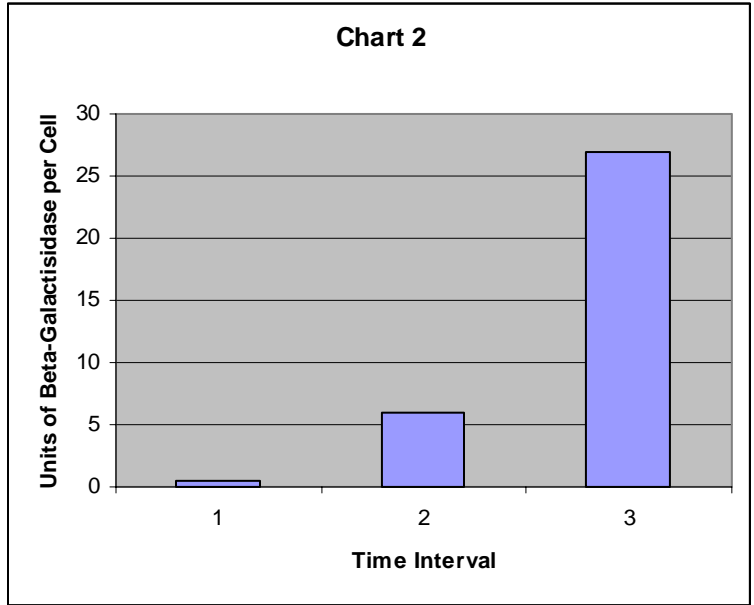
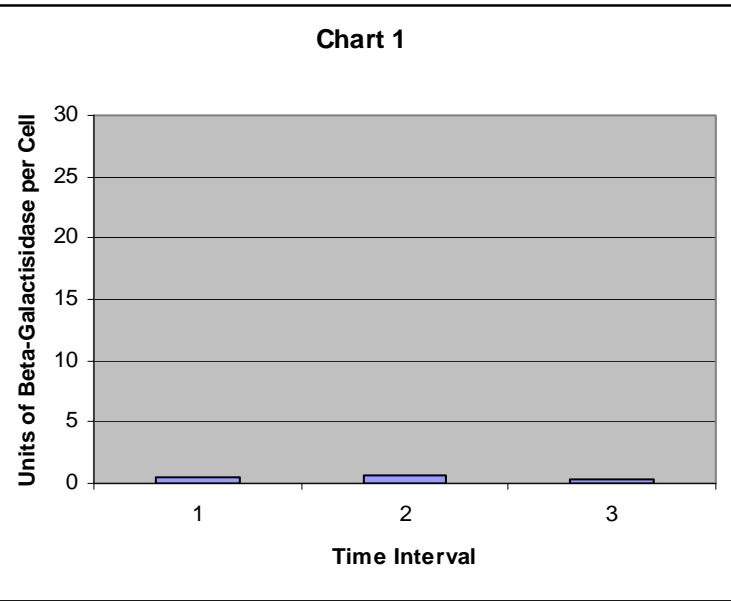
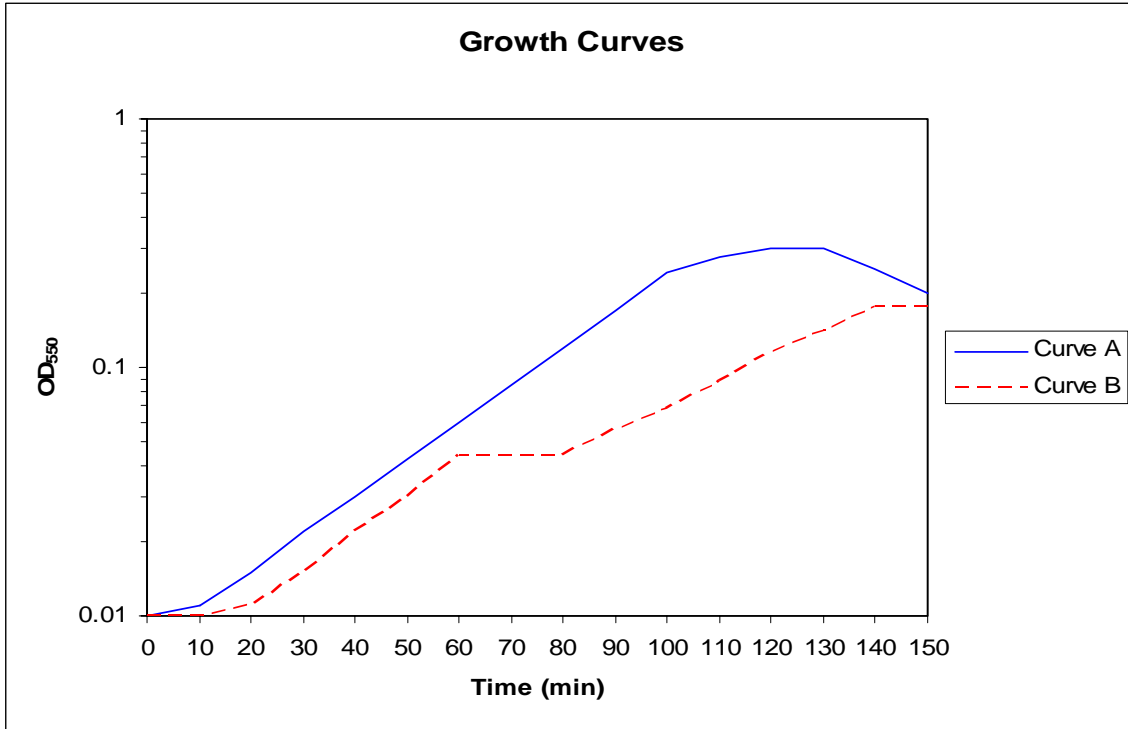


I. (8 points.) In order to function properly, a cell must assign locations to every component it contains. Fill in the table below by naming the subcellular localization of the cell components in the first column.

Cellular Component	Location in the Cell
DNA	
Electron Transport Chain	
Photosynthesis	
Cytochromes	
Glycolysis	
Ribosomes	
Peptidoglycan	
Ion Channels	



II. A. (2 points) The data in the charts above correspond to the growth curves in the graph above. The charts measure units of β -galactosidase per cell. Curve A above is wild type *E. coli* grown on glucose. Curve B above is wild type *E. coli* grown on glucose and lactose. Match the curves to their corresponding charts.

Chart 1 contains enzyme induction data of Curve _____.

Chart 2 contains enzyme induction data of Curve _____.

B. (6 points) Given the different mutations in column one, determine how the β -galactosidase level would change in reference to the curve and time interval provided if the mutant is grown to the same time interval. Circle one answer in each box.

<i>E. coli</i> Mutants	Curve A (Glucose), Time Interval 1	Curve B (Glucose and Lactose) Time Interval 3
Deletion of <i>lac</i> promoter	Higher Lower Same	Higher Lower Same
A Transposon inserts into the <i>lac</i> permease gene.	Higher Lower Same	Higher Lower Same
Deletion of <i>lac</i> repressor	Higher Lower Same	Higher Lower Same

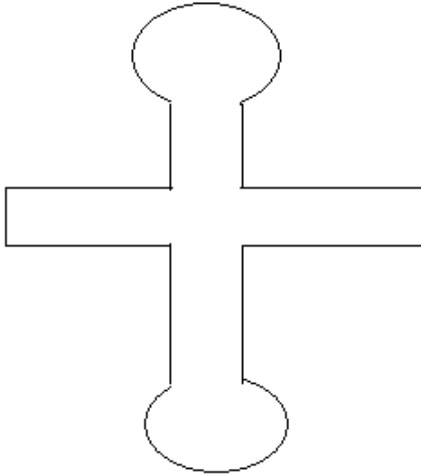
III. (5 points) Phenylalanine is an amino acid. Fill in the following table with regard to the enzymes of phenylalanine biosynthesis.

<i>E. coli</i> genotype	Enzymes of phenylalanine biosynthesis made		Can the bacteria grow in the absence of phenylalanine?
	in the presence of phenylalanine	in the absence of phenylalanine	
wild type			
delete repressor and region 1 of the attenuator			
replace the promoter and region up to the ATG of the first gene with P _R from λ			
P O ^C Δ att ABCDE			
merodiploid R ⁺ P O ⁺ att ⁺ AB ⁻ CDE R ⁻ P O ^C att ⁺ ABCD:: Δ Tn5E			

P= promoter O^C = operator constitutive att=attenuator Δ = deletion

IV. (4 points) You are trying to determine the expression pattern of highly transcribed Gene X. To do this, you are going to use a fluorescent protein (Gfp) driven by Gene X's promoter. Construct a functional transcriptional unit out of the fragments of DNA below that will allow you elucidate the expression pattern of Gene X. You will not use every fragment.

A.



- B.

A.	GFP ORF	A.
----	---------	----
- C.

GAATTC (EcoRI Site)

- D.

AGGAGGA (RBS)

- E.

The 15 bp of DNA upstream of Gene X's ATG

- F.

The 300 bp of DNA upstream of Gene X's ATG
--
- G.

CCACCAGTGACCACC (cAMP BS)

- H.

AAAAAAAAAAAAA

- I.

ATG

V. (7 points) Cellulase is an important virulence factor in *Erwinia carotovora*. In order to study it more closely you want to isolate the gene that codes for this protein. The easiest way to do this is by creating knockout mutations via transposon mutagenesis. If grown on media containing cellulose, wild type *E. carotovora* colonies will digest cellulose which can be detected by incorporating the stain cellufluor in the agar. Wild type colonies degrade the cellulose leaving a dark ring (no stain for cellulose) around the colony. Fill in the outlined protocol below to generate and isolate mutants using the following tools. You will not use all items on list.

E. carotovora FV89 (pir⁻, tra⁻, tet^r)
E. carotovora BM17 (pir⁺, tra⁺, tet^r)
E. coli NN13 (pir⁺, tra⁺, and contains pGI309)
E. coli UT56 (pir⁺, tra⁻, and contains pGI309)
Complex Media
Minimal Media
Ethanol
Glucose
Lactose
Tetracycline
Kanamycin
Carbenicillin
Rifampicin
Hygromycin
Soytone
Cellulose
Aniline Blue
Cellufluor
UV Light
Infrared Light

- 1) Grow up a liquid culture of _____ using _____ for selection.
- 2) Grow up a liquid culture of _____ using _____ for selection.
- 3) Plate both bacterial strains on _____. Allow bacteria to grow and conjugate overnight.
- 4) Elute bacteria from bacterial lawn and spread mixture on _____.
- 5) Isolate those colonies that (do) or (don't) have a dark ring around them. (circle one)

VI. (9 points) Indicate for each of the following their role in energy production and usage by putting check marks in the appropriate columns.

A. Check one box only per line.

reaction	generates net energy	consumes net energy	energetically neutral
cyclic photophosphorylation			
photosystem II			
$N_2 \rightarrow NH_3$			
$CO_2 \rightarrow C(H_2O)$			
glucose \rightarrow pyruvate			

B. Check as many boxes as apply.

compound	can be used as a source of energy	can be used as a place to dump electrons
NO_3^-		
NO_2^-		
H_2		
CH_4		
CO_2		
glucose		
acetate		
CH_3OH		

VII. (5 points) Circle all those of the following reactions which a prokaryote which was unable to make cytochromes could carry out (note this question will be graded as number correct minus number wrong).

aerobic respiration

cyclic photophosphorylation

anaerobic respiration

oxygenic photosynthesis

glycolysis

CO_2 fixation

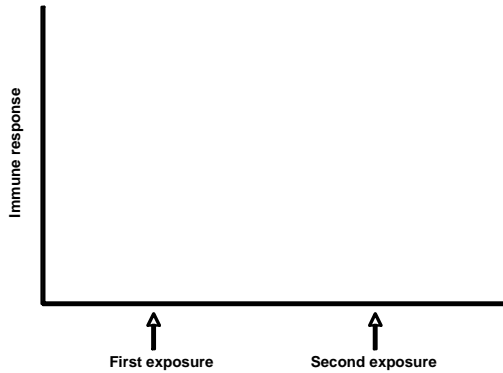
TCA cycle

N_2 fixation

methanogenesis

energy production using bacteriorhodopsin

VIII. (7 points) Use the axes below to graph typical immune system responses for A) innate, B) primary and C) secondary immunity. **Be sure to label your response curves.**



Complete the following table with respect to the immune system response.

Type of exposure	Major immune response
an organ transplant	
viral infection	
a novel toxin	
hay fever	

IX. A. (6 points) You are a doctor with Doctors Without Borders working in the refugee camps in the area of the recent earthquake in the foothills of the Himalayas near the border of Pakistan and India. As winter is starting what diseases are you particularly worried about in your camp? Name 2 different diseases with **different routes of transmission**.

disease	why it is likely to be a problem	route of transmission
1.		
2.		

B. (3 points) Fill in the following table to indicate which of the following diseases could be eradicated.

disease	could this disease be eradicated? Yes or no.	reasons why or why not
Salmonella food poisoning		1. 2.
measles (rubella)		1. 2. 3.
syphilis		1. 2.

X. (6 points) You are working on a problem for the US Department of Agriculture concerning the stability of the genomes of plant viruses. They are particularly interested in 3 viruses which have the following characteristics:

Virus 1: ds DNA virus replicates in the nucleus and has a broad host range

Virus 2: ss RNA virus, + strand one strand, uses a polyprotein strategy for expression, has a narrow host range infecting only corn

Virus 3: ss RNA virus + strand, 4 pieces, has a broad host range including all grasses and many weeds

For each of these viruses indicate whether you think they would be likely to show significant genetic drift and genetic shift and explain why.

Virus	Likely to show genetic drift	Why or why not?	Likely to show genetic shift ?	Why or why not?
1.				
2.				
3.				

XI. (6 points)

You wish to clone a toxin gene from *Clostridium difficile* in order to make a toxoid to use in an experimental immunization. You have available antibodies to the toxin protein. You digest *C. difficile* DNA with each of several different restriction enzymes being careful to do partial digestions and clone the DNA in a multiple copy vector which you transform into *E. coli* (the map of the vector is shown above). You try to identify a clone carrying the toxin gene using the antibodies you have available but you are unable to find the clone. You also have antibodies available for a cytochrome protein and you identify this clone with no difficulty. Give 3 possible reasons for your failure to find a clone of the toxin gene and suggest a way to test one of them.

Possible reasons for the failure to identify a clone of the toxin gene.

1.

2.

3.

Now you wish to test to determine if one of your hypotheses is correct.

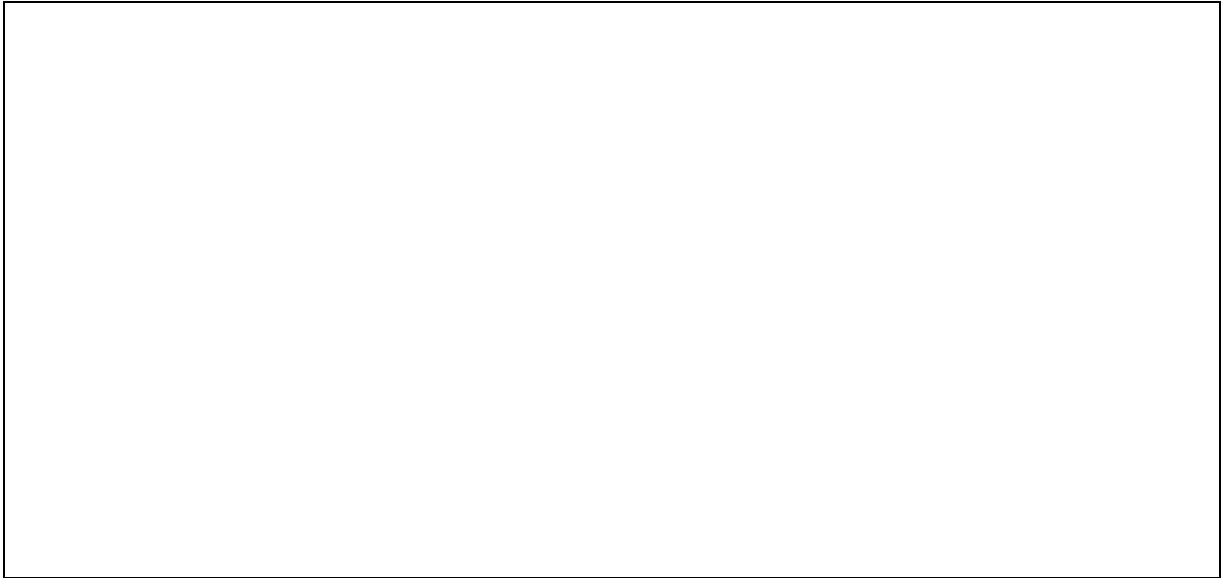
Which hypothesis are you testing? _____

Briefly describe the test you would use.

XII. (10 points) Fill in the following table for two **different** symbiotic interactions **other than that of rhizobia with legumes**.

symbiotic interaction	eukaryotic host	prokaryotic symbiont	benefit to prokaryote	benefit to host	modifications to host involved in symbiosis
1.					
2.					

XIV. A. (5 points) Explain how a prokaryote such as *Halobacterium* can get energy from light without using photosynthesis.

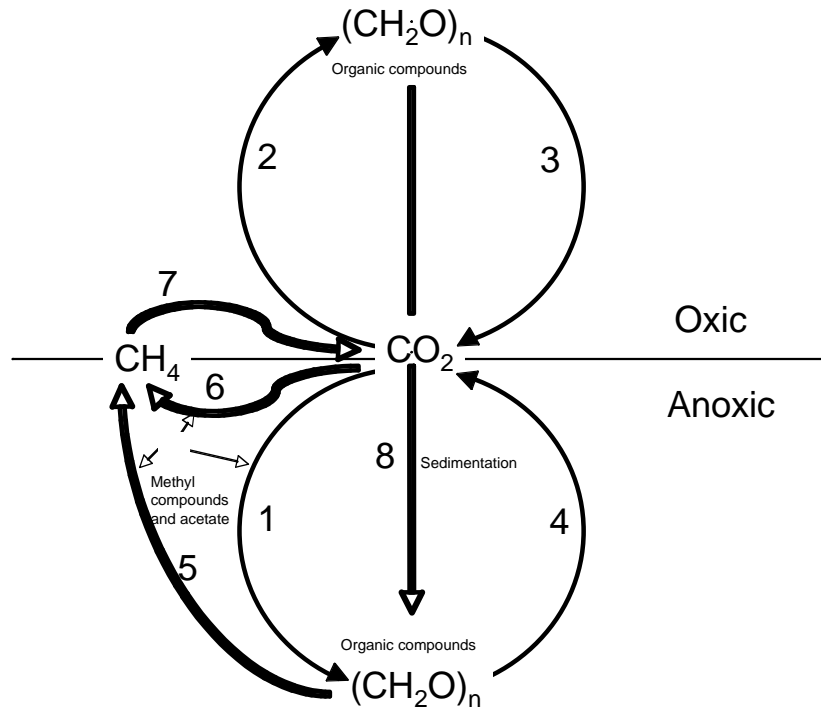


B. (4 points) You isolate a microorganism from a hot spring and want to determine if it is a eubacterium or archaeobacterium. Describe two different tests you could perform (excluding analyses of cell wall composition) that would allow you to determine what type of bacterium you have.



- 1.
- 2.

XV. (11 points) Below is a diagram of the carbon cycle



Name a type of prokaryote that carries out reaction 1 _____

Name a type of prokaryote that carries out reaction 2 _____

Name a type of prokaryote that carries out reaction 4 _____

Are there any eukaryotes that carry out reaction 1? _____

Which reactions can be carried out by archaeobacteria? _____

Which reactions can be carried out **only** by archaeobacteria? _____

If organisms which can carry out reaction 2 had never evolved, what would be the major consequence? _____

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

Which groups of organisms have photosystem I? _____

Which groups of organisms have photosystem II? _____

Which photosystem yields more ATP and reducing power? _____

XVI. (4 points) Fill in the following table.

Characteristic	Eubacteria	Archaea	Eukaryotic cells cytoplasm and nucleus	Eukaryotic cells chloroplasts and mitochondria
structure of lipids				XXXXXXXXXX
some of these organisms can fix nitrogen				XXXXXXXXXX
an inhibitor of protein synthesis				
structure of cell wall for those organisms that have them (if none have a cell wall state none)				

We have enjoyed teaching you this semester and wish you a good holiday and success in your future endeavors.