Name _____
First Last
(Please Print)

PID Number _____-_____

FINAL EXAMINATION

BIOLOGY 108

FALL, 2002

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

Signature_____

1_____

2_____ 15._____

3_____ 16_____

4_____ 17._____

5_____ 18._____

6_____ 19._____

7_____

8_____

9_____

10_____

11_____

12_____

13_____

14_____

1. (6 points) Bacteria differ in the ways in which they obtain energy. What are the three fundamentally different types of energy available to bacteria?

type of energy	example of a bacterium which uses this type of energy

2. (4 points) Order the following compounds by their probable rate of degradation in soil under aerobic conditions:

fastest_____slowest

Of C and F which would be degraded most rapidly under anaerobic conditions? _____

3. (4 points) In studying a superfund site you find that one of the major xenobiotic compounds in this site (2, 3, 6 trichlorobenzoic acid) is being degraded slowly. You attempt to isolate bacteria from the site which are able to use this compound as a carbon source but are unsuccessful. What are two major possible different reasons why this might not be possible? Give only a **brief (less than 10 word)** reason. If your answers are more than 10 words **only** the first 10 words will be graded.

Reason 1_____

Reason 2_____

4. (4 points) Circle all of the following conditions which would be likely to support the growth of a bacterium isolated from inside a rock in the dry valleys of Antarctica (high elevation, very dry, no eukaryotes around).

nutrient broth at 37^{o}	minimal salts plus glucose	light and minimal salts
nutrient broth at 90^{o}	minimal salts plus Fe^{+3}	minimal salts plus FeS
nutrient broth at 4^{o}	minimal salts plus Fe^{+2}	minimal salts plus Na_2SO_4

5. (5 points) You know that *E. coli* can take up either reduced S compounds or sulfate for growth. You wish to obtain a mutant in the sulfate uptake system in *E. coli*. For this purpose you propose to use chromate which you believe is taken up by the same system as sulfate. Chromate is toxic to most living cells.

If sulfate and chromate are taken up by the same system what will happen if you attempt to grow *E. coli* on a mixture of sulfate and chromate?

What will happen to bacterial growth if after a few hours you add methionine to the medium containing sulfate and chromate?

You make a transposon mutant which you hope is in the sulfate uptake system. What do you expect to be the growth characteristics of the mutant on the following media? (Indicate dead, alive but no growth, slow growth, or normal growth)

medium	growth
sulfate	
sulfate plus chromate	
sulfate plus chromate plus methionine	

6. (4 points) Some bacteria are capable of forming spores.

What conditions trigger a bacterium such as *Bacillus subtilis* to form spores (Be brief, less than 10 words) _____

Gene expression during spore formation is carefully regulated. What is the major mechanism used to do this? (Be brief, less than 10 words) _____

7. (6 points) What would be the effect on the amount of ß-galactosidase made by cells incubated in minimal salts with lactose of each of the following mutations:

mutant	amount of ß-galactosidase made (indicate none, a little, normal or excess)
repressor which can't bind lactose	
operator which can't bind the repressor	
promoter which can't bind RNA polymerase	
deleted RBS	
deleted *lacY* (lactose permease)	
deleted *lacA* (lactose transacetylase)	

8. (6 points) In all organisms, DNA replication is necessary for cells to divide. Circle all components of the DNA replication process listed below (note: not all components are listed)

DNA polymerase III	template strand	sigma factor
promoter	primers	single strand binding protein
lagging strand	helicase	DNA polymerase I

In a bacterium with a temperature sensitive primase, when the bacterium is shifted to a higher temperature, which molecules will continue to be synthesized? (Circle the correct answer.)
A. none
B. DNA synthesis will proceed uninterrupted
C. the leading strand will continue uninterrupted and nothing will be synthesized on the lagging strand
D. small fragments of both strands will be made
E. the leading strand will continue uninterrupted and small fragements on the lagging strand will be completed

If a compound which blocks the initiation of DNA replication is added to a bacterium: (Circle the correct answer.)
A. nothing will happen, the bacterium will be undisturbed
B. the bacterium will finish its current round of replication, but will not start any new rounds of replication and will not continue to divide.
C. the bacteria will cease DNA synthesis, all growth will stop, and the bacteria will not divide.
D. bacterial DNA synthesis will be error prone, and there will be lots of mutations

9. (9 points) The activity of biosynthetic pathways such as that leading to the synthesis of tryptophan can be regulated at several levels. You obtain several mutants which overproduce tryptophan when they are fed this amino acid. What are three mutations at **different sites** which could account for this phenotype?

1.

2.

3.

Given the following reagents how could you test to determine which site was actually mutated?
tryptophan
a radioactive precursor of tryptophan whose incorporation into tryptophan you can measure
a clone of the tryptophan operon on a plasmid
the ability to delete any specified part of the trp operon on this clone and still have a usable clone
a clone of the trpR gene on a plasmid

To determine if site 1 (as listed above) was mutated do the following:

To determine if site 2 was mutated do the following:

To determine if site 3 was mutated do the following:

10. (5 points) Fill in the following table. What will be the phenotype (clear plaques, cloudy plaques, or no plaques) of *E. coli* infected with each of the following phages?

P_R cro t_{R1} CII t_{R2} Q t_{R3} P_{Rlate} SR...A...

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xis att int CIII t_{L1} N P_L CI P_{RM} P_{RE}

	E. coli strain	
? genotype	K12 (-)	K12(?)
wild type ?		
? P_{RE} no longer functional		
? Q^{--}		
? with P_R and P_L replaced with P_{trp} grown in complex medium		
? with P_R and P_L replaced with P_{lac}, grown in minimal medium plus lactose		

11. (10 points) Influenza is an enveloped virus. How does it get (make) this envelope? _____
Fill in the following table.

type of virus	enzymes the virus must encode (if none, state none)	enzymes the virus must bring into the host cell with it (if none, state none)
ds DNA, replicates in the nucleus		
ds DNA, replicates in the cytoplasm		
ss RNA + strand		
ss RNA - strand		

12. (16 points) While on a field trip to Utah you isolate a gram-negative bacterium from the edge of the Great Salt Lake which can grow in 2.5 M NaCl. This bacterium will also grow in ordinary Luria broth and in minimal salts medium with organic acids. You wish to determine the genes required for this bacterium (GSL-1) to grow at high salt concentrations. Your first thought is to try transposon mutagenesis.

You have the following available:
Luria agar and broth
NaCl
Luria agar and broth with 2.5 M NaCl
minimal salts medium (liquid and solid) with organic acids
proline (pro, an amino acid)
glucose
tetracycline, ampicillin, chloramphenicol
the plasmids pUNC and p108 each in *E. coli* $tra^{+}pro^{-}$
purified plasmid DNA of pUNC and p108

Fill in the spaces in the following protocol for obtaining your mutants.
1. Introduce _____ into GSL-1 by _____ it with _____.
2. Select for the bacteria you want by plating on _____.
3. Determine which mutants are in genes required for growth in high salt by _____

_____.

Your transposon mutagenesis protocol is unsuccessful; you isolate no bacterial colonies in step 2. Although the protocols were done correctly and a parallel mutagenesis carried out at the same time using Rhizobium as a recipient was successful. Suggest a possible reason for this failure.
_____.

Since transposon mutagenesis didn't work you decide to try introducing genes from GSL-1 into *E. coli* to see if you can make *E. coli* salt resistant. For this purpose you
1. make a library of GSL-1 DNA in _____ by _____.
You introduce these clones into *E. coli* by _____ and plate on _____ to select for bacteria which received clones which made them salt resistant.
Unfortunately this protocol too fails although you are able to obtain clones which make pro^{-} *E. coli* able to grow without added proline suggesting that the procedure was carried out correctly. suggest a possible reason for the failure of this experiment._____
You now decide that you need to know more about GSL-1. You start to find out to what other bacteria it is related. What is the easiest method to use to do this? _____
You discover that GSL-1 is not a member of the proteobacteria. To what group is it likely to belong?
_____
You now redesign your protocols using a more suitable host than *E. coli* and everything works well!

13. (8 points) You are a doctor in rural North Carolina. You see a patient brought in by a relative. The patient is a young Hispanic man who is having difficulty moving and speaking. All his muscles are tense. He has a nasty wound on his leg where a dog bit him a few days ago. The surface of the wound is surrounded by an inflamed area and pus and covered with a scab. What is the major disease from which this young man is suffering? _____

What is the principle virulence factor of the causative organism? _____
What is the mechanism of action of this virulence factor?

How would you treat this disease?

Are there any public health measures you should take? If so, what?

14. (5 points) Why is it that we have been able to use the same immunization for yellow fever for the last 50 years but can not use the same immunization for influenza for more than one or two years?

15. (6 points) Fill in the following table with respect to symbiotic interactions between bacteria and eukaryotes.

eukaryotic site	symbiotic bacteria	adaptation of eukaryote to symbiosis	advantage to eukaryote
cow's rumen			
deep sea vent tube worm			
soy bean roots			

16. (6 points) Different bacteria have evolved to grow in different environments. For each kind of bacterium shown below list one category of genes you would expect to be frequent and one category you would expect to be rare in that organism. (An example of a category of gene is “sugar transporters”; you may not use this example in the table).

organism	categories of genes which may be frequent	categories of genes which may be rare
obligate human pathogen which lives only in the blood		
facultative enteric pathogen which can live in the gut or in the environment		
bacterium which lives in mine tailings		

17. (5 points) How can a prokaryote such as *Halobacterium* get energy from light without using photosynthesis?
Describe the process briefly.

18. (5 points) Answer the following questions using the diagram of the nitrogen cycle shown below.

Are there any eukaryotes which can carry out reaction 1 or 11? yes or no (circle one)

Why do organisms carry out reaction 3? _____

Why do organisms carry out reaction 7? _____
Are there any eukaryotes which can carry out reaction 9 or 10? yes or no (circle one)

Which major groups of organisms can carry out reaction 4? _____

19. (8 points) Fill in the following table with respect to the characteristics of major groups of living things and their organelles.

	eubacteria	archaea	eukaryote nucleus and cytoplasm	eukaryote chloroplast and mitochondria
presence of nucleus (yes or no)			XXXXXXXX	XXXXXXXXX
structure of lipids				XXXXXXXXXX
this group contains organisms which can grow at temperatures above 90^{o}C (yes or no)				XXXXXXXXXX XXXXXXXXXX XXXXXXXXXX
has introns in many genes				
susceptible to antibiotics which act on the cell wall such as penicillin (yes or no)				
susceptible to antibiotics which act on ribosomes such as streptomycin (yes or no)				
initiation of protein synthesis by F-met (yes or no)				
susceptible to diphtheria toxin (yes or no)				

I have enjoyed teaching you this term and wish you success in your future endeavors.
Have a good holiday!