Name_____ **Answer Key**___

Student ID#_____

Bacterial Genetics,
BIO 4443/6443
Fall Semester 2002
Exam I

1.) What is a sigma factor? Why does the cell contain multiple sigma factors? (5pts)

Sigma
Is a subunit of the RNA polymerase that recognizes the promoter region on the DNA and is required to RNA initiation. It is thought be released from the RNA polymerase holoenzyme after initiation has occurred. Different sigma factors recognize different promoter regions and help to regulate the transcription of different sets of genes.

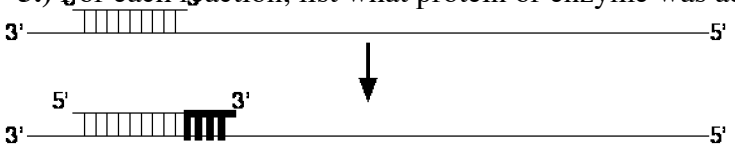
2.) Purified Polymerase III will incorporate the wrong nucleotide once in every 10^6 bases. Yet when we measure the rate of misincorporation in the cell, we observe that a base is misincorporated at a rate of only once in 10^{10} bases. Describe two mechanisms that account for this difference. (6pts)

DnaQ, proof-reading exonuclease subunit of the polIII holoenzyme cleaves mismatched bases.

Methyl-directed mismatch repair system can excise mismatched base pairs from the daughter strand and then re-replicate the region shortly after replication occurs.

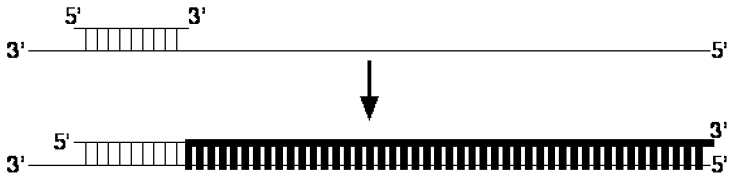
In a test tube, you mix a primed, DNA substrate and dNTPS (all four of the deoxyribonucleotide triphosphates). You add an unknown protein to your reaction mixture and observe the product shown.

3.) For each reaction, list what protein or enzyme was added to the reaction. (9pts)



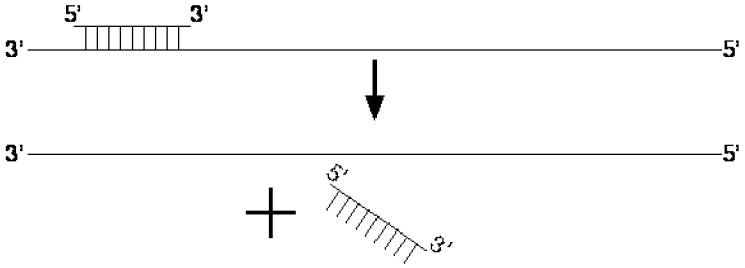
any polymerase such as PolI PolII or PolIII

Enzyme Added:_____



a polymerase and processivity factor, (clamp, B subunit dnaN gene product)

2 Enzymes Added:_____



Any DNA helicase such as the *dnaB* gene product

Enzyme Added:_____

You add an antibiotic that inhibits translation to a bacterial culture.
4.) How would you expect this to affect factor independent transcriptional termination? Why? (3pts)

It should not affect the transcription of these genes early on. Termination by this mechanism relies upon a hairpin and stretch of UUUUs in the RNA transcript that interacts with the RNA polymerase on its own to terminate transcription.

5.) How would you expect this to affect factor dependent transcriptional termination? Why? (3pts)

(Either increase or decrease was acceptable with justification)
It should increase the frequency that transcription terminates since, in the absence of translation, the termination factor, such as rho, would always have access to its binding site on the RNA transcript (in the case of rho, it bind to the rut sequence). When translation is actively occurring, the ribosomes may cover or hide the binding site preventing the termination factor from functioning. (Or you could say it will decrease frequency that transcription terminates if you thought that translation was needed to make the termination factor (such as Rho protein)

6.) A new polymerase was purified from a bacteria living in the nose of an Antarctic penguin. It is interesting because the polymerase appears to be equally active at 4C and 43C, a very wide temperature range.

You have isolated three mutations that each map to the 4^{th} codon of the polymerase gene. Define what each mutation is, and determine which mutations are the most likely to inactivate the bacterial polymerase. (6pts)

Missense mutation: a base pair change that changes the codon for one amino acid to another amino acid. Of the three mutations, this is the least likely to inactivate the polymerase since the vast majority of the protein will remain unchanged.

Nonsense mutation: a base pair change that changes the codon for an amino acid into a stop codon. This will almost certainly inactivate the protein since only the first four amino acids of the protein are produced.

Frameshift mutation: a base pair insertion of deletion in the coding region of the protein. Since the frameshift occurs so early in the protein, it will alter all the amino acids after the first four codons and will also almost certainly inactivate the protein.

7.) A strain of *Samonella enterica* has approximately $6*10^6$ base pairs in its genome, and it can replicate and divide every 30 minutes. Assuming that it has one bidirectional origin of replication (just like *E. coli*), what's the minimum rate that the replication fork must be moving in bases per minute? (Show your work) (6pts)

(6*106 bp/genome) / 1 (genome/30minutes) / 2 replication forks =
200,000 bp/minute / 2 replication forks =
100,000 bp/min/replication fork

8.) Briefly describe (or draw) the events involved in the initiation of translation.
Include the following terms, if appropriate (not all of them are appropriate).
TATA Box, 30S Subunit, 50S Subunit, RNA Polymerase, Shine Delgarno, rho, formyl-methionine, P site, A site, RF1, IF3 and IF1, IF2, EF-Tu, Ef-G, t-RNA, sigma factor. (8pts)

1. The **30S subunit** binds to the **Shine Delgarno** sequence on the RNA. (facilitated by **IF3** and **IF1**)
2. At the start codon, a charged **tRNA** carrying **formyl-methionine** is brought into the **P site** of the30S Subunit (facilitated by **IF2**)
3. The **50S subunit** then tops it off, and it translation can proceed.

9.) Briefly describe (or draw) the events involved in base excision repair when uracil is found in DNA. Include the following terms, if appropriate (not all of them are appropriate).
Polymerase I, Polymerase III, Ligase, UvrA, UvrB, UvrC, UvrD, AP Endonuclease, Uracil DNA glycosylase, Photolyase, dRpase (deoxyribophosphodiesterase), MutS, MutL, MutH. (8pts)

1. **uracil glycosylase** cleaves the damaged base at the glycosidic bond.
2. an **AP endonuclease** cleaves the DNA backbone. (if there os not a 3'OH, **dRpase** is also needed)
3. **Polymerase I** resynthesizes the damaged region
4. **Ligase** seals the nick

To demonstrate how to determine whether DNA replicates conservatively or semi-conservatively, you decide to reproduce the Meselson-Stahl experiment for your microbial genetics class.

You and a graduate student who works with you decide to each do the experiment (just in case one person messes up).

A.) For one control, you grow an *E.coli* culture in media containing heavy isotopes of nitrogen (^{14}N) and carbon (^{13}C) to label the DNA during growth.

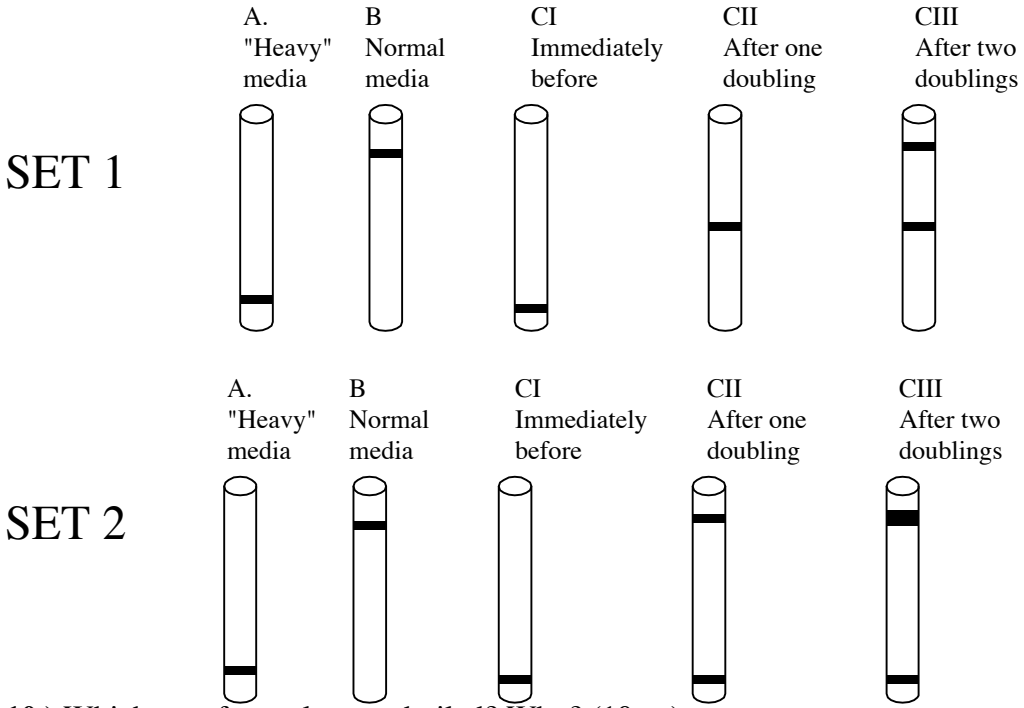
B.) For another control, you grow an *E.coli* culture in normal media.

C.) Then for your experimental analysis, you grow a third culture in the media containing heavy isotopes. Then, after several generations of growth, you transfer the bacteria into normal media and prepare samples at various times (I. immediately before transfer, II. after one doubling time in the normal media, and III. after two doubling times in the normal media).

You lyse the cells, isolate the DNA, and load your samples into tubes containing a neutral CsCl solution of the appropriate density.

The graduate student's samples look fine. However, you accidentally boiled your samples when you were trying to get the CsCl to dissolve. (Boiling will denature the DNA, all the double stranded DNA will melt apart into single stranded DNA). A little disappointed with yourself, you decide to centrifuge both the boiled and nonboiled sets of samples anyway.

Both sets of samples are shown below, along with where the DNA banded in each tube.



10.) Which set of samples was boiled? Why? (10pts)

Set 2 was boiled. After one generation, all the DNA should be hybrid, one heavy strand: one light strand following semiconservative replication. So molecules will have an intermediate density (set 1). If these samples were boiled and all the DNA is in single stranded form, the heavy and light DNA strands are separated from each other. So all the heavy strands will "float" at a lower, denser portion in the gradient and all the light strands will float" at a higher, less-dense portion in the gradient (set 2)

The Defense Department brings you an unknown bacteria that was isolated from an abandoned biological warfare plant in an undisclosed country. All tests suggest that the strain is completely nonpathogenic but the Defense Department is still a little worried about it. They ask you to determine if it can be killed with any of the common antibiotics. You test to see if it will grow on penicillin or streptomycin and find that the bacteria is indeed resistant to penicillin but it is still sensitive to streptomycin.

Content that the bacteria is not a threat, they leave the bacteria with you and you decide to learn more about the resistance genes in this bacteria. You decide to see if you can isolate mutants that are sensitive to penicillin.

11.) Briefly describe how you would isolate penicillin sensitive mutants. (6pts)

1. Plate cells on nonselective media (no antibiotics).
2. Replica plate the cells onto media plates that contain penicillin
3. Colonies that did not grow on the penicillin plates but did grow on your nonselective plates are your mutants

12.) What are two techniques you could use to increase the frequency that you recover penicillin sensitive mutants? (4pts) mutagenize the cells, use an enrichment strategy, or grow the cells for several generations prior to selecting.

13.) Does this screen involve a positive selection or a negative selection? (2pts)

negative selection

You are curious to know if the strain has a particularly high mutation rate, and decide to measure the mutation rate for streptomycin resistance.

You inoculate 21 individual 1ml cultures with the streptomycin sensitive bacteria and allow them to grow overnight. In the morning you plate serial dilutions of the 21st culture and determine that your bacteria have grown up to a concentration of $1*10^{10}$ bacteria/ml (pretty concentrated). You then plate the remaining 1ml cultures onto separate plates containing streptomycin, place them in the incubator overnight, and count how many colonies grow up on each plate the following day. Your results are below

plate#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Streptomycin resistant colonies	1	1	1	2	2	13	150	12	13	0	0	0	0	0	0	0	0	0	0	14

14.) Does this represent a positive or negative selection? (2pts)

positive selection

15.) Based upon your results, what is the mutation rate for generating resistance to streptomycin in this bacteria?

Remember that the mutation rate is equal to the number of mutational events per cell division, a = m/N. Assume that the probability of having i mutations per culture is represented by Poisson expression, $P_i = (m^i e^{-m})/ i!$. Show your work. (10pts)

10 out of 20 cultures had 0 mutations. So the probability of having zero mutations (P_i) is 10/20, and i= 0 mutational events per culture in this situation.

$P_i = (m^i e^{-m})/ i!$
$(10/20) = (m^0 e^{-m})/ 0!$
$0.5 = (1 e^{-m})/ 1$
$-\ln(0.5) = m$
$0.69 = m$

0.69 mutational events per culture of 1 x 10^9 cells
a=m/N
a=0.69 mutational events/1 x 10^{10} cells
a=6.9 x 10^{-11} StrR mutational events per cell division

Looking back at your notebook, you see that a mutation generating sensitivity to penicillin occurs once in every 100,000 cell divisions in this strain.

16.) How does this compare with the mutation rate for *Str*^{R}? Why are these mutation rates so different if you are using the same strain of bacteria? (6pts)

Target size. The chance that a mutation occurs which results in sensitivity to penicillin is much higher than the chance that a mutation will result in resistance to streptomycin because any mutation, anywhere in the penicillin resistance gene that inactivates it will result in a sensitive phenotype. In contrast, you probably need a mutation in a very specific location of the *rpsL* gene to create a protein that now unaffected (not inactived) by Streptomycin.
(There are many more ways to break a car, than there are to fix a broken one)

Streptomycin is an antibiotic that binds to the bacterial ribosome (encoded by the *rpsL* gene) and prevents translation. You think it would be interesting to know how many different mutations in the *rpsL* gene can confer streptomycin resistance. To get at this question, you decide to sequence the *rpsL* gene from some of the *Str*^{R} colonies that grew on your selective plates. YOU KNOW THIS IS GOING TO BE A LOT OF WORK so you hire an undergraduate to help you.

The undergraduate is a little lazy, but manages to sequence the five colonies that grew on plates 1, 2, 3, and 4. You are much more ambitious and sequence all 150 of the colonies that grew on plate 7.

17.) Who is more likely to isolate a larger number of different *rpsL* mutations? Why? (6pts)

The undergraduate is more likely to isolate a larger number of different *rpsL* mutations. Since all 150 of your colonies came from the same culture, they more than likely all arose as a result of a single mutation in one original parent cell.

Since the undergraduate sequenced colonies from 4 separate cultures, at least 4 independent mutations must have occurred.

Remember, on average, only 0.69 mutational events occurred in each culture.