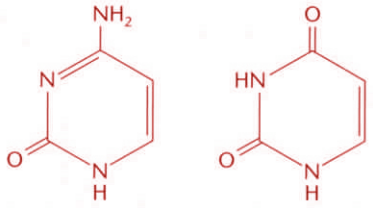
Name_____

Student ID#_____ Answer Key

Bacterial Genetics,
BIO 4443/6443
Fall Semester 2003
Exam I

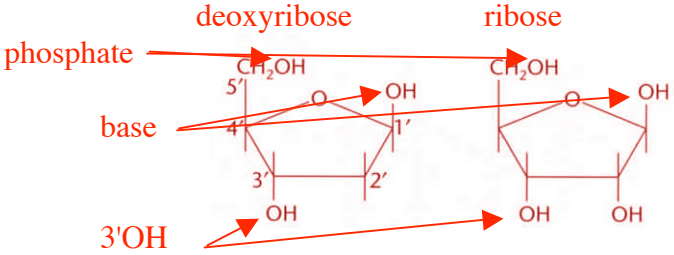
1.) Draw the structure of cytosine and uracil. (5pts)

cytosine thymine



2.) Draw the structure of ribose and deoxyribose. On each molecule, indicate where the 3'OH is located and where the phosphate and base attach on a nucleoside triphosphate (5pts).

deoxyribose ribose



3.) Purified Polymerase III incorporates the correct base 9,999 times for every 10,000 bases it polymerizes. *Escherichia coli* K12 has 4.5 X 10^6 basepairs in its genome. How many errors does polymerase III make each time the cell replicates? (4pts)

$(4.5 * 10^6$ bases / genome) * (1 error/ 10000 bases) =
$(4.5 * 10^6$ ~~bases~~ / genome) * (1 error/ 10000 ~~bases~~) =
450 errors / genome

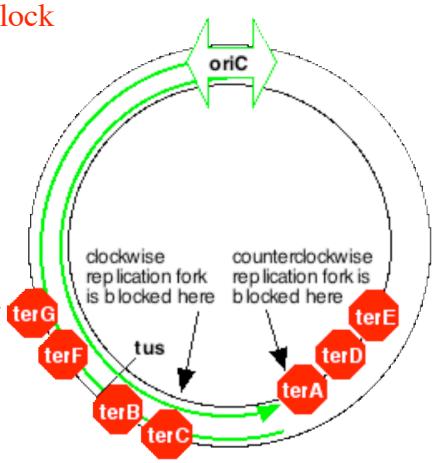
4.) The actual error (or mutation) rate per cell division in *E. coli* has been estimated to be approximately one base for every 10^{10} bases polymerized. Name two mechanisms that help increase the accuracy of replication and briefly describe how they function (6pts).

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

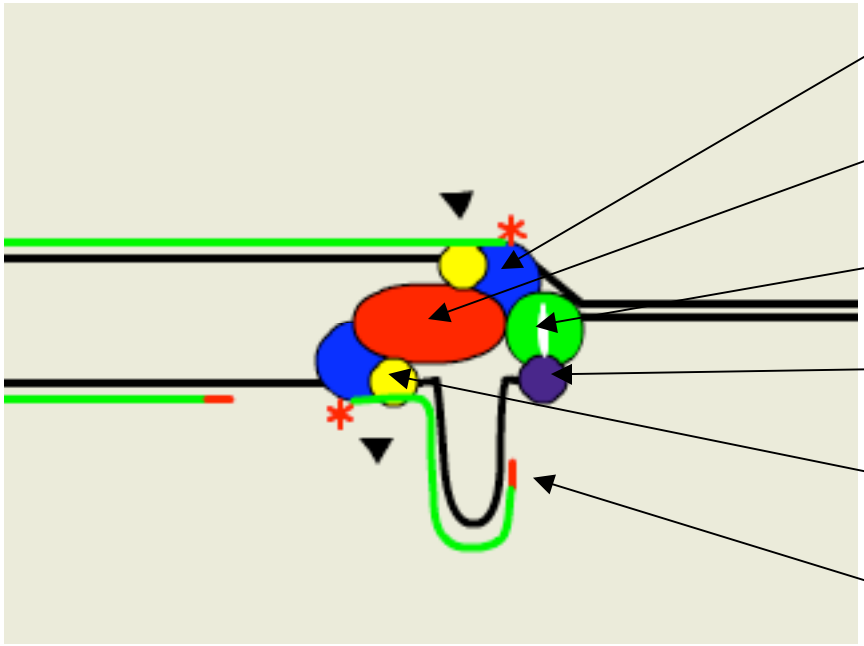
Methyl-directed mismatch repair system can excise mismatched base pairs from the daughter strand and then re-replicate the region shortly after replication occurs.

5.) How is Tus thought to promote the termination of replication? What happens to replication termination in a *tus* mutant? (5pts)

Tus protein binds to *ter* sequences in the terminus region an acts to block replication forks approaching from one direction while allowing replication forks approaching from the other direction to pass. The *ter* sequences are oriented on the chromosome so that replication forks are "trapped" or blocked once they have replicated slightly more than halfway around the chromosome. This is thought to help ensure that replication is completed in the termination region of the chromosome. The inability to complete replication is probably lethal for the cell. However, *tus* mutants replicate and grow normally suggesting that Tus is not essential for replication to terminate.



5.) A movie of an active replication fork is playing on the screen in the front of the room. Based on the activities of each component in the movie, label the indicated components of the holoenzyme on the diagram below (12pts).



6.) Briefly describe the protein(s) and DNA sequences that determine where an RNA polymerase initiates transcription on a particular stretch of DNA? (6pts)

A sigma factor bound to an RNA polymerase will bind to specific sequences in the promoter region of the DNA that is to be transcribed. Different sigma recognize different promoter sequences. The most common sigma factor, □70, the sequences at -10, the TATAA box, and the -35 region are important for recognition.

7.) What DNA sequences are important for factor independent transcriptional termination? How are these thought to promote transcription termination? (4pts)

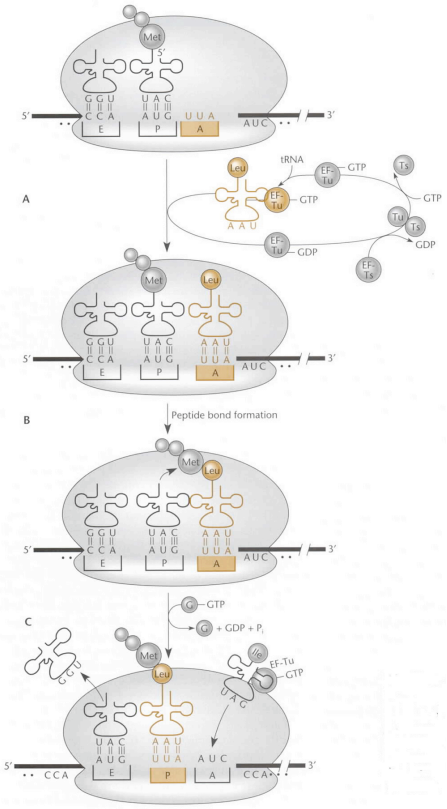
Termination by this mechanism relies upon an inverted repeat sequence that is followed by a stretch of UUUUs in the RNA transcript. Transcription of the inverted repeats produces a hairpin in the RNA that destabilizes the RNA polymerase enough to dissociate it when followed by a string of UA base pairs in the transcription bubble. The UA base pairs are less stable than GC base pairs due to the lower number of hydrogen bonds formed between these base pairs.

8.) What DNA sequences are important for factor dependent transcriptional termination? How are these thought to promote transcription termination? (4pts)

Factor dependant termination occurs when a protein factor, such a Rho, binds to a specific sequence on the RNA transcript. In the case of Rho, the *rut* sequence is bound. The Rho factor is an RNA helicase that, in effect, chases or follows the RNA polymerase and dislodges it at specific pause sites downstream on the transcript. Since Rho can only bind to *rut* when translation is not "hiding" the rut sequence, this also provides a mechanism to regulate the transcription of polycistronic messages.

9.) Briefly describe (or draw) the events involved during translation (you do not need to include events that are associated specifically with initiation or termination).
Include the following terms, if appropriate (not all terms are appropriate).

TATA Box, 30S Subunit, 50S Subunit, 70S Subunit, RNA Polymerase, Shine Delgarno, rho, ATP, GTP, formyl-methionine, P site, A site, RF1, IF3 and IF1, IF2, EF-Tu, EF-G, t-RNA, sigma factor. (12pts)



A. **EF-Tu loads a charged tRNA into the empty A site** of the 30S ribosome subunit using GTP as an energy source. (EF-Ts then exchanges the GDP for GTP on Ef-Tu to "recharge" the enzyme for the next round.

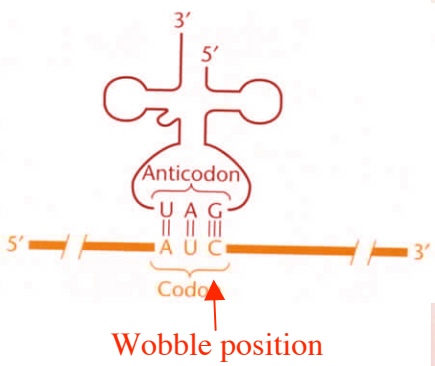
B. **The polypeptide attached to the tRNA in the P site Is transferred (bonded) to the amino acid-tRNA in the A site** using a peptidyltransferase activity found on the 50S subunit.

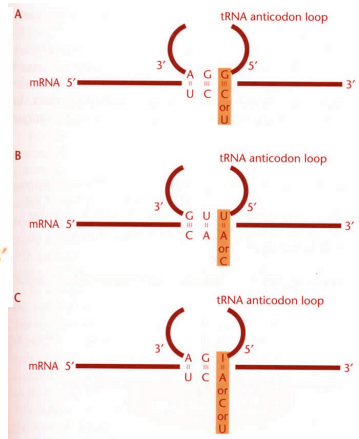
C. **GTP charged EF-G powers the ribosome forward moving the tRNA containing the polypeptide into the P site.**

The cycle can repeat.

10.) A translated portion of an mRNA reads 5'AUC AUU AUA3'. Although each codon contains a different sequence, each encodes an isoleucine in the protein product and is read by the identical tRNA molecule. What allows one tRNA to recognize all these sequences? (4pts)

The wobble position, due to either the curvature of the tRNA or a modified base can pair with more than one type of base and allows for a single tRNA to recognize more than one codon.





11.) What are nonsense codons? (4pts)

Three codons in the genetic code, UAA, UAG, and UGA, do not have corresponding tRNAs that recognize them. Instead, these sequences are usually used to terminate translation. They translate as "stop" codons.

The mitochondria in your cells actually contain their own DNA. They replicate and divide independently from the DNA in the nucleus, almost like smaller, symbiotic organisms living inside our own cells. Several recent articles indicate that mitochondrial replication is unique in several ways. For instance, rather than coordinately replicating both the leading and lagging strand simultaneously, it is reported that they replicate the entire leading strand first, and then replicate the lagging strand after the leading strand is complete.

Thinking this through, you decide that this could also mean that mitochondrial replication occurs conservatively, rather than semiconservatively. You decide to test this idea and examine how mitochrondria replicate using a variation of the Meselson-Stahl experiment.

You carefully examine the mitochondrial replication cycle in your cultured cells and determine that they double (or replicate) once every 24 hours.

As controls, you grow your two different cell cultures for several generations.

A.) One culture is grown in normal media

B.) And the other culture is grown in media containing 5-bromouracil, which is an analog of thymine that has a much higher buoyant density.

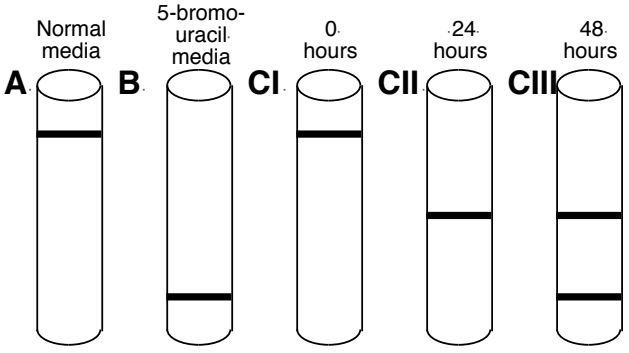
C.) For your experimental analysis, you grow a third cell culture in normal media. You then transfer the cells into media containing 5-bromouracil and collect them at the following times: I) immediately before transfer, II) after 24hours, and III) after 48 hours.

You isolate the mitochondrial DNA from each sample and then centrifuge them to equilibrium in neutral CsCl gradients.

You lyse the cells, isolate the DNA, and load your samples into tubes containing a neutral CsCl solution of the appropriate density. Your results are shown below, along with where the DNA banded in each tube.

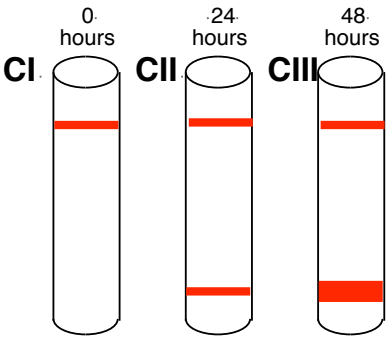
12.) Based on these results, do mitochondria replicate conservatively or semiconservatively? (4pts)

semiconservatively



13.) Indicate where the bands would be expected to appear if the mitochondria replicated through the alternative mode? (6pts)

If conservative…..



14.) The restriction endonuclease, Eco RI, recognizes the six basepair sequence 5'GAATT*C3'/3'CTTAA*G5'. If the *E.coli* genome contains 4.5 million bases, how many times EcoRI restriction sites would we expect to find on the *E.coli* genome? Show your work (5pts).

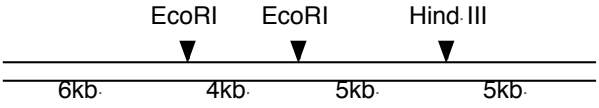
There is a 1 in 4 chance that each base in the sequence is correct, compounded by the length of the sequence.
(1/4) (1/4) (1/4) (1/4) (1/4) (1/4) =base pairs
$(1/4)^6$ =
1/4096
So if the DNA sequence were completely random, we would expect to encount an Eco RI site once every 4096 bases, on average.

4,500,000 bp/genome * 1EcoRI site/4096 bp =
4,500,000 bp/genome * 1EcoRI site/4096 bp =
1099 Eco RI sites / genome

You've isolated an unknown bacteriophage that has a linear genome that is 20,000 bp long and contains 2 EcoRI restriction sites and 1 Hind III site at the positions shown below.

You isolate and purify the viral DNA and divide it into four tubes.

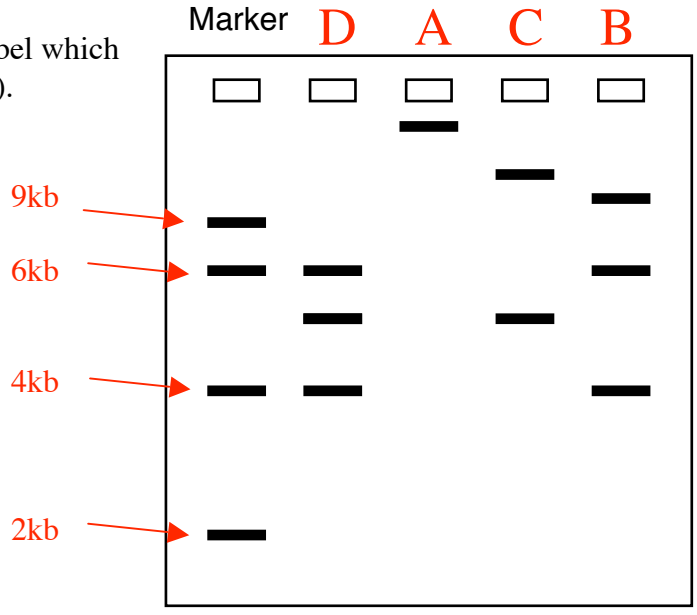
Tube A you add nothing
Tube B you add EcoRI.
Tube C you add Hind III
Tube D you add both EcoRI and HindIII.



You'ld like to isolate the different fragments for cloning and further characterization, but as you are getting ready to load your gel, you realize that you forgot to label your tubes! Although you are extremely discouraged about your sloppy lab practices, you are not too worried because you have a tube with "DNA markers" that contain four fragments of known DNA sizes, a 9 kb fragment, a 6 kb fragment, a 4 kb fragment, and a 2 kb fragment.

So you load your marker in the first lane and then your unlabeled samples in the following lanes of the agarose gel. Following electrophoresis you observe the bands as shown below.

15.) In the space above each lane, label which tube was loaded into each lane (6pts).
Smaller fragments run faster, so…



16.) Briefly describe how the process of PCR can be used to amplify a given DNA sequence? (8pts)

PCR, polymerase chain reaction, takes advantage of the exponential power of growth each time molecules replicate.
Cycle 1.
A.) To amplify the sequence of gene X, two short primers are synthesized, one homologous to the beginning of gene X and a second that is homologous to the complementary strand of the end of gene X.

B.) Then, the primers are added in excess to a solution containing a (thermostable) polymerase, dNTPs, and the DNA to be amplified (sometimes only the DNA of one cell).

C.) The solution is heated (to denature the DNA), and cooled (to allow the primers to anneal to the template). The polymerase then extends the primers replicating the sequence.
Now there are two copies of gene X.

Cycle 2. The process is repeated. Now there are four copies of gene X.

Cycle 3. The process is repeated. Now there are eight copies of gene X.

Cycle N. The process is repeated… Now there are 2^N copies of gene X.

After 40 cycles, you have gone from having 1 copy of the gene to having 1,099,511,627,776 copies of the gene.

Bonus
When the US government funded the human genome project, how long and how many scientists did they expect it to take to sequence the entire human genome? How long and how many scientists did it take for Celera to sequence the human genome, once they started? What were the primary scientific advances, ideas, or breakthroughs that allowed them to do this (Be as specific as possible)? 20pts.
They expected that in order to complete the sequence of the entire human genome, more than 3000 scientists would have to work on the project for more than 20 years.

Celera, once the initiated the sequencing of humans, were able to complete the project in 9 months with a staff of 50 scientists on the project.

Celera pushed forward the automation and advancement of the DNA sequencing process such that it became much more efficient. The increased computing power that became available during this time period also made the concept of "shot gun" sequencing possible for very large genomes such as the human genome.

Basically, shot gun sequencing involves just randomly sequencing stretches of the genome (500 base pair/sequence reaction) until everything has been done many times over. Computers are then used to match the ends of the sequences and reconstruct the genome into its proper order.