Molecular Biology Through Discovery
Problem Set 4: DNA Replication

1. A linear piece of DNA has just finished replicating from an origin of replication approximately in the center of the molecule. Draw a simple picture of the finished replication products in a mutant *E. coli* strain that lacks the enzyme DNA ligase. Provide zoomed in views of regions of interest to illustrate pertinent detail. Also provide a legend to your drawing and be sure to indicate the directionality of each strand. (If you have to make any assumptions to answer this question, please state them.)

2. The E. coli chromosome is 4.5 million nucleotides. Its replication requires about 40 minutes. Human DNA polymerase proceeds at about 20 times slower than E. coli DNA polymerase. At that pace, how long would it take to replicate an average human chromosome? Any problem with your answer? Any solution?

3. You have an idea of how to accomplish something that nature has not been able to do: make an enzyme that can convert atmospheric N_2 to NH_3 in the presence of oxygen. This would have enormous practical implications. For example, it might be possible to engineer the protein into crop plants and eliminate the need for fertilizer!

The first step is to get a copy of a gene that encodes the normal, oxygen-sensitive enzyme (called nitrogenase). The cyanobacterium Anabaena PCC 7120 (nicknamed A7120) has such a gene, called ALL1440. Take a look at the sequence of the genome near the gene by going to CyanoBIKE and using the SEQUENCE-OF function to display the sequence of A7120. Then type ALL1440 into the **Go to** box and press **Enter**.

Your plan is to use Polymerase Chain Reaction (PCR) to amplify the gene in sufficient amounts to clone it… Don't know how PCR works? Try the following sites (you can easily find many more resources on your own):

basic description: http://www.youtube.com/watch?v=eEcy9k_KsDI
more flash: http://www.youtube.com/watch?v=2KoLnIwoZKU

3a. From the genome sequence you displayed, devise primers that will enable you to use Polymerase Chain Reaction (PCR) to amplify the gene, from its start codon to its stop codon, and nothing else. To check to see if your primers work, use the RUN-PCR tool, available in CyanoBIKE by bringing down the RUN-FILE function from the INPUT-OUTPUT menu (see *Analysis Tool* from the September 20 calendar for details), type in the entry box "run-pcr.bike" (press **Enter**), select the SHARED option, and execute the function. This will bring the RUN-PCR function into your FUNCTION menu. Bring down the function from the FUNCTION menu, enter the sequences of your two primers (between "…"), and enter A7120 as the genome. Then execute the function. If your primers are good, you should amplify the gene ALL1440 and nothing else. ***Provide the sequences of the primers and the output of the function.***

3b. Modify your primers so that you get about 10 amplification products, including ALL1440. ***Provide the sequences of the primers and the output of the function.***

4. You are a Crime Scene Investigator investigating a murder so mysterious you don't even know who was murdered. Keen observer that you are, you notice a tiny droplet of blood at the putative crime scene. If there's enough DNA in it, you might be able to amplify a 1000-nucleotide diagnostic region and get important clues as to who the victim is/was. You're worried though -- it's a mighty small drop of blood. Is there enough DNA there? You need just 10 copies of the gene for PCR to have a chance.

4a. How much DNA (in grams) do you need to find in the droplet? ***Provide details of your calculation plus any assumptions you made.***

4b. If, after amplification, you need 100 ng of DNA to detect the region of interest, then how many rounds of PCR amplification will you need? ***Provide details of your calculation plus any assumptions you made.***