Introduction to Molecular Biology
Problem Set: DNA Replication

1. Which of the characteristics at the right are true for both DNA Polymerase and DNA Primase? Which are true for DNA Primase but not DNA Polymerase?

   A. Needs a primer
   B. Makes a strand 5' to 3'
   C. Needs ribonucleotides

   D. Needs a template
   E. Makes a strand 3' to 5'

2. 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.)

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, 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.*