

## Lab 8

# DNA Sequencing

### A. Expt. #15: PCR-Based DNA Sequencing-II

### B. Sequence Analysis of your Clones

### A. Expt. #15: DNA Sequencing of Your Clones-II

Each person did four DNA minipreps in Expt. #11. Each of these minipreps was subjected to PCR and restriction digests and run on an agarose gel in Expt. #14. Today you will be sequencing only those DNAs that contained a cDNA insert of 200 bp or greater. (Some DNAs may contain a very small insert and are therefore not worth being sequenced.) This is the same protocol used in Expt. #10.

- 1. Label 0.2-ml PCR tubes for each of your DNA samples to be sequenced. Label the sides of the tubes near their tops.** (You may also label the tops of the tubes, but any marker label on the top of the tube will likely get rubbed off while in the PCR machine).
- 2. Add 1.6  $\mu$ l of the M13 Forward primer (1 pmol/ $\mu$ l) to each of these tubes.**
- 3. Add 2.4  $\mu$ l of dH<sub>2</sub>O sterile to each tube**
- 4. Add 2  $\mu$ l of miniprep DNA to each tube.**  
**In order for the sequencing reaction to work efficiently, the total volume of the primer and template DNA must be 6  $\mu$ l.**
- 5. After you have added all of the above ingredients to each sequencing reaction tube (each tube should now have a total volume of 6  $\mu$ l), place your tubes in the thermocycler machine.**  
Your samples will be submitted to the DNA Sequencing Facility at UMDNJ for DNA sequencing. The waveform results of your DNA sequences will be posted within one week on the 315 webpage:  
**<http://mbb.rutgers.edu/315.html>**

## B. Sequence Analysis of Your Clones

In Expt. #10 each student sequenced those plasmid DNAs that had inserts of 200 bp or larger. The DNA sequence data obtained from the UMDNJ sequencing facility has been obtained and placed on the server in a folder marked with your group name. We hope that you have produced good sequence data. Although it is gratifying to see sequence data, it is the interpretation of this data that is of the greatest significance. Therefore, after you have finished setting up the other experiments for the day or if you have a few moments between procedures, you can begin your computer-based bioinformatics analysis. Follow the Outline of IMBBR Bioinformatics Steps below to get started on the computer-based searches. In addition, consult Lecture 5-7 for more detailed information on bioinformatic analysis. Do not hesitate to ask an instructor for help.

### Outline of IMBBR Bioinformatics Steps using the DSAP

We recommend that you follow the outline listed below for each of your DNA sequences. Following this order will help you find a match in the most efficient manner and will hopefully reduce the number of searches that will yield non-significant results. Each of these steps is described in more detail in Chapters 5-7 of the lecture notes. Use the 315 DNA Sequence Analysis Program to go through the different steps and record your answers. A link to this program can be found on the course web site. **Make sure all the information is organized in your notebook and that the Clone Log table is completed.**

### Outline of DNA Sequence Analysis Procedure:

1. **Clone Name** – Each clone has a unique name.
2. **What is the size of the insert?**
3. **Are both the M13 Forward and Reverse (???) sequences good?** Analyze the waveforms.
4. **Determine where the insert starts and ends.** Remove vector and bad sequences
5. **Determine if the M13 Forward and Reverse sequences overlap using the BLAST2seq program.**
  - a) If they do not overlap each sequence will analyzed separately
  - b) If the sequences do overlap you will need to build a contig – a composite that combines the two sequences.
6. **Search the *C. elegans* genomic database to identify matches to your sequence.** Determine the possible functions of the protein.

- 7. Search the NCBI database to identify DNA and protein matches to your sequence. In other organisms.**
- 8. Search the literature and protein databases to determine the function of the protein coded by your gene.**
- 9. Determine and verify the protein sequence coded by your gene.**
- 10. Submit the DSAP analysis for verification and publication at NCBI.**
- 11. Use Wormbase, MEDLINE and WormPD to find data and literature on one of your clones for your oral report.**
- 12. Perform Sequence Motif and structural analysis of related proteins.**