**Week 13: Compare Expected DNA to Actual DNA Sequence**

Learning Objectives for DNA Control Element Discovery

*Skills*

* Read DNA chromats using free software.
* Compare two sequences using CLUSTAL omega.

*Cognitive*

* Employ a scientific approach to answering biological questions and test hypotheses.
* Analyze experimental data and reach logical conclusions.
* Describe the big idea of information based on lab experiences.
* Review the information contained within promoters and RBSs.
* Use protocols for molecular biology to clone DNA.
* Interpret Synergy data for fluorescence and optical density.
* Summarize the results from two rounds of experimentation with DNA control elements.

**Pre-Lab**

Before you come to lab

1) Read about DNA chromatograms and how to interpret them.

2) Answer each of these four questions in two sentences or less.

A) How close to the primer is the first readable base when doing Sanger sequencing?

B) How many copies of a plasmid are inside a single *E. coli* cell if the plasmid uses a “pUC” origin of replication?

C) Why would it be reasonable to expect the DNA from all the plasmid copies in a mini-prep to have exactly the same sequences of bases?

D) Why does PCR use two primers but Sanger sequencing only uses one primer?

**Information: Interpret DNA Sequencing Results**

In Lab

3) Download all the sequencing files and find your files. You will want to open the files called name.seq and name.ab1. The dot seq file gives you letters whereas the dot ab1 gives you the chromatograms. You can use the chromats to correct any errors the software made in the .seq file.

4) Use Clustal omega to compare your expected DNA control element sequence with your actual sequences that you had synthesized. You should trim off any Ns at the beginning and the end of your sequence. How well do the two sequences match? If your sequence has any internal N bases that you want to clarify, you can evaluate the chromat (.ab1) yourself and override any Ns based on the quality of the chromat. You have to submit your sequences using “fasta format” (see image below for example).



5) Finalize all your data collection, figure preparation and methods. Each person will write his or her own laboratory report using the guidelines below.

**Guidelines for Lab Reports**

developed by A. Malcolm Campbell at Davidson College

*Writers that use many words for explaining any subject, doth, like the* [*cuttlefish*](https://www.youtube.com/watch?v=GDwOi7HpHtQ)*, hide for the most part in his/her own ink.* Paraphrased from John Ray, British naturalist, who said this in 1692.

 Your lab report should tell a story. Therefore, it needs to have a gripping beginning, as substantial middle and an instructive ending. If you are a great experimentalist but you cannot write well, you will be limited in your success to the degree that your writing is unclear. Lab reports are written in a format with the following sections. Note that the order they appear in your report does not match the order in which they should be written. You have access to the [grading rubric I use to evaluate lab reports](http://www.bio.davidson.edu/113/grade_Rubrics.html).

 **Table 1.** Sections of a lab report as they appear and are written.

|  |  |
| --- | --- |
| **Order of Appearance in Lab Report** | **Order of Writing Each Section** |
| Title/Authors | Results |
| Abstract | Discussion |
| Introduction | Introduction |
| Methods | Methods |
| Results | References |
| Discussion | Abstract |
| References | Title/Authors |

**Results**

 The best place to start writing the results section is with your data. Generate any graphs, tables or other visual summaries of the data before you start writing. The results section should tell the reader what experiments you did without going into most of specific details covered in the methods section. It is OK to say you did triplicates and averaged the results, but you don’t need to tell us in results the concentrations, or volumes *etc*. Your writing should tell the reader what to look at. Describe the results in words and support your words by calling attention to the visualization of your data (Figures and Tables). Remember that the summarized data (not all the raw data) belong in your Results section. Figures must have figure legends that allow a reader to know what experiment you did and any quantitative analysis such as average triplicates +/- standard error of the mean. Figures must be well labeled including axes. Results do NOT convey the significance of your research since that’s what Discussion does.

**Discussion**

 Discussion can be thought of at a triangle with a point at the top and a broad base at the bottom (Figure 1). Start your discussion with the original hypothesis and refer back to figures. This is where you can say what implications your results have for your hypothesis. Was your hypothesis supported or disproven? If it was disproven, try to explain why. If your hypothesis was supported, what experiments might you do next to further challenge your hypothesis? At the end of your Discussion, you want to tie your research back into the big idea or core concept being explored by your experiment. How does this research pertain to bigger questions in biology? You can have figures in Discussion if they are summary in nature, but they should not include data. A figure could be a speculative model explaining your hypothesis, or it could illustrate a core concept with your specific case incorporated visually. It is common to include references in the discussion that support your results and interpretation of your results. These references might be used again in the introduction.

**Figure Legend 1.** Visual representation of a lab report. The three main section of a lab report are intellectually shaped like a piece of hard candy. The introduction starts wide and narrows to your research question and hypothesis. The Results contains the main parts of your report with figures, tables and descriptions of the data. The Discussion begins narrowly with your question and hypothesis and widens by connecting your research to big ideas and core concepts.

**Introduction**

 Now that you have written the Results and Discussion, you are in a better position to introduce your lab report. The introduction can be thought of as an “upside down” triangle with a large base at the beginning and the small point at the bottom (see Figure 1). Start with general statements, core concepts and big ideas, then narrow to explain how your research question and hypothesis fit into these bigger issues. The introduction is the most important part of your story telling. You have to quickly grab the reader’s attention and explain why you did this work and why they should keep reading. Provide the appropriate context by citing relevant scientific publications, including any research papers you used and your textbook.

**Methods**

 Methods must cite your lab manual and any scientific publications you used developing your hypothesis and experimental design. You should be very careful to provide enough information about variations you performed from the generalized method described in the lab manual. When possible, use concentrations of reagents instead of volume. You do not need to tell the reader details that are obvious or irrelevant such as how your wrote labels on the tubes, *etc*. You should supply sufficient information that the reader could reproduce your results. It is common to cite references in Methods such as the lab manual and scientific papers that you used to design your methods. Be sure to describe any quantitative methods you used when crunching the numbers and generating error bars or significant differences.

**References**

 Plagiarism is claiming ideas or wording as your own when in fact you got them from someone else. The only exception is common knowledge such as DNA is the heritable material. Any ideas or directions you found in another source (textbook, scientific paper, web site, video, etc.) must be cited in the Reference section. If you read three papers but only used one of them for your experiment, only cite the one relevant papers but not the other two. To go in the Reference section, a source must be cited in the lab report. Failing to cite the original sources of information implies the information originated with you during your research. That’s why you don’t cite your own figures since those *did* originate from you and your lab group.

**Abstract**

 An abstract is one paragraph composed of 250 words maximum. Like any story, abstracts have a beginning, middle and end. Tell us what you did and why. Tell us what you learned and how. Tell us the significance of your results then stop. An abstract is a synopsis of your entire research project.

**Title and Authors**

 The title should be descriptive but not so jargon-filled that a typical college student would be unable to understand it. Avoid cute titles that are too vague to be informative. Authors should include everyone in your lab group who contributed intellectually to the effort. If you are writing the lab report for an individual grade, your name is listed first. Group lab reports list the names alphabetically. The author list should fit this format: Polly Merase, and Lye Gation. Biology Department, Davidson College. Davidson, NC. 28035.