Preface

A Project-Based Curriculum

The field of biotechnology has evolved from the principles and applications of molecular biology. Much of biotechnology focuses on the production and characterization of biological molecules, particularly DNA, RNA, and protein. However, like most of biological research, biotechnology typically involves a project that requires the integration of many techniques. Most undergraduate laboratory courses typically involve a series of independent and unrelated laboratory activities that provide information and experience in some particular aspect of the subject. This approach, however, fails to convey how these principles and techniques relate to each other.

This manual presents both the principles of molecular biology that make biotechnology possible and the techniques that come from the practical application of some of these principles. You will characterize the enzyme α-amylase in this project-based course. You will study two important aspects of biotechnology using the α-amylase gene and α-amylase protein: (1) the nature of proteins and their action and (2) the principles and methods for cloning and expressing a gene. Thus, both the analysis of protein and the analysis of DNA are integrated within a single project.

The project begins with a study of the characteristics of the protein α-amylase and its function. You will start by examining the enzymatic action and some commercial uses of α-amylase proteins. Since production of higher yields of α-amylase is the goal of this project, it is logical to understand the protein and the significance of its thermostable characteristic before you begin the work of cloning the gene. Once the commercial importance of this enzyme is established, you will proceed to clone the α-amylase gene of *Bacillus licheniformis* and introduce it into *Escherichia coli,* where the gene will be expressed. At the end of the course, you will return to the study of proteins as you quantitate the production of α-amylase by *E. coli.* In addition, you will use computer modeling software to study the three-dimensional structure of DNA and an α-amylase protein. Exercises in Appendix I will introduce you to some bioinformatics techniques that use sequence databases as well as sequence analysis software.

In this curriculum, you will gain experience in many of the techniques that are important in the field of biotechnology as part of an integrated project. You will begin with a problem, a hypothesis, and a goal that will be achieved by the end of the project. In solving the problem and achieving the goal, you will not only learn the principles of DNA, RNA, and protein structure as well as techniques for analysis of DNA and proteins, but you will also integrate this knowledge as you complete the project. This is particularly apparent near the end of the project when you repeat several earlier performed techniques. This serves to reinforce what you have already learned but also allows you to appreciate how techniques can be used in different contexts to give different information, all of which contribute to the larger overall project.

The final aspect of this curriculum that makes it unique is that the project has a potential practical application in the commercial field of biotechnology. You will be asked to evaluate the success of your project not only in terms of the molecular biology but also in terms of practical considerations such as production and cost.

At the beginning of each lab in this manual, there is a Background section that provides sufficient information for you to understand what you are going to do and why. These sections were not designed to be all-inclusive; your instructor may provide additional information (e.g., alternate detection methods, other thermostable DNA polymerases, etc.). Although not explicitly addressed in the manual, your instructor may have you prepare your own solutions or pour your own media plates (recipes and protocols are provided in the companion Instructor's Manual).

Your Laboratory Notebook

It is important that you keep an organized and accurate record of your activities, results, and observations in a notebook. Your laboratory notebook should contain an up-to-the-minute record of your laboratory activities. This means that you write directly in your notebook, not on separate pieces of paper that you plan to copy into your notebook at a later time. Your notebook is not meant to be a work of art, but it should be legible and organized. Each entry in your laboratory notebook should be dated. Each laboratory exercise should be titled and have the following sections:

Introduction. Briefly explain the purpose of the experiment, i.e., what you are doing and why.

Procedure. This is a record of your laboratory activities, i.e., what materials you used and how you did the experiment. A flow chart diagramming the steps of the experiment is helpful. Provide all of the relevant details, such as volumes, concentrations, time, temperature, the order in which samples were loaded on a gel, etc. Include all of your calculations. (Calculations should be done in your notebook and not in this manual or on a scrap of paper.)

Results. Include all of your experimental results. Present your data in tables, drawings, charts, photographs of gels, photocopies of blots, etc. Throughout this manual, directions regarding your results are provided, e.g., "include this table in your notebook" or "label and place this photograph in your notebook."

Data Analysis. Interpret your results. The Data Analysis sections of this manual tell you what to do. For example, plot your data to generate a standard curve, determine which proteins were detected on your Western blot, estimate the size of the DNA fragments detected via Southern hybridization, determine the number of transformants, etc. Again, show your calculations where relevant.

Summary/Conclusions. Concisely state your major conclusions.

A Sample Lab Schedule

To help you integrate the parts of this project, a sample lab schedule is presented as follows. This sample schedule is for a course that meets twice a week for a semester.

SESSION	LAB #	LAB ACTIVITY
1	Lab 1	Streak bacterial cell plates
2	Lab 1	Streak enzyme, saliva, and detergent plates; analyze all plates Micropipetting exercise (Appendix I)
3	Lab 2	Generate maltose standard curve
4	Lab 2	Measure α-amylase activity and quantitate protein
5	Lab 3	Test effects of temperature and pH on α-amylase activity

6	Lab 5A	Prepare samples for SDS-PAGE
7	Lab 4	Protein computer lab
8	Lab 5A	Load and run SDS-polyacrylamide gels; stain one gel with Coomassie Blue
	Lab 5B	Transfer proteins in second gel to membrane
9	Lab 5B	Western blotting
10	Lab 6	DNA computer lab
11	Lab 7	Isolate chromosomal DNA from *Bacillus licheniformis*
12	Lab 7	Check chromosomal DNA on agarose gel
	Lab 8	Assemble PCR reactions to make biotin-labeled α-amylase probe
13	Lab 8	Run PCR products on agarose gel
14	Lab 9A	Assemble restriction cleavage reactions of chromosomal DNA for Southern analysis
15	Lab 9A	Run restriction fragments of chromosomal DNA on agarose gel
	Lab 9B	Denature DNA and set up capillary transfer of DNA to membrane
16	Lab 9B	Affix DNA to membrane
	Lab 9C	Set up Southern hybridization
17	Lab 9C	Detect hybridized DNA
18	Lab 10A	Set up *Hin*d III cleavage of chromosomal DNA
	Lab 10B	Set up *Hin*d III cleavage of plasmid DNA
19	Lab 10A, B	Run cleaved chromosomal DNA and plasmid DNA on agarose gel
	Lab 10C	Assemble ligation reaction
20	Lab 10D	Transformation (spread on agar starch plates)
21	Lab 10E	Primary screening: stain plates, pick colonies, dilute and spread on agar starch plates
22	Lab 10E	Secondary screening: stain plates, pick positive colonies
	Lab 11A	Set up PCR reactions
23	Lab 11A	Run PCR products on agarose gel
	Lab 11B	Set up cultures for mini-prep isolation of plasmid DNA
24	Lab 11B	Isolate plasmid DNA
	Lab 11C	Set up *Bgl* II cleavage reaction (to determine size and orientation of insert)
25	Lab 11C	Run *Bgl* II-cleaved plasmid DNA on agarose gel
	Lab 11C	Set up restriction cleavages of plasmid DNA for mapping
	Lab 11D	Set up cultures for permanent stocks
26	Lab 11C	Run cleaved plasmid DNA on agarose gel
	Lab 11D	Prepare permanent stocks
	Lab 11E	Denature DNA and set up Southern transfer
27	Lab 11E	Affix DNA to membrane and set up Southern hybridization
		Construct plasmid map
28	Lab 11E	Detect hybridized DNA
		Complete plasmid map
29	Lab 12	Inoculate *E. coli* and *Bacillus* cultures
30	Lab 12	Assay enzyme activity of cloned α-amylase gene