Biotechnology

Proteins to PCR

A Course in Strategies and Lab Techniques

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To Dimples, Bristol Bay, and Johnny
D. Burden

To my parents
D. Whitney

Preface

The working environment for pharmaceutical and biotechnology scientists has seen major changes in the early 1990s. No longer are research budgets endless. The federal government, pharmaceutical industry, and academia have all tightened their belts, and downsizing has become a dreaded word in the private sector. Consequently, for those who are entering the field of biotechnology, or for those who are looking to advance their careers, the need to be valuable is never more apparent. This manual is intended to make its readers more valuable in the biotechnology laboratory.

In 1990, the Biotechnology Training Institute was established to meet the ongoing educational needs of scientists. As researchers acquired new responsibilities or wished to simply update skills, we became a source for their training. We have examined the training needs of over a thousand researchers and have used this knowledge to help develop this manual. In addition to techniques and skills, one common weakness of many of our clients is their lack of understanding of the research process, i.e., where does it start and where does it end? Similarly, from our personal experiences in teaching undergraduates, we felt that there is a

lack of teaching material that covers current techniques but also gives a comprehensive view of the research process. We believe this manual addresses both needs.

We do expect students using this manual to have some background in the sciences. Obvious prerequisites should include general biology and chemistry. Course work in organic chemistry would certainly be a plus. However, microbiology and biochemistry are not necessarily prerequisites since this manual could easily accompany such lecture courses. Although this manual does provide background information and in-depth explanations on strategies and techniques, other books with a broader approach to biotechnology may be helpful references. Three good general texts are:

Glick B, Pasternak J (1994): *Molecular Biotechnology: Principles and Applications of Recombinant DNA*. Washington: ASM Press

Old RW, Primrose SB (1989): *Principles of Gene Manipulation: An Introduction to Genetic Engineering*, 4th Ed. Oxford: Blackwell Scientific Publications

Watson J, Tooze J, Kurtz D (1983): *Recombinant DNA: A Short Course*. New York: Scientific American Books

The notes and laboratory exercises in this manual evolved from several courses offered at the Biotechnology Training Institute, including Protein Purification and Characterization, Techniques of Molecular Biology, Introduction to liquid Chromatography, DNA Sequencing, and Applications of the Polymerase Chain Reaction. Tried and tested experiments from each program were assembled around a common theme. These experiments not only teach valuable skills, but also demonstrate the research process used in biotechnology laboratories.

With an understanding of academic budgets, we also wanted to develop instructional materials which were both affordable and flexible. We have endeavored to choose experiments that reflect current methodologies while minimizing cost. We have experienced the frustration of using a prepared laboratory text while not having access to the specific materials required to perform the experiments. Therefore, we have overcome this problem by focusing on a readily available organ-

ism (i.e., *Saccharomyces carlsbergensis*, or brewer's yeast) and one of its corresponding enzymes. The experiments on this organism and enzyme are not limited to the materials suggested and can be easily adapted to the desired technical level and available budget. Similarly, the subsequent cloning experiments suggest that use of particular vectors and strains, but, as indicated, alternative materials can be used to successfully perform the laboratory exercises.

We would like to thank the corporate sponsors of the Biotechnology Training Institute for providing the materials and expertise for the development of our programs, and thus for the materials in this manual. These sponsors include:

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Chapter 1

Introduction to the Biotechnology Laboratory

1.1 OVERVIEW

Introductory biology texts often present the biologist as a naturalist, such as Darwin or Lamark, who through careful observations develops theories and draws conclusions about living organisms. Originally these scientists kept biology and its subtopics as pure areas of study and resisted the multidisciplinary nature of modern science. For instance, in the 1830s Cagniard de Latour and von Liébig argued that fermentation was a biological phenomenon, not chemical. At this time biochemistry had yet to evolve, and the notion that biology and chemistry overlapped had not been fully realized. As the biology of the cell was discovered, several different scientific disciplines found it necessary to communicate in order to answer questions. In 1953, for instance, the structure of DNA was elucidated only after Watson and Crick pooled the information produced by biologists, chemists, and physicists. Since that time, the various scientific disciplines have continued to actively interact. However, it wasn't until the 1970s and 1980s that biology and business wholeheartedly converged to produce today's biotechnology industry.

Although biotechnology has existed for many years (e.g., the baking and brewing industries, and the microbial production of enzymes and vitamins), the explosion in interest and investment seen during the 1980s was unparalleled.

Science has become highly interdisciplinary, and, consequently, scientists require a diverse array of skills to accomplish their research. Where once a biologist might have relied on visual observation of an organism for a behavioral study, today the same research could combine visual observations with molecular techniques. It is commonplace to see a biochemist relying on recombinant proteins for analysis, a molecular biologist on a computer for data analysis, and a microbiologist on a DNA sequence for the detection of a pathogenic microorganism. Unfortunately, these disciplines are often segregated in the classroom, and true integration does not occur until graduate research. Similarly, in industry the narrow focus of research technicians will often prevent their exposure to or participation in duties outside of their immediate job responsibilities.

Our goal is to remove the barriers between scientific disciplines and to demonstrate the diverse techniques and strategies used in the biotechnology laboratory. To accomplish this, you will weave through a series of interrelated experiments designed to mimic the discovery process. The discovery process in this manual will focus on purifying and characterizing a protein and then cloning its associated gene. This manual will not only act as a source of techniques and methods involved in protein and nucleic acid research, but it also will serve as a reference and describe the research process itself.

The initial experiments presented in this first chapter will involve the common techniques of media preparation, handling and observation of yeast and bacteria, and the culturing of yeast for protein production. These exercises will lead directly to subsequent experiments on the purification and characterization of the enzyme α-galactosidase.

1.2 BACKGROUND

The process involved in creating a biotechnological product or service can take many years and involve a battery of scientists and administrators. In this, most researchers are involved in only a small portion of the discovery process. For example, in pharmaceutical research, one research

team might investigate the mechanism of a disease, another could screen therapeutic natural products, a third may clone a gene encoding a valuable product, and a fourth group could be responsible for producing that product. The clinical side of this process also requires a large number of individuals and research groups.

A typical research group is staffed with junior and associate scientists and supervised by senior scientists. In many institutions, including academia, it is common for juniors and associates to be so focused in their research responsibilities that they are oblivious to other elements of the discovery process. These researchers in the trenches, however, are often an untapped resource in regard to examining and planning the research process. Administrators are now realizing that input from all participating individuals helps to avoid problems and results in a better product.

The information and experiments presented in this manual are interrelated and are presented in such a way as to be representative of an actual research project. Individuals who successfully complete the experiments in this manual will not only possess a comprehensive set of skills, but also will have repeated the equivalent of over thirty years of research previously performed by a host of individuals in numerous laboratories. Therefore this manual will serve both to illustrate the classical discovery process in biotechnology and as a source of pertinent information and techniques for individuals interested in biotechnology.

Scope of Biotechnology

Biotechnology is often thought to have arisen in the 1970s as a result of the discoveries of cloning and monoclonal antibodies. Actually biotechnology has been in use for centuries if it is viewed as any technology that employs biological systems (e.g., organisms, cells) or components (e.g., enzymes, antibodies) to achieve an applied goal. Conceptually, both making bread and medical research rely on biotechnology. It is the newsworthy (sensationalistic) and economic aspects involving genetic engineering that typically receive the greatest attention, however, biotechnology has subtly found many applications, some of which are summarized in Table 1.1.

Biotechnology is very broad based. Therefore, to be successful, a scientist will require a working knowledge of several scientific disciplines,

Table 1.1 Examples of Biotechnology

Field	Application	Comment
Agriculture	Crop improvement	Down regulation of genes encoding metabolic enzymes leads to greater shelf life of produce.
	Milk production	Recombinant bovine growth hormone (somatotropin) is fed to dairy cows to increase milk production.
	Insect resistance	Genes encoding naturally occurring insecticides can be transferred from bacteria into plants.
Environmental	Waste degradation	Microbial communities can be used to degrade sewage and industrial wastes.
Pharmaceuticals	Antibody production	Activated B-cells can be cultured in vitro for antibody production. Recombinant antibodies can also be produced by *E. coli*.
	Recombinant vaccines	Genes for selected peptides can be cloned and then used to produce large quantities of pathogen-free vaccines.
Medicine	Gene therapy	Modified DNA can be introduced into gametes and used to replace defective genes.
	Pathogen detection	Molecular probes can be used to rapidly detect species and strains of infecting organisms.
Energy	Alcohol production	Ethanol-producing microbes can be engineered to yield large quantities from low-cost substrates such as cellulose.

such as microbiology, biochemistry, immunology, etc. These skills can be combined to produce the novel products and processes often associated with biotechnology. For instance, it is impossible to design and produce a diagnostic test kit for Hepatitis B without knowledge of immunology, microbiology, and enzymology (Figure 1.1). Realistically, many scientists pool their individual expertise in the development of

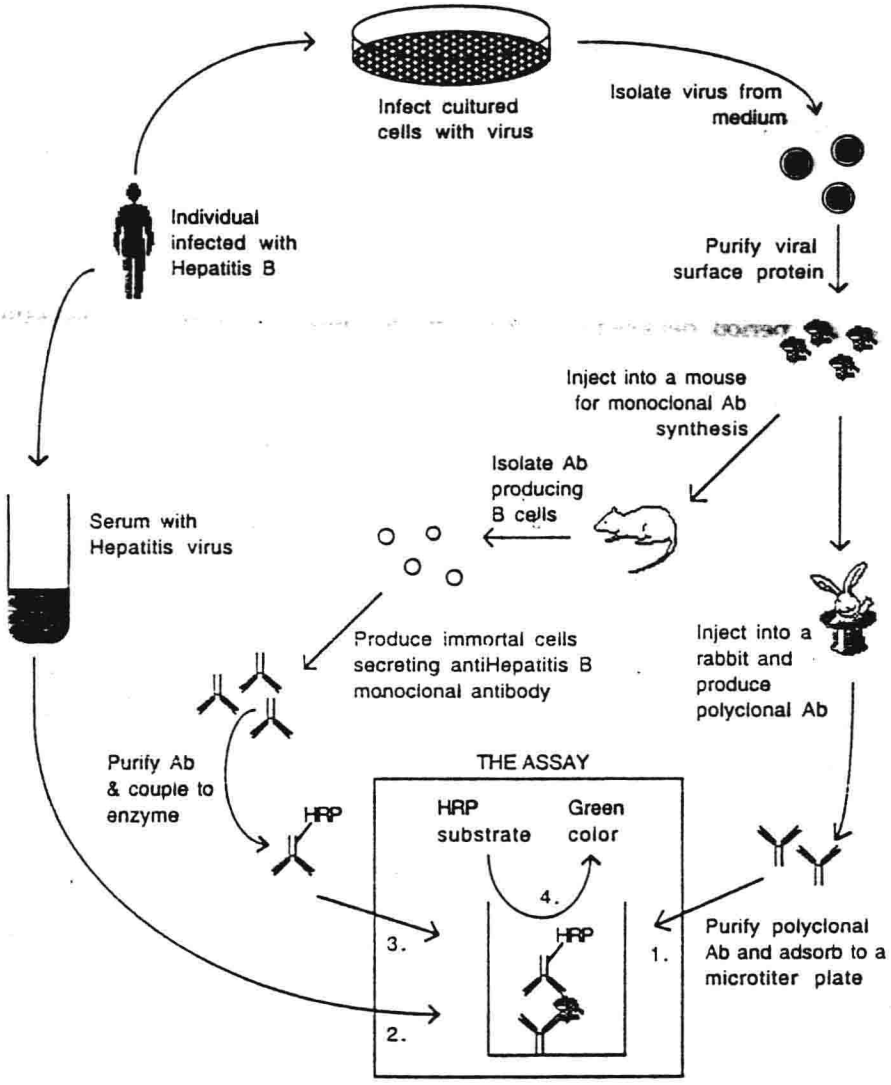

Figure 1.1 Hypothetical schematic of steps and components used in a diagnostic test kit for Hepatitis B. The steps in the assay are (1) adsorbing a polyclonal antibody to a microtiter well; (2) adding serum with Hepatitis B antigen; (3) binding an antiHepatitis B monoclonal antibody-horseradish peroxidase (HRP) enzyme conjugate; and (4) indirectly detecting the Hepatitis B by the addition of a colorimetric HRP substrate. The presence of Hepatitis B is indicated by the development of a green color.