RECOMBINANT DNA TECHNOLOGY AND APPLICATIONS

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Recombinant DNA Technology and Applications

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Preface

Genetic engineering is a multidisciplinary field involving two distinct classes of professionals. The first, involved in cloning and cell culturing, is composed of genetists, molecular biologists, biochemists, and cell biologists; the second, involved in large-scale facility design, bioprocessing, and manufacturing, is composed of technologists and engineers. Each group uses specific terminology and jargon that is rarely integrated together. In addition, matters are often complicated by legal, ethical, regulatory, and policy issues.

This book represents an attempt to bridge the gap between the two groups of professionals. Each chapter presents state-of-the-art knowledge with a strong focus on underlying fundamentals. The initial chapters stress molecular aspects. Then, the emphasis gradually moves to more applied and technical issues. Some of the topics were selected to be at the crossroads between two distant sides (plasmid stability, protein refolding and protein recovery), so as to allow for gradual changeover. The true interdisciplinary nature of this book is reflected by the diverse disciplines it includes: genetic manipulation, microbial physiology, plant tissue culture, plant-microbe interactions, genetics, molecular and cell biology, protein chemistry and recovery, chemical engineering (bioprocessing, plant design, control), laws, government regulations, and policy issues.

There are five parts in this book. Part 1 contains six chapters that review the basic techniques, with some present and perspective applications, essential for cloning cells in microorganisms, mammals, plants, and insects. In Part 2, there are three chapters that discuss various applications of recombinant and other related technologies (such as gene probes and monoclonal antibodies) for medicine. Part 3 includes four chapters devoted to the improvement of product expression in prokaryotes through genetic, phenotypic, and environmental means as well as product recovery and purification which to a certain degree serves as a bridge between two ends, i.e., biological and engineering. Part 4 presents the practical ways of handling recombinant organisms in a real device or plant. Finally, Part 5 provides some "soft" societal issues, which are never as clear as those of science and technology: offering many possible alternatives in social matters, be it regulatory, patent, or policy issues.

In the overall attempt of placing a product in a market, the limiting, bottlenecking steps move typically from scientific to technical to societal issues; thus, this book follows that pattern.

Also, the book provides a coherent picture of the topic of recombinant DNA

technology. The contributors are well-known experts and active scientists in their respective fields. They provide an overall review with an attempt to identify the future direction or the potential for application. The book, however, does not intend to cover all aspects of this proliferating segment of biotechnology.

We are very grateful to all contributors for their diligent work, effort, patience in dealing with editors, and meeting deadlines. Also, the editors acknowledge the effort of the reviewers listed here: Juan A. Asenjo, *University of Reading;* Rakesh K. Bajpai, *University of Missouri;* Wayne M. Barnes, *Washington University;* Donald W. Bowden, *Collaborative Research, Inc.;* Donald A. Cooksey, *University of California;* Bruce E. Dale, *Texas A&M University;* Judy Diaz-Collier, *Monsanto Company;* Malcolm Fraser, *University of Notre Dame;* Kevin Glenn, *Monsanto Company;* W. Fred Hink, *Ohio State University;* Paul Hippenmeyer, *Monsanto Company;* Juan Hong, *University of California;* James B. Kaper, *University of Maryland;* Paul Kiefer, *Monsanto Company;* Dhinakar S. Kompala, *University of Colorado;* Jack H. Ladenson, *Washington University;* Verne A. Luckow, *Monsanto Company;* Victor M. Morales, *National Research Council Canada;* Garry K. Patterson, *University of Missouri;* Donald W. Peterson, *Leydig, Voight & Mayer;* Aleš Prokop, *Washington University;* Janos Scharer, *University of Waterloo;* Richard A. Schoenfeld, *Genzyme Corporation;* Lisa Steiner, *Massachusetts Institute of Technology;* Kathryn C. Zoon, *Food and Drug Administration.*

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Part

1

Cloning Techniques and Applications

Chapter

1

Cloning in Bacteria: Vectors and Techniques

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Introduction

In the past decade there has been a near-explosive increase in both the number of investigators using in vitro gene manipulation and the range of power and usefulness of those techniques. Gene cloning has become much easier since the early days of molecular biology. The variety of restriction and modification en-

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Abbreviations: Ap^R, ampicillin resistance; β-Gal, β-galactosidase; BHR, broad-host-range; bp, base pair(s); CM^R, chloramphenicol resistance; dCTP, deoxyribo-cytosine 5'triphosphate; fl, filamentous; IPTG, isopropyl-β-D-thiogalactopyranoside; MCS, multiple cloning site; RBS, ribosomal binding site; Tc^R, tetracycline resistance; vector 1/2, there are two vectors in the series vector 1 and vector 2.

zymes that are commercially available, as well as kits for hybridization, nucleotide sequence determination, in vitro site directed mutagenesis, λ in vitro packaging, etc., increases each year. Several other tasks required during the cloning process have become routine; examples are plasmid DNA purification, restriction mapping, hybridization or immunodetection, oligonucleotide synthesis, and, up to a certain point, nucleotide sequence determination. That has not, by any means, made molecular cloning less of a challenge; it has only expanded its range of possibilities. There are still many cloning projects that require ingenuity.

The most basic needs for cloning are a vector, a host bacterium, and a selection or screening strategy for the phenotypic expression of the desired gene. A vector is an autonomous replicating DNA molecule into which a desired gene can be introduced and stably maintained in the host. That allows further manipulations and analysis of the gene of interest. The type of vector is important for the success of the cloning, but the key factor is the selection, or screening method, that can be applied to distinguish the clone with the desired gene from the background. The most commonly used screening procedures are hybridization to a probe, immunodetection of the gene product, and detection of the activity of the gene product or change in the expression of a gene in the vector (insertional inactivation or insertional activation). Protein activity can be detected by an enzyme assay or through complementation of a metabolic defect of the host. [For a review on protein detection methods, consult (22).] Very often the choice of the vector depends on the available selection.

There are now several books and laboratory manuals that give step-by-step instructions for cloning (3, 20, 36, 117, 119, 122) or that list cloning vectors with maps and brief descriptions of their uses (79). For an in-depth review on cloning vectors see Rodriguez and Denhardt (85). Another recent review on cloning techniques and analysis of bacterial genes is that of Chater and Hopwood (17). One of the most widely used practical manuals is undoubtedly that of Sambrook, Fritsch, and Maniatis (91). The size of this work has quadrupled since the first edition in 1982, a consequence of the expansion of the field. Nevertheless, the book remains a must in every molecular cloning laboratory.

In spite of the number of recent articles on this subject, the pace of development of cloning techniques and vectors warrants another publication. We will, however, try to avoid duplication of information as much as possible. This chapter will be a wide overview of recent advances with a few specific examples that will direct readers to more detailed publications; these will help them in their quest for the proper vector and technique for a particular cloning project.

Vectors for *Escherichia coli*

Since cloning was developed in *E. coli,* most vectors, as well as techniques, are restricted to this bacterial species. Usually the cloning is performed by ligation of DNA fragments to a vector molecule and transformation of the recombinant plasmid into *E. coli,* and it is in this bacterial species that the desired clones are selected and manipulated (e.g., subcloned, mutated, and sequenced). They can then be introduced into other microbes if necessary.

The introduction of foreign DNA into *E. coli* is not devoid of problems. The