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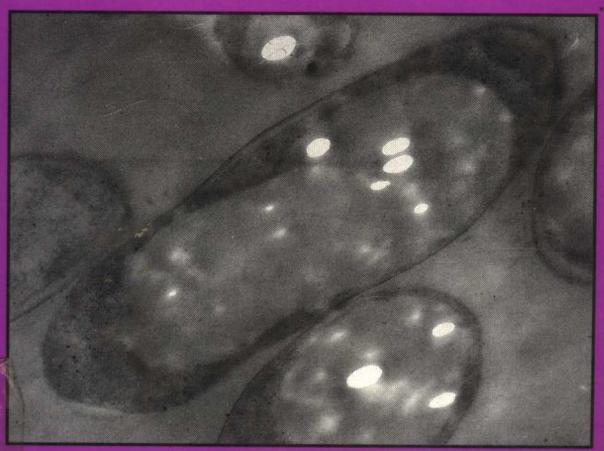
RECOMBINANT PROTEIN PROTOCOLS

Detection and Isolation

Edited by

Rocky S. Tuan



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METHODS IN MOLECULAR BIOLOGY

Recombinant Protein Protocols

Detection and Isolation

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Preface

A major success story of modern molecular biology is the development of technologies to clone and express specific genes. Current applications of recombinant gene products cover a wide spectrum, including gene therapy, production of bioactive pharmaceuticals, synthesis of novel biopolymers, agriculture and animal husbandry, and so on. Inherent in bringing these applications to fruition is the need to design "expression constructs" that will permit the ready and specific detection and isolation of the defined recombinant gene products.

Recombinant Protein Protocols grows out of the need for a laboratory manual on the detection and isolation of recombinantly expressed genes that covers both the background information and the practical laboratory recipes for these analyses. In this book, detailed and contemporary protocols are collected to provide the reader with a wide-ranging number of methodologies to enhance the detection and isolation of their gene product(s) of interest. A large number of molecular tags and labels and their usage are described, including enzymes, ligand-binding moieties, immunodetectable molecules, as well as methods to detect interactive proteins, and gene expression-mediated alterations in cellular activity. Chapters on *in situ* detection of gene expression deal with technologies that are currently being applied to the study of gene function and activity. Highlights of applications for recombinant gene expression technologies are provided to give readers exciting perspectives on the future of such technologies.

Throughout *Recombinant Protein Protocols*, the authors have consistently striven for a balanced presentation of both background information and practical procedures for each of the methodologies treated. The reader is first guided through the necessary supporting background information and then presented with step-by-step specifics for each protocol, including reagents, instrumentation, and other requirements. It is anticipated that this highly practical format, a feature of the *Methods in Molecular Biology* series, will permit the reader to bring new concepts into personal practice in a most efficient manner.

The practice of molecular biology as a means to express recombinant genes continues to gain attention in basic biomedical research, as well as the

biotechnology and pharmaceutical industries. For this reason, it is anticipated that the subjects covered here will continue to be developed, serving as the basis for more sophisticated and efficient methodologies in the future.

The preparation of *Recombinant Protein Protocols* would not have been possible without the outstanding work of the contributing authors, all of whom have been most tolerant of my persistent reminders. Dr. John M. Walker, the mastermind of the *Methods in Molecular Biology* series, was instrumental in initiating and guiding the project. The staff at the Humana Press showed great patience and provided excellent guidance and assistance. My wife, Cecilia, and my newborn son, Chuck, both tolerated my indulgence in the project, and always gave me the necessary emotional support throughout the preparation of the volume. Finally, the excellent secretarial assistance of Margaret Feoli, Susan Lowenstein, and in particular, Lynn Stierle, is gratefully acknowledged.

Rocky S. Tuan

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Contents

I

INTRODUCTION

1

Overview of Experimental Strategies on the Detection and Isolation of Recombinant Proteins and Their Applications

Rocky S. Tuan

1. Introduction

Recent advances in recombinant DNA technology have permitted the direct cloning of DNA fragments (either derived from naturally occurring or artificially designed gene sequences) into various cloning vectors including bacteriophages, plasmids, and viruses. Such recombinant constructs represent the basic reagents of molecular biology. A major application utilizing cloned DNA sequences is the expression of the cloned DNA into a protein product, i.e., the expression of recombinant genes. Because the cloned DNA sequences may be modified or altered, recombinant expression technology thus enables the investigator to "custom-design" the final protein product. Furthermore, most expression vectors are designed to allow the linking of various "tags" to the expressed recombinant protein to facilitate subsequent detection and isolation. This chapter provides a brief overview of the technologies currently employed in "tagging" expressed recombinant proteins and the corresponding detection and isolation methodologies, as well as some of the applications utilizing recombinant gene products.

2. Molecular Tags and Reporters

The basic strategy in "labeling" or "tagging" a cloned sequence is to place either upstream or downstream a translationally in-frame sequence corresponding to a polypeptide domain or protein that exhibits highly active or distinct properties not found in the host cell. In this manner, the recombinant hybrid protein, containing the tag and the desired expressed gene product, may be detected and/or isolated on the basis of the unique properties of the tag. In

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some instances, an additional sequence corresponding to a specific protease cleavage site is inserted between the tag and the cloned sequence, such that treatment of the final recombinant hybrid protein with the appropriate protease produces the desired gene product from the tag. Chapter 2 (Groskreutz and Schenborn) in this book provides further background for the general rationale used in constructing an expression vector.

2.1. Enzymes

Owing to their ability to catalyze specific reactions yielding distinct, detectable products, enzymes are probably the most popular molecular tag for expression of recombinant genes. The most commonly used enzymes include: chloramphenicol acetyltransferase (CAT); firefly luciferase; β-galactosidase; alkaline phosphatase; and β-glucuronidase. Some of the key reasons for selecting these enzymes as functional labels include high signal-to-background ratios of the catalyzed reactions, high stability of enzyme activity, and the high sensitivity for detection. A number of methods are currently in use for the detection of enzyme activity, including standard colorimetric assays, more sensitive fluorescence- or luminescence-based procedures, chromogenic histochemistry, and immunohistochemistry or solution-phase immunoassays such as radioimmunoassay or enzyme-linked immunosorbent assay (ELISA). The specific characteristics of some of these enzymes and their respective detection protocols are presented in detail in a number of chapters in this book (Chapters 3, 4, 5, and 6).

2.2. Ligand-Binding Labels

Another type of molecular interaction that has been exploited to generate detectable activities in recombinant gene expression includes those involving specific, high-affinity ligand binding. In this manner, the recombinant product possesses the ability to interact with a specific ligand, which ideally is not a property of the host cell proteins. Using a labeled ligand, the corresponding recombinant product may be clearly identified. Alternatively, another reagent, either a protein or a chemical (which is itself labeled), may be used to detect the bound ligand, and thus the recombinant protein. In many instances, the ligand may be immobilized onto a solid support, such as chromatography resins and gels, to develop affinity fractionation methods for isolation and purification of the desired recombinant product. Examples of these protocols may be found in a number of chapters in this book, dealing with specific ligand-binding entities such as: maltose-binding protein, which allows purification of the chimeric protein on amylose columns; Protein A, which recognizes the Fc domain of immunoglobulin G; streptavidin, which binds with extremely high affinity and specificity to biotin; and hexahistidine peptide se-

quence, which has high metal affinity, i.e., applicable for affinity purification on nickel-nitrilotriacetate column. These topics are covered in detail in Chapters 9, 10, 11, and 12.

2.3. Expression-Coupled Gene Activation

Another means to detect recombinant gene expression, which has recently gained substantial popularity, is the coupling of recombinant gene expression to the transactivation of another unique gene. This approach, an example of which is the yeast two-hybrid system, is particularly useful for the detection of interacting proteins, and the assay is performed in vivo rather than in vitro, thus permitting the detection of such proteins in their native, biologically active state. The yeast two-hybrid system takes advantage of the fact that many eukaryotic transcription activators are made up of structurally separable and functionally independent domains. For example, the yeast transcriptional activator protein GAL4 contains a DNA-binding domain (DNA-BD), which recognizes a 17 base-pair DNA sequence, and an activation domain (AD). Upon DNA-BD binding to the specific upstream region of GAL4-responsive genes, the AD interacts with other components of the machinery to initiate transcription. Thus, both domains are needed in an interactive manner for specific gene activation to take place. In the popular yeast two-hybrid system, the two GAL4 domains are separately fused to genes encoding proteins that interact with each other, and these recombinant hybrid proteins are expressed in yeast. Interaction of the two-hybrid proteins brings the two GAL4 domains in close enough proximity to form a functional gene activator, resulting in the expression of specific reporter gene(s), thereby rendering the protein interaction, i.e., expression of the desired recombinant protein, phenotypically identifiable. In practice, the target protein gene is ligated to the DNA-BD in the form of an expression vector. The gene of interest, whose activity includes interaction with the target protein, is ligated into an AD vector. The two hybrid plasmids are then cotransformed into specialized yeast reporter strain. Expression of the desired gene thus activates a known GAL4 responsive gene(s) and confers specific phenotype to the host cell, which can be selectively identified. Protocols utilizing the two-hybrid system and its variants are described in several chapters in this book (Chapter 12, 15, and 16).

2.4. Immunospecific Detection

Another type of recombinant label or tag consists of components to which specific antibodies are available. In this manner, immunoassays and immunoaffinity chromatography may be used efficiently to detect and isolate, respectively, the recombinant protein. Momand and Sepehrnia (Chapter 14) illustrate how this principle may be exploited using recombinant p53 as an example, and