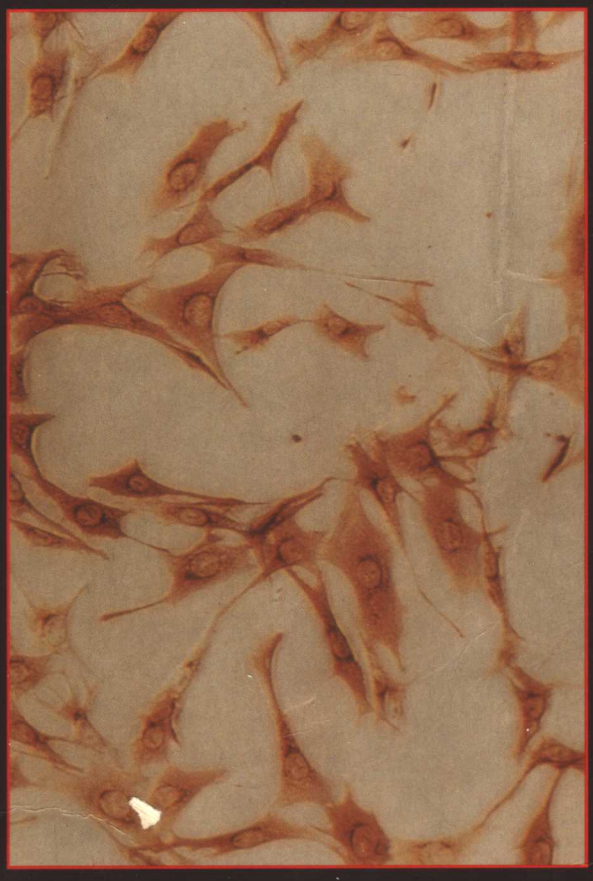
Gene Expression Technology



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Volume 185

Gene Expression Technology

EDITED BY

David V. Goeddel

MOLECULAR BIOLOGY DEPARTMENT
GENENTECH INC.
SOUTH SAN FRANCISCO, CALIFORNIA

COEDITORS

Scott D. Emr

DIVISION OF BIOLOGY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

Dennis J. Henner

CELL GENETICS DEPARTMENT
GENENTECH, INC.
SOUTH SAN FRANCISCO, CALIFORNIA

Larry Gold

DEPARTMENT OF MOLECULAR, CELLULAR
AND DEVELOPMENTAL BIOLOGY
UNIVERSITY OF COLORADO
BOULDER, COLORADO

Arthur D. Levinson

CELL GENETICS DEPARTMENT
GENENTECH, INC.
SOUTH SAN FRANCISCO, CALIFORNIA

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Contributors to Volume 185

Article numbers are in parentheses following the names of contributors.
Affiliations listed are current.

LARS ABRAHMSÉN (13), *Department of Biomolecular Chemistry, Genentech Inc., South San Francisco, California 94080*

PAULINA BALBAS (3), *Departamento de Biologia Molecular, Centro de Investigacion sobre Ingenieria Genética y Biotechnologia, Universidad Nacional Autonoma de Mexico, Cuernavaca, Morelos, Mexico*

CLINTON E. BALLOU (36), *Department of Biochemistry and Molecular Biology, University of California at Berkeley, Berkeley, California 94720*

RAMA M. BELAGAJE (8), *Division of Molecular and Cell Biology, Lilly Research Laboratories, Indianapolis, Indiana 46285*

ANNE BELL (29), *Zymo Genetics Inc., Seattle, Washington 98105*

FRANCISCO BOLIVAR (3), *Departamento de Biologia Molecular, Centro de Investigacion sobre Ingenieria Genética y Biotechnologia, Universidad Nacional Autonoma de Mexico, Cuernavaca, Morelos, Mexico*

DAVID BOTSTEIN (23), *Genentech Inc., South San Francisco, California 94080*

RALPH A. BRADSHAW (33), *Department of Biological Chemistry, College of Medicine, University of California at Irvine, Irvine, California 92717*

ANTHONY J. BRAKE (34), *Chiron Corporation, Emeryville, California 94608*

JAMES R. BROACH (22), *Department of Biology, Princeton University, Princeton, New Jersey 08544*

COLIN R. CAMPBELL (41), *Department of Genetics, University of Illinois College of Medicine, Chicago, Illinois 60612*

DAVID F. CARMICHAEL (16), *Synergen Inc., Boulder, Colorado 80303*

A. CHAMBERS (27), *Department of Biochemistry, University of Oxford, Oxford OX1 3QU, England*

CHUNG NAN CHANG (35), *Protein Design Laboratories, Palo Alto, California 94304*

CHRISTINA Y. CHEN (35, 37), *Department of Cell Genetics, Genentech Inc., South San Francisco, California 94080*

VANESSA CHISHOLM (35, 37), *Department of Cell Genetics, Genentech Inc., South San Francisco, California 94080*

A. MARK CIGAN (30), *Laboratory of Molecular Genetics, National Institutes of Health, Bethesda, Maryland 20892*

WILLIAM CLEVENGER (25), *Department of Molecular Biology, Immunex Corporation, Seattle, Washington 98101*

DORIS COIT (28), *Chiron Corporation, Emeryville, California 94608*

D. COUSENS (27), *Wellcome, Beckenham, Kent, England*

HERMAN A. DE BOER (9), *Gorlaeus Laboratories, University of Leiden, 2300 RA Leiden, The Netherlands*

DANIEL DIMAIO (45), *Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut 06510*

THOMAS F. DONAHUE (30), *Department of Biology, Indiana University, Bloomington, Indiana 47405*

ANDREW J. DORNER (44), *Department of Molecular and Cellular Genetics, Genetics Institute Inc., Cambridge, Massachusetts 02140*

DONALD J. DOWBENKO (35), *Department of Molecular Immunology, Genentech Inc., South San Francisco, California 94080*

JOHN W. DUBENDORFF (6), *Department of Biology, Brookhaven National Laboratory, Upton, New York 11973*

JOHN J. DUNN (6), *Department of Biology, Brookhaven National Laboratory, Upton, New York 11973*

SCOTT D. EMR (21), *Division of Biology, California Institute of Technology, Pasadena, California 91125*

TINA ETCHEVERRY (26), *Department of Fermentation Research, Genentech Inc., South San Francisco, California 94080*

DAVID V. GOEDDEL (1), *Molecular Biology Department, Genentech Inc., South San Francisco, California 94080*

LARRY GOLD (2, 7), *Department of Molecular, Cellular and Developmental Biology, University of Colorado at Boulder, Boulder, Colorado 80309*

SUSAN GOTTESMAN (11), *Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892*

JAY D. GRALLA (4), *Department of Chemistry and Biochemistry and the Molecular Biology Institute, University of California at Los Angeles, West Los Angeles, California 90024*

FRANCIS J. GRANT (29), *Zymo Genetics Inc., Seattle, Washington 98105*

ROBERT HAMILTON (35), *Department of Fermentation Research and Process Development, Genentech Inc., South San Francisco, California 94080*

DENNIS J. HENNER (5, 17, 20), *Department of Genetics, Genentech Inc., South San Francisco, California 94080*

RONALD A. HITZEMAN (35, 37), *Department of Cell Genetics, Genentech Inc., South San Francisco, California 94080*

KATHRYN J. HOFMANN (24), *Department of Virus and Cell Biology, Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania 19486*

JAMES E. HOPPER (24), *Department of Biological Chemistry, The Milton S. Hershey Medical Center, Pennsylvania State University, Hershey, Pennsylvania 17033*

BRUCE H. HORWITZ (45), *Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut 06510*

ANNA S. HUI (9), *Department of Pharmacology and Cell Biophysics, University of Cincinnati, Cincinnati, Ohio 45267*

ELIZABETH W. JONES (31), *Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213*

RANDAL J. KAUFMAN (39, 42, 44), *Department of Molecular and Cellular Genetics, Genetics Institute Inc., Cambridge, Massachusetts 02140*

KIMBERLY KELSAY (29), *Department of Biochemistry, University of British Columbia, Vancouver, British Columbia V6K 157, Canada*

RICHARD L. KENDALL (33), *Department of Biological Chemistry, College of Medicine, University of California at Irvine, Irvine, California 92717*

WAYNE A. KEOWN (41), *Department of Genetics, University of Illinois College of Medicine, Chicago, Illinois 60612*

A. J. KINGSMAN (27), *Department of Biochemistry, University of Oxford, Oxford OX1 3QU, England*

S. M. KINGSMAN (27), *Department of Biochemistry, University of Oxford, Oxford OX1 3QU, England*

TADAHIKO KOHNO (16), *Synergen Inc., Boulder, Colorado 80303*

WILLIAM J. KOHR (35), *Department of Protein Chemistry, Genentech Inc., South San Francisco, California 94080*

MICHAEL KRIEGLER (40), *Cetus Corporation, Emeryville, California 94608*

RAJU S. KUCHERLAPATI (41), *Department of Genetics, University of Illinois College of Medicine, Chicago, Illinois 60612*

STUART F. J. LE GRICE (18), *Department of Infectious Diseases, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106*

ARTHUR D. LEVINSON (38), *Department of Cell Genetics, Genentech Inc., South San Francisco, California 94080*

CHUNG LIU (35), *Department of Protein Chemistry, Genentech Inc., South San Francisco, California 94080*

HONG MA (23), *Division of Biology, California Institute of Technology, Pasadena, California 91125*

VIVIAN L. MACKAY (29), *Zymo Genetics Inc., Seattle, Washington 98105*

JENNIE P. MATHER (43), *Department of Cell Culture Research and Development, Genentech Inc., South San Francisco, California 94080*

TOMAS MOKS (12), *Department of Biochemistry, The Royal Institute of Technology, S-100 44 Stockholm, Sweden*

LAWRENCE M. MYLIN (24), *Department of Biological Chemistry, The Milton S. Hershey Medical Center, Pennsylvania State University, Hershey, Pennsylvania 17033*

VASANTHA NAGARAJAN (19), *Central Research and Development Department, E. I. du Pont de Nemours and Co., Wilmington, Delaware 19880*

BJÖRN NILSSON (13), *Department of Biochemistry and Biotechnology, Royal Institute of Technology, S-100 44 Stockholm, Sweden*

PETER O. OLINS (10), *Biological Sciences Department, Monsanto Company, St. Louis, Missouri 63198*

ROGER PAI (35), *Department of Product Recovery, Genentech Inc., South San Francisco, California 94080*

VIRGINIA L. PRICE (25), *Department of Molecular Biology, Immunex Corporation, Seattle, Washington 98101*

SHAUKAT H. RANGWALA (10), *Biological Sciences Department, Monsanto Company, St. Louis, Missouri 63198*

MARK E. RENZ (35), *Department of Developmental Biology, Genentech Inc., South San Francisco, California 94080*

ALAN B. ROSE (22), *Department of Biology, Princeton University, Princeton, New Jersey 08544*

ALAN H. ROSENBERG (6), *Department of Biology, Brookhaven National Laboratory, Upton, New York 11973*

STEVEN ROSENBERG (28), *Protos Corporation, Emeryville, California 94608*

BRIGITTE E. SCHONER (8), *Division of Molecular and Cell Biology, Lilly Research Laboratories, Indianapolis, Indiana 46285*

RONALD G. SCHONER (8), *Division of Molecular and Cell Biology, Lilly Research Laboratories, Indianapolis, Indiana 46285*

LOREN D. SCHULTZ (24), *Department of Virus and Cell Biology, Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486*

ANDRZEJ Z. SLEDZIEWSKI (29), *Zymo Genetics Inc., Seattle, Washington 98105*

THOMAS J. SILHAVY (15), *Department of Biology, Princeton University, Princeton, New Jersey 08544*

NANCY J. SIMPSON (35, 37), *Department of Cell Genetics, Genentech Inc., South San Francisco, California 94080*

ARJUN SINGH (35), *Department of Cell Genetics, Genentech Inc., South San Francisco, California 94080*

ANDREAS SOMMER (16), *Biogrowth Inc., Richmond, California 94806*

JOAN A. STADER (15), *School of Basic Life Sciences, University of Missouri, Kansas City, Missouri 64110*

C. A. STANWAY (27), *Institute of Molecular Medicine, Headington, Oxford, England*

TIM STEARNS (23), *Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, California 94143*

GARY D. STORMO (7), *Department of Molecular, Cellular and Developmental Biology, University of Colorado at Boulder, Boulder, Colorado 80309*

F. WILLIAM STUDIER (6), *Department of Biology, Brookhaven National Laboratory, Upton, New York 11973*

WAYNE E. TAYLOR (25), *Department of Chemistry and Biochemistry, California State University, Fullerton, California 92634*

PATRICIA TEKAMP-OLSON (28), *Chiron Corporation, Emeryville, California 94608*

ROBERT C. THOMPSON (16), *Synergen Inc., Boulder, Colorado 80301*

MATHIAS UHLÉN (12), *Department of Biochemistry, The Royal Institute of Technology, S-100 44 Stockholm, Sweden*

KEITH D. WILKINSON (32), *Department of Biochemistry, Emory University School of Medicine, Atlanta, Georgia 30322*

M. WILSON (27), *Delta Biotechnology Nottingham, England*

MARLIS WORTHINGTON (25), *Department of Biochemistry, University of Washington, Seattle, Washington 98195*

RYO YAMADA (33), *Department of Biological Chemistry, College of Medicine, University of California at Irvine, Irvine, California 92717*

DANIEL G. YANSURA (5, 14), *Department of Cell Genetics, Genentech Inc., South San Francisco, California 94080*

CARLI YIP (29), *Zymo Genetics Inc., Seattle, Washington 98105*

ELTON T. YOUNG (25), *Department of Biochemistry, University of Washington, Seattle, Washington 98195*

Preface

The articles in this volume were assembled to enable the reader to design effective strategies for the expression of cloned genes and cDNAs. More than a compilation of papers describing the multitude of techniques now available for expressing cloned genes, this volume provides a manual that should prove useful for solving the majority of expression problems one is likely to encounter.

The four major expression systems commonly available to most investigators are stressed: *Escherichia coli, Bacillus subtilis,* yeast, and mammalian cells. Each of these systems has its advantages and disadvantages, details of which are found in Chapter [1] and the strategic overviews for the four major sections of the volume. The papers in each of these sections provide many suggestions on how to proceed if initial expression levels are not sufficient.

DAVID V. GOEDDEL

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Section I

Introduction

[1] Systems for Heterologous Gene Expression

By DAVID V. GOEDDEL

This volume concentrates on the four major expression systems that are commonly available to most investigators: *Escherichia coli, Bacillus subtilis,* yeast, and mammalian cells. Each of these systems has its advantages and disadvantages, some of which I briefly outline in this introductory article.

One expression system which is not covered in this volume is the baculovirus system in insect cells since it has been primarily developed by one group, and a detailed manual describing the system is available from that laboratory.[1] There are also examples in the literature of heterologous gene expression in a variety of other organisms, including *Streptomyces,* a number of fungi, pseudomonads, and others. Although these systems might become relatively more important in the future, we feel their utility has not at present been demonstrated broadly enough to recommend their routine use for heterologous protein production.

In general, the expression of each cDNA or gene presents its own peculiar set of problems that must be overcome to achieve high-level expression. The synthesis of foreign proteins is still largely empirical. There is no set of hard-and-fast rules to follow. In fact, a particular protein is almost as likely to be the exception as it is to follow any set of rules. Keeping this caveat in mind, I will make some generalizations which I hope will aid the reader in selecting an initial expression system.

Types of Proteins to Be Expressed

For the purpose of gene expression in heterologous cells, proteins can be arbitrarily grouped into four broad classes. The first class covers small (less than ~80 amino acids) peptides. These are most easily expressed as fusion proteins, usually in *E. coli.* The second class are polypeptides that are normally secreted proteins (e.g., enzymes, cytokines, hormones) and range in size from about 80 to 500 amino acids. This class of proteins is often the most straightforward to express (in all four systems) and secretion or direct expression should be considered the method of choice. In particular, direct expression in *E. coli* has proved extremely effective for the subset of proteins in the 100–200 amino acid size range. A third class consists of very large (greater than about 500 amino acids) secreted proteins and cell

[1] V. A. Lukow and M. D. Summers, *Bio/Technology* **6,** 47 (1988).

surface receptor proteins. Unless the protein of interest is of microbial origin, one generally has the most success with this class using a mammalian cell expression system. The fourth group encompasses all nonsecreted proteins larger than ~80 amino acids. Many proteins fall into this class; however, relative to secreted proteins, much less work has been directed toward overexpression of proteins in this category. Therefore, the selection of an appropriate expression system should be based on the intended use for the protein (see below).

For What Purpose Is the Expressed Protein Needed?

Probably the most common reason for expressing a new gene or cDNA has been to verify that the correct sequence has, in fact, been isolated. If verification is all that is required, and if the protein being expressed is from a higher eukaryote, then transient expression in mammalian cells is the preferred expression route. Transient expression is not only easy to perform, but also gives answers quickly and has a very high probability of yielding a biologically active protein. Historically, *E. coli* has also been used with great success for identification of mammalian proteins, often yielding biologically active protein, and, nearly always, immunoreactive material. For verification of microbial proteins, the generalization "make yeast proteins in yeast and bacterial proteins in bacteria" can be made.

A relatively large amount of substantially pure protein is desirable for *in vivo* biological experiments or structural determination. In this case, it is worth taking the time and effort to evaluate more than one expression system to find the optimal one for that protein. Bacteria, yeast, and mammalian cells have all been used to produce clinical grade proteins in large amounts.

Escherichia coli can be considered a good bet for the preparation of proteins, or portions thereof, to be used for the generation of antibodies. It is usually possible to express large quantities of antigen rapidly in *E. coli,* even though the overexpressed protein may not be correctly folded. In Section II of this volume there are articles on *E. coli* that outline, in addition to the old and still reliable *trp* vector systems, various novel methods for the direct and fusion expression of polypeptides suitable for antibody preparation.

An increasingly common need for the expression of cloned genes is to obtain mutagenized protein for structure–function experiments (see [45] in this volume). The selection of the appropriate expression system for such studies is usually based on the ease of generating many defined mutants in an assayable form. Therefore, *E. coli* is an obvious first choice.