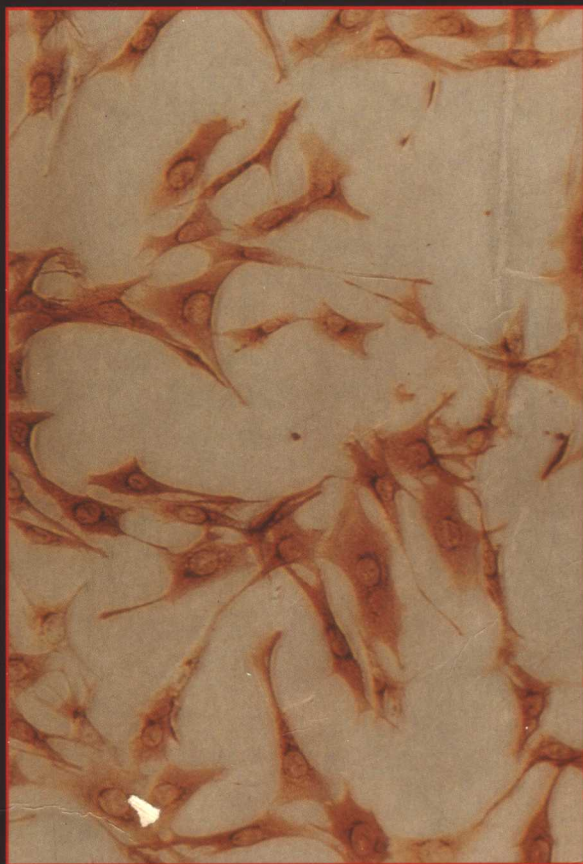


# Gene Expression Technology



edited by **David V. Goeddel**

**Methods in Enzymology**

*Methods in Enzymology*

*Volume 185*

# *Gene Expression Technology*

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## 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. GOEDEL



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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.<sup>1</sup> 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

<sup>1</sup> 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  $\sim 80$  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.