
Preface

Since newly created beings are often perceived as either wholly good or bad, the genetic alteration of living cells impacts directly on a symbolic meaning deeply imbedded in every culture. During the earlier years of gene expression research, technological applications were confined mainly to academic and industrial laboratories, and were perceived as highly beneficial since molecules that were previously unable to be separated or synthesized became accessible as therapeutic agents. Such were the success stories of hormones, antibodies, and vaccines produced in the bacterium *Escherichia coli*.

Originally this bacterium gained fame among humans for being an unwanted host in the intestine, or worse yet, for being occasionally dangerous and pathogenic. However, it was easily identified in contaminated waters during the 19th century, thus becoming a clear indicator of water pollution by human feces. Tamed, cultivated, and easily maintained in laboratories, its fast growth rate and metabolic capacity to adjust to changing environments fascinated the minds of scientists who studied and modeled such complex phenomena as growth, evolution, genetic exchange, infection, survival, adaptation, and further on—gene expression.

Although at the lower end of the complexity scale, this microbe became a very successful model system and a key player in the fantastic revolution kindled by the birth of recombinant DNA technology.

Without the information provided by years of basic research on *E. coli*, the successful application of gene expression would not have taken place with such extraordinary speed. *E. coli* is still unmatched for gene expression work; foreign genes can be introduced into its genome, or that of its plasmids and viruses, with relative ease and predictability; several of its primary metabolic regulatory networks have been unveiled; and its complete genomic DNA sequence is available since 1996. Although it suffers from deficiencies and limitations in the biosynthesis of complex proteins, after 30 years it remains the preferred model system in which to try out new strategies.

Other more complex biological systems for gene expression were developed in parallel to surpass the limitations of *E. coli*. Several bacteria, fungi, plant, and animal cells, as well as complete eukaryotic organisms are today in full use in laboratories and industries throughout the world as protein factories, and gene expression is now understood as a more integrated process. A protein expression system contains at least four general components: (i) the genetic elements necessary for transcription/translation and selection; (ii) in the case of vector-based systems, a suitable replicon: plasmid, virus, bacteriophage, etc.; (iii) a host strain containing the appropriate genetic traits needed to function with the specific expression signals and selection scheme; and (iv) the culturing conditions of the transformed cells or organisms. Bioengineering-related downstream operations are also considered in the original expression system design, so that purification of the product is made easy.

Nowadays, expression systems have evolved well beyond the era of the early high-level “one gene makes one protein” expression schemes. Well-characterized, efficient, and flexible expression vectors for these applications are available from companies, whereas novel approaches by the scientific community point toward a finer and more precise regulation of gene expression in the production of nonprotein molecules via metabolic engineering: chromosomal editing, promoter replacement vectors, and chloroplast transformation/ expression systems are just some of the promising examples of emerging applications of older technologies.

Since understanding and engineering the metabolism of a cell offers an unparalleled flexibility for the use of biological systems as environment-friendly factories for biomolecules, enormous efforts toward this goal are underway. Moreover, gene expression is also developing beyond living cells, with in vitro systems for gene expression and protein folding now evolving in parallel as novel strategies to overcome certain very specific problems.

Public concern about the biosafety of genetically engineered cells and organisms is also impacting the design of molecules and expression strategies. Therefore, chromosomal editing for the removal of undesirable DNA, the use of safer selection schemes and replicons, are some of the actively developing fields of application and research.

But the increasing number and varieties of both applications and transgenic organisms, especially plants, needed to be tested out of containment, an issue that continues to raise serious concerns about their possible environmental impact. To date, these concerns remain only partially answered. There are as yet no clear indicators that transgenic plants impose health risks on people and animals, or even threaten the environment farther than what regular agricultural practices do. Tons of herbicides, insecticides, and fertilizers poured yearly on behalf of productivity have indeed poisoned the environment and originated degenerative diseases in millions of people. Modern biotechnology, through gene expression, offers just such an alternative to help overcome some of the negative consequences that are the inevitable byproducts of industrialization. Transgenic plants may not be the ultimate answer, and long-term monitoring of secondary effects is absolutely necessary. However, the field of research and development is one of the most active and promising in terms of applications.

Transgenic animals are also generally confined to closed research environments, although concerns remain about the potential risk from escapes into the wilderness and also their probable impact on the biological chain and environment.

Scientific proof and experimentation is the best tool humans have developed to help reduce the fear and anxiety raised by the unknown consequences of using recombinant organisms sparingly and without custody in open spaces. Thus, the relevance of *Recombinant Gene Expression* becomes enormous, because it offers the many different views of scientific experts about how best to enhance the biosafety of recombinant organisms, without compromising their efficiency and productivity.

History has demonstrated that significant advances in scientific endeavors are always accomplished faster if, and only if, the work is supported by efficient tools and methodologies. Tools and methods are usually subject to evolution themselves, so their refinement also becomes a very important aspect of scientific progress. Conse-

quently, the relevance of publishing a collection of protocols dealing with the different systems and applications of gene expression becomes enormous, because these are the key creation pathways to which biological scientific growth is anchored.

We are indebted to our authors, all of them experts on a particular expression system. Thanks to this truly interdisciplinary group of international scientists, the present book contains an original collection of protocols for gene expression as well as some overviews and troubleshooting guides for the biological systems addressed. Thus, the main objective of *Recombinant Gene Expression* has been enlarged, since it contains much more than a first quality collection of hands-on protocols. It will capture the attention of a wider variety of experts and experts-to-be, impelled by the curiosity of looking through the experts' eyes at the evolution and advancement of different fields of application.

While organizing a book on such an extensive topic as gene expression, it proved indispensable to pick and choose from the endless variety of strategies, vectors, promoters, and so on, so our coverage is far from complete. Some expression systems were omitted because of size limitations, and even within the areas presented, unavoidably, some research approaches were unevenly treated.

The information we provide in *Recombinant Gene Expression* is organized in sections by biological host: bacteria, fungi, plants and plant cells, and animals and animal cells, presenting a review and several protocol chapters for each. In the accompanying table, the reader will find a comprehensive glimpse of the contents and organization of our book, beyond the subject index. Although every chapter refers to the basic components of an expression system, as mentioned above, only those chapters containing detailed protocols for transformation or selection schemes are quoted in the table. Finally, every single chapter offers the valuable expertise of scientists and their personal views of strategy planning, as well as a variety of approaches that will surely be useful and inspiring to you, the reader.

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Table 1
Recombinant Gene Expression Contents by Chapter

Group	General Info	Vectors					Transformation Transfection Encapsulation	Selection schemes	Growth
		Chromosomal editing/ delivery	Chromosomal promoter replacement	Translational fusions	Extra- chromosomal expression	Chloroplast delivery			
Prokaryotes	1-4, 12,13	7-10	7	5-7	5,6,11,12, 14,15	—	13-15	6,7,12, 13,15	2,3
Fungi	1-3, 16,21	20,22	17	18,19	18,19	—	18-20,22	20,22	2,3
Plants and plant cells	3,23, 24,27	24,26	—	24	—	25	24-26	25,26	3,24
Animal cells and animals	1-3, 28,33	29-32	—	29-31	—	—	29-32	29-32	2,3



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Reviews and Protocols

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