Book Reviews

DNA Cloning 2. A Practical Approach. Expression Systems, 2nd edn.; Edited by D.M. Glover and B. D. Hames, Oxford University Press; Oxford, 1995; xviii+255 pp. £25.00. ISBN 0-19-963478-5

This book represents volume 2 out of 4 revised volumes that cover DNA cloning techniques. The first edition dates a decade back – a rather long period of time in the field of recombinant DNA technology. The present book consists of 7 chapters that each comprehensively describe different aspects of the subject and which may be read independently. Each chapter contains several detailed and useful protocols that describe the procedures thoroughly and mention several pitfalls that may be encountered with the techniques. In spite of the fact that it is a multi-authored book each chapter is presented in a stringent way with only few repetitions among the chapters. Only chapters 2, 3 and 4 seem to overlap to a certain extent as all three chapters describe the expression and purification of proteins from *Escherichia coli*.

Chapter 1 describes the screening of λ expression libraries with antibody and protein probes. Detailed protocols are presented for each screening technique with comments on how to increase the efficiency. The use of labelled protein probes for screening are exemplified with the use of the glutathione *S*-transferase (GST) fusion protein labelled with ^{32}P.

Chapter 2 deals with expression of foreign proteins in *E. coli* using plasmid vectors and with purification of specific polyclonal antibodies. Several prokaryotic expression vectors are described, including plasmids using the T7 promoter, e.g. pT7-7 and pET, and the *tac* promoter, e.g. pGEX and pMAL. This is followed by a good description of expression strategies detailing the putative encountered problems which tend to increase with the size and the toxicity of the protein. Fourteen protocols describe virtually any technique used – from the preparation of vector DNA over screening for recombinant clones to induction of protein expression and purification of untagged proteins by SDS-PAGE. Finally, a section is devoted to the production of polyclonal antibodies emphasizing the advantages obtained by affinity purified antibodies specific to a recombinant protein.

Chapter 3 covers the purification of over-produced proteins from *E. coli* cells. It is emphasized, as anyone working with protein expression will learn, that each protein has its own 'personality' with its own demands for purification procedures. A description of factors that affect the amount and physical properties of the overproduced proteins in *E. coli* follows. This discussion include problems with inclusion body formation, which type of codons that are used, which type of expression vector that is used, effect of growth temperature and type of growth media used. A large section devoted to the purification of proteins follows with emphasis on the use of affinity chromatography and especially with the metal chelate affinity chromatography technique using fusion proteins with six histidines which may be used for purification under both native and denaturing conditions. Other systems that are described include the use of making fusion proteins with glutathione *S*-transferase, the maltose binding protein, staphylococcal protein A, β-galactosidase as well as coupling with various epitopes or biotin. Finally, this chapter contains a section on the processing of fusion proteins and the various possibilities on how to cleave off the fusion part. Also, in vitro re-folding of proteins is covered here. This represents a tremendous problem where a trial and error way still represents a main part of the process.

Chapter 4 is about the production of monoclonal antibodies against proteins expressed in *E. coli*. The techniques for cloning and expression are only briefly described and the authors refer to chapters 2 and 3. Instead this chapter focuses on a detailed description, with several protocols describing the techniques involved, of the production of monoclonal antibodies and the application of these.

Chapter 5 deals with the expression of cloned genes in yeast. Since yeast is a eukaryote it is much more prone to fold foreign eukaryotic proteins than *E. coli* but it is almost as easy to manipulate genetically as the bacteria. Yeast may therefore be the first alternative choice if *E. coli* is unsuccessful. It is stressed that eukaryotic intercellular proteins are often soluble and active in yeast, proteolytic degradation is a minor problem compared with *E. coli*, posttranslational modifications are often carried out although it also has some drawbacks as the yeast system sometimes glycosylate proteins differently. Detailed protocols which described various expression systems and techniques for culturing, transforming and storage of yeast cells follow. Also, different techniques analyzing the synthesis of mRNA and proteins including strategies for problem diagnosis and optimization of the system are given.

Chapter 6 describes the interaction trap which is a two-hybrid system for cloning of cDNAs encoding proteins that interact with a protein with a known coding sequence or for studying the interaction between two known proteins. The general principles of the two-hybrid cloning systems are described. The interaction trap system used to find proteins interacting with a protein fused to LexA, the bait protein, is described in more detail. Plasmids that direct the synthesis of LexA fusion proteins are given together with various tests for the function of the bait protein. A detailed description on how to perform an interactor hunt concludes the chapter.

Chapter 7 covers the baculovirus expression system, a convenient and efficient way to over-produce posttranslationally modified proteins in a eukaryotic cell. The baculoviruses have no known non-arthropod hosts and are therefore considered safe for laboratory use. The most intensively studied is *Autographa californica* (AcMNPV). The biology of the virus is described together with advantages and disadvantages offered by the system. As with many other systems some protein modifications do occur efficiently while others are less efficiently performed. The construction and screening of recombinant viruses have become more efficient with the introduction of *LacZ*-based systems and with the use of linearized genomic DNA. Recombinant viruses may now also be generated in yeast. The techniques of how to culture and infect cells are covered by eight protocols, and a few additional protocols are reserved for the techniques involving the handling of yeast.

All in all this book is very detailed and informative and contains much information with key literature citations of use for any scientist working in the area of heterologous protein expression. The book can thus be highly recommended.

Bent Honoré

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PII S0014-5793(96)00892-7