

Book Reviews

PCR 2. A Practical Approach; Edited by M.J. McPherson, B.D. Hames and G.R. Taylor, Oxford University Press; Oxford, 1995. xxv + 332 pp. £25.00 (pb). ISBN 0-19-963424-6

The Practical Approach Series has by now a very well justified reputation for useful books directed to specific practical methods. The context of reviewing any new book in this series is, therefore, not only whether it is a useful addition to one's bookshelves but is it of the standard and of the quality that we have come to expect from this series. The new book, PCR 2, A Practical Approach, is clearly a worthy new addition to this family.

PCR 2, A Practical Approach, is a compilation of up-to-date applications of PCR. Rather than being a revised version of its predecessor, 'PCR, A Practical Approach', it explores new areas where PCR plays an integral methodological role. It shares with its predecessor an emphasis on the practical applications of the techniques, along with detailed experimental protocols and useful troubleshooting tips. The book is divided into 15 independently written chapters, but the editors have performed a fine job in preventing unnecessary repetition.

The book starts with a comprehensive overview of the important features of the PCR technique. All parameters are examined and guidance is provided to optimise amplifications. It is an excellent and up-to-date summary of present understanding of the PCR process and will benefit all users of the technique. The following two chapters describe the use of non-standard phosphoramidites for primer synthesis and 5' non-isotopic labelling of primers. Both chapters provide detailed methodologies, and will be useful to the growing number of laboratories that synthesise their own primers. Solid phase PCR is covered in the next chapter, with emphasis on the use of this technique in cDNA synthesis. Methods are provided for attachment of specific oligonucleotides to a solid phase, use of these primers in cDNA synthesis and the handling of the cDNA in PCR reactions. This methodology will be of great interest to those researchers that must work with small amounts of RNA, as the coupled cDNA can be used repeatedly in PCR reactions, thereby allowing the generation of solid phase 'cDNA libraries'. Solid phase sequencing of PCR products is covered in Chapter 5, while Chapter 6 describes the cloning of full length cDNA and members of multigene families. The experimental procedures are clearly described, and the chapter would be of value to those undertaking this type of experiment. However, it was surprising that blunt-ended sub-cloning of PCR products was recommended, as it is an experiment that is very often doomed to failure particularly when small amounts of product are available. The advent of TA vectors has made sub-cloning of PCR products easy, and it seemed unfortunate that the authors did not include this procedure. Chapters 7 and 8 detail approaches for the quantitation of mRNA levels. Chapter 7 describes PCR/TGGE (temperature gradient electrophoresis) while Chapter 8 provides information on PCR MIMICS, competitive fragments for use in quantitative PCR. Both chapters provide detailed background information and the rationale behind the methods described. The methods are clear and detailed, useful and informative examples of the applications of the technique are given. These chapters would be of considerable benefit to those interested in quantitative PCR.

Chapter 9 deals with in vitro expression of proteins from PCR products, whereby the product can be amplified, translated and assayed in a single day. The most commonly used in vitro translation systems, including *E. coli* S30, rabbit reticulocyte and wheat germ extracts, are

detailed. Careful consideration is given to the preparation of the PCR products, from promoter to termination signal. Furthermore, attention is drawn to areas where inefficient translation and contamination of the protein may occur. Finally the chapter lists some of the applications of in vitro translated products. The following chapter analyses PCR-based approaches to human genome mapping. Initially screening of YAC libraries is described. To alleviate the time-consuming process of further sub-cloning short, overlapping fragments, a new strategy of vectorette PCR is detailed. The chapter is rich in protocols, but for a novice to the area, background reading on genome mapping would be recommended. Chapter 11 provides a good introduction to the commonly used technique of fingerprinting DNA using arbitrary primers and more recently, the technique applied to RNA. Of interest is the increasingly important area of differential RNA expression. The author is clearly aware of the difficulty that the PCR will preferentially target the abundant RNA populations and that a differential display of poorly expressed messages may be difficult to obtain.

Analysis of known and unknown mutations are detailed in Chapters 12 and 13 respectively. The author describes numerous permutations and combinations on the amplification refractory mutation system (ARMS), including the use of fluorescent primers which has the potential to drastically speed up genetic testing. DGGE, SSCP, and chemical cleavage may be used to detect unknown mutations, and while convincing photographs are presented in the chapter, it is worth commenting that these techniques are not applicable to every situation and may be technically difficult. Chapter 14 highlights the problems of in vivo study of ligand/DNA interactions. Detailed protocols, from preparation of nuclei to linear PCR are useful, while inclusion of a standard DNA sequencing protocol may be unnecessary. Finally, ligation-mediation PCR serves as a very useful alternative to genomic library screening for promoter isolation. Although the author has dealt with many common problems in the troubleshooting section, it is also worth noting that technical problems may also be encountered in the critical step of ligation of the linker molecule.

This contents list gives a flavour to a prospective buyer (presumably, a post-graduate researcher) of the range of methods which are covered in this book. However, it does not carry with it an adequate feeling of the usefulness of the practical methods which are presented in a very clear box format such that it is possible to use them without having read the illuminating additional text. The first chapter includes a section on troubleshooting. One minor quibble is that it would have been very appropriate to continue this style in all of the other chapters. Many of the methods which are described are clearly complex. Things can go wrong and do. It would be a useful addition to the book if it was possible to address such problems on a chapter by chapter basis. However, this is a small point; this is an outstanding, up-to-date and useful book. The authors are to be commended. It will turn out to be a well leafed book in many laboratories. It shows once again the resilience and benefit of this extremely useful series of books.

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