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Book Reviews

Vectors: A Survey of Molecular Cloning Vectors and Their Uses. Edited by R. L. RODRIGUEZ and D. T. DENHARDT. London: Butterworth Scientific. 1987. 578 pages. £40.00 ISBN 0 409 90042 7.

In compiling this excellent book, Ray Rodriguez and David Denhardt (credited on the back cover as 'co-inventor of... pBR322' and 'originator of Denhardt's Solution' respectively), have provided a valuable resource for biotechnologists and other biologists involved in molecular cloning. Here in one state-of-the-art volume can be found a detailed and comprehensive survey of cloning vehicles. Certainly, as a part-time inventor of vectors (though not yet an originator of Solutions), I welcome it as a more convenient source of information than the stack of papers I used to rely on. This is not a work aimed at those unacquainted with the field. Each chapter assumes some familiarity with basic cloning techniques, so it will be most useful to those with a grounding in the uses of cloning vectors, but who wish to increase their knowledge or get up to date. Perhaps an introductory chapter should have been included to make it more accessible to a wider readership. The book is divided into four parts (bacterial, fungal, insect/animal cell and plant cloning vectors) with a total of 27 chapters contributed by suitable experts. The bacterial part, by far the largest at over half the book, is subdivided into four sections covering general, specialized and broad-host-range vectors for Gram negative bacteria, and vectors for Gram positive species. Some may argue there is too little emphasis on eukaryote and especially mammalian vectors, but I think the allocation of space simply underlines the relative maturity of the prokaryotic systems. The same bias is evident in the two-volume *Cloning Vectors. A Laboratory Manual* (eds Pouwels *et al.*, 1985, Amsterdam: Elsevier, with which the present volume compares favourably. The latter treats the underlying biology of the host–vector systems much more coherently, whereas the 1985 work is rather diffuse and is better suited for quick reference.

Inevitably, the first chapter is about the plasmid pBR322, the Model T Ford of plasmid cloning vectors. Despite (or because of) its ten-year age, it is still popular. There is no compelling scientific reason for choosing it as a vector now, but the fact that it is tried and tested, and that it is adequate for many applications, will ensure its continued use. The authors concentrate on pBR322 as the prototype of the many hundreds of its derivatives, which are listed in a comprehensive table. Two points: firstly, in these days of public sequence databases, it seems unnecessary to print the entire pBR322 sequence again (both strands). The map on page 18 is more useful for most purposes. Secondly, I spotted one error in their brief mobilization section: in fact, neither the orientation of *bom*, nor transcription through it, are important for mobilization (Boyd & Sherratt, 1986, MGG, **203**, 496).

Chapter 2 concerns that other main progenitor of cloning vehicles, bacteriophage lambda, and serves as an update on the more comprehensive review by N. E. Murray in *Lambda II* (eds Hendrix *et al.*, 1983, New York: Cold Spring Harbor). Of particular note is the sophisticated Lambda ZAP vector (10 kb capacity). It has a polylinker cloning site flanked by opposing phage T3 and T7 promoters, with partial M13 origins arranged so that straightforward *in vivo* manipulations result in excision of a plasmid containing the cloned insert from the recombinant phage. The resulting plasmid can then be induced to extrude single-stranded DNA (ssDNA) ready for sequencing, obviating all subcloning steps. The T3 and T7 promoters allow maximal transcription of the insert in either orientation.

The blurring of the distinction between phage and plasmid continues as a theme in the next four chapters. Many researchers continue to rely on filamentous phage derived vectors (M13mp8, etc.) as a source of ssDNA for sequencing and site-directed mutagenesis. These chapters describe how, by including the M13 (or similar) origins of replication in conventional plasmid vectors to produce so-called 'phagemids', it is possible to generate ssDNA by superinfection rather than by subcloning from plasmid to M13 vector. Some vectors are described that have two phage origins in opposite orientation, so that selection of the superinfecting phage permits the polarity of the extruded ssDNA to be chosen at will. The section ends with a chapter on cosmids and their uses. A serious omission here is any mention of the lambda replication origin cosmids constructed by Little & Gross (PNAS, 1985, **82**, 3159), which are apparently more stable with large inserts than ColE1-origin cosmids.

Chapters 7–14 describe specialized bacterial cloning vectors. There are vectors for making cloning easier (positive selection vectors, cDNA cloning vectors and vectors for selecting promoters and terminators) and

vectors which facilitate the expression of cloned DNA (vectors with very strong and/or regulatable promoters and adjustable copy number vectors). The choice of strong promoters on vectors is now very wide: as well as the traditional *lac*, *trp*, P_L and P_R promoters, expression vectors are now available with the *tac* (hybrid *trp/lac*) and T7 promoters, and many others. The T7 promoter is active only in the presence of T7 RNA polymerase, so is especially useful where even residual expression of a cloned DNA segment is deleterious to the cell. Also included is a chapter on the rather idiosyncratic suppressor tRNA vectors which are used as recombinational probes *in vivo* to detect specific inserts in lambda libraries of genomic DNA. One of the common aims of cloning experiments is the expression of foreign (especially eukaryotic) proteins in *E. coli*. This can often be achieved most easily by fusion with a native protein such as β-galactosidase. Thus, while gene and protein fusion vectors are covered in various chapters, I feel that a full chapter devoted to their use would have been justified here.

For those concerned with the cloning and expression of DNA in bacterial species other than *E. coli*, the next two sections (four chapters) describe many of the available vectors. The broad-host-range vectors discussed in the first section (for Gram negative bacteria) are based mainly on IncP and IncQ replicons, and it is gratifying to see what a wide range of versatile vectors have been constructed from them for exploring the bacterial world outside the *E. coli* paradigm. The chapter from Kado's group describes the development of more specialized stable plasmid vectors for Rhizobiaceae derived from pTAR, a natural *Agrobacterium* plasmid. The second section deals with vectors for Gram positive species, mainly *B. subtilis*. Development of *B. subtilis* vectors has mirrored that in *E. coli*, so there are now available both phage and plasmid vectors. As most *B. subtilis* plasmid vectors are unstable, phage cloning is said to be the method of choice. Jeff Errington in Chapter 17 describes how the high natural transformability of *B. subtilis* permits cloning techniques such as prophage transformation, that are not feasible in *E. coli*.

The development of transformation systems and the cloning and characterization of chromosomal replication origins (ARS sequences) has allowed the construction of a large range of vectors for fungi, the subject of Part II. Transformants are still obtained if there is no fungal replication origin, but the authors stress the utility of vectors able to replicate autonomously rather than by integration into the host chromosome. Most vectors have naturally been developed for yeast, which has the added advantage that its 2-micron plasmid can also serve as a vector backbone. Almost all are shuttle vectors able to replicate in *E. coli* for ease of manipulation: they therefore generally contain the pBR322 moiety. The introduction of centromere sequences seems to have solved many of the stability problems of the early yeast vectors. For other fungi, vector development is not so advanced: transformation systems have only recently been devised. However, the construction of useful shuttle vectors for both *Neurospora crassa* and *Aspergillus nidulans* is described here, and they offer the hope that molecular sense will soon be made of the wealth of classical genetic data amassed for these species.

Part III deals with insect and other animal cell vectors. For *Drosophila*, vectors are described which make use of the biology of the P-element transposon to allow integration of cloned DNA segments into the germ line of flies. No vectors analogous to the yeast ones capable of stable, autonomous replication in *Drosophila* have yet been constructed. Nevertheless, a plasmid containing a cloned *copia* element which appears to replicate extrachromosomally has been described (Sinclair *et al.*, 1983, Nature, **306**, 198), so in principle such vectors could be made. Another chapter concerns vectors derived from baculovirus, which infects insects such as the charmingly named alfalfa looper. They are expression vectors and make use of the very efficient promoter of the polyhedrin gene, the product of which can constitute 20% of cell protein. These vectors are sure to make a major impact in the field of vaccine development in the next few years. The rest of Part III discusses shuttle vectors derived from viruses for use with mammalian systems. Many such vectors are by nature lytic or integrative, but episomally replicating vectors based on bovine papilloma virus are also described. As Anthony Ridgway states at the end of Chapter 24, the prospects for gene therapy are obvious.

The fourth and final part summarizes vectors for use with plants. Nature has already provided, in the shape of the Ti plasmid, an elegant system for readily inserting DNA from bacteria into plant genomes. Not surprisingly, then, this system has been extensively modified as described here to support the channelling of cloned DNA by mobilization from *E. coli* through *Agrobacterium tumefaciens* and thence into plants. Given the unwieldy 200–250 kilobase size of the Ti plasmids, special tricks have had to be used to introduce foreign DNA into them and hence into plants. Transposition from a smaller vector is one trick; integration by homologous recombination is another. More recently, the observation that mini T-regions can be complemented by Ti plasmid *vir* genes in *trans* has allowed the construction of small, 'binary' vectors which facilitate cloning (chapter 26). The discovery (too recent to appear here) by Buchanan-Wollaston *et al.* (*Nature*, 1987, **328**, 172) that a small IncQ plasmid (RSF1010, 8.7 kb) can also be mobilized into plant cells opens up the possibility that even simpler cloning systems for plants may be devised.

I have a few pedantic complaints about style.

Foremost among these is the format of the references. Firstly, I would have preferred one set of references collated from all the chapters and placed at the end of the book. Secondly, references should include the titles of papers. It is infuriating to have to hunt down a paper before discovering it has only tangential relevance to the topic being pursued. The index also has the quirk of repeating the words 'plasmid' and 'phage' before every example of these, when simple indentation would have sufficed – as indeed it does elsewhere. Each chapter is subdivided into small numbered sections: these might usefully have been collected into a contents listing, in the style of the *Annual Reviews*. But these are minor quibbles. Overall, 'Vectors' merits a place in any library catering for cloners. I am sure that many noteworthy vectors have been omitted, but I believe that most of the current vector concepts are covered. It is, however, a rapidly moving field: I look forward to the next edition, which will need to appear in about five years.

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Maximising Gene Expression. Edited by WILLIAM REZNIKOFF and LARRY GOLD. London: Butterworths. 1987. 375 pages. £65. ISBN 0 409 90027 3.

This book in the Biotechnology Series from Butterworths, which has Julian Davies as general editor, has the practical aim, kept in rather distant view, of helping the biotechnologist to achieve maximal expression of genes of special interest in suitably artificial genetic and environmental backgrounds. The eleven chapters in the book will not make the biotechnologist's task seem easy, but they contain much of interest to geneticists and molecular biologists who are not busy modifying some genome for the genefit of the human race.

In the first two chapters William Reznikoff and William McClure discuss the promoters of *E. coli* and Kevin Struhl examines the comparable elements of yeast, as the best-known representatives of prokaryotes and eukaryotes, and this sets the scene for the later studies. Transcription initiation in *E. coli* occurs at frequencies ranging from one per 5–10 seconds (for rRNA genes) to once per generation (*lacI*) or even less, a difference of near 10000-fold. Analysing this variation has raised many difficult problems, e.g. relating *in vitro* and *in vivo* data and sorting out the so-called tight-binding sites which behave as promoters only *in vitro*, determining the precise 5′ end of each mRNA, analysing the successive chemical steps in initiation, predicting promoter strength from DNA sequence, and so on. The importance of conservation of certain bases in the −10 and −35 regions of the promoter are well documented from comparisons of 112 *E. coli* promoters; and I was disappointed not to find an explanation of the very slow rate of *lacI* gene expression. In this context a comparison of the *E. coli* *lacZ* and *lacI* promoter sequences with those of Klebsiella, recently sequenced by Buvinger & Riley (*J. Bacteriol.* **163**, 858–862 (1985)), should be of interest in view of the very different levels of activity of these two operons.

Yeast genes are not organized in operon-like clusters, and their promoters (if that is the correct word for them) are hardly comparable with those of *E. coli*. They are larger, variable in size, and can be as far as 450 base pairs upstream of the RNA start; and the molecular mechanism of transcription initiation and its regulation must be qualitatively different in yeast. Yeast cells must have many specific transcription factors because upstream elements are required for transcription but can consist of different DNA sequences, can act at long and variable distances upstream and can act even when inverted with respect to the TATA box and the initiation site.

B. Wasylyk discusses promoter elements and *trans*-acting factors of protein coding genes in higher eukaryotes, where the situation is even more complex than in yeast and includes data on SV40 and adenovirus promoters. The promoter region consists of several components: the cap site element at the start site of transcription, the TATA box at around −30, the upstream elements between about −40 and −110, and enhancer elements that are located either farther upstream or even downstream of the initiation site. The TATA box directs initiation to the correct position 30 base pairs downstream, and also affects promoter efficiency. Upstream elements determine efficiency but not accuracy of initiation, and upstream elements are interchangeable between different promoters in some cases. Enhancers appear to form, quantitatively, the most important promoter elements and have no fixed position relative to other promoter elements. Wasylyk discusses current models of their action.

David Kennell examines an old mystery, the mRNA instability in bacteria. In *E. coli* each message decays at a unique rate, with functional half-lives ranging from 30 seconds to more than 8 minutes at 37 °C and rather longer times at lower temperatures. These rates form a marked contrast to the relative stability of many messengers in higher eukaryotes, but are clearly what the bacterium needs to enable it to adapt to rapid environmental changes. One way of increasing gene expression would be to reduce the decay rate of one or a group of messenger RNAs, but this has not been achieved experimentally and no specific enzymes for mRNA degradation have been identified. Kennell has been unable to detect any characteristics of sequenced messengers which could make them more, or less, resistant to the many RNAses present in the cell.