in media supplemented with uracil. All of the protocols listed in these chapters are simple and well presented.

The remainder of the book focuses primarily on production of heterologous proteins. In Chapter 4, Higgins *et al.* describe compact expression vectors that contain a zeocin-resistance cassette as a dominant selectable marker. The subsequent chapters will be helpful for investigators trying to optimize the production of their favourite protein. Romanos *et al.* explain how to generate *Pichia* strains containing multiple copies of a gene of interest; Gleeson *et al.* describe the use of protease-deficient strains for maximizing protein recovery; and Stratton *et al.* summarize strategies for high-cell-density fermentation. These chapters are all well written, although it should be noted that much of the same information can be found in online manuals available at the Invitrogen Web site. A more specialized topic is the glycosylation profiling of proteins secreted by *Pichia*. Glycosylation patterns can influence the activity and antigenicity of secreted proteins, and this important issue is discussed by Cremata *et al.* A chapter on nucleic acid isolation will likely prove less useful because most researchers now employ commercial kits for this purpose. Several additional chapters focus on strategies and results concerning the expression of specific heterologous genes in *Pichia*. While much of this information strikes me as superfluous, some of it might be helpful to workers undertaking a troubleshooting analysis of their own gene expression data.

Finally, two chapters deal with cell-biological topics. Subramani and colleagues have spearheaded the use of *Pichia* for studying peroxisome biogenesis, and they provide a nice overview that includes protocols for immunofluorescence, subcellular fractionation and the generation of temperature-sensitive alleles. The final chapter, from Reiländer and colleagues, gives a method for immunoelectron microscopy of *Pichia* cells.

Like all such publications, *Pichia Protocols* is already outdated in certain respects, and it should be used in conjunction with recent journal articles. A survey of the *Pichia* literature reveals new expression vectors, improved methods for immunofluorescence imaging, and new findings about protein glycosylation and chromosome structure. For those seeking more comprehensive data, another important source is the patent literature. Because *Pichia* was first studied by industrial rather than academic researchers, much of the available information is found not in standard journals or molecular biology databases but, rather, in patent summaries. Two good examples are the sequences of the *Pichia URA3* and *PEP4* genes. It would have been useful if *Pichia Protocols* had included an appendix listing relevant patents. But overall, this book is a first-rate guide that will be an essential reference for anyone who works with *Pichia*.

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Separation helps

Cell Separation, A Practical Approach (Practical Approach Series No. 193)

edited by Derek Fisher, Gillian E. Francis and David Rickwood, Oxford University Press, 1998. £65.00 (267 pages) ISBN 0 19 963580 3

The book describes how to prepare and handle homogeneous suspensions of single cells originating from body fluids or tissue samples. It collects in a single volume a variety of the techniques that previously could be obtained only from separate sources and is a good cookbook for the scientist whose everyday work deals with the cuisine of cell separation. Advanced students specializing in cell biology might find this book useful as well.

The book is organized into seven chapters. Every chapter is written by a different author or group of authors. The benefit of such an approach is that a topic is described by specialists in each field. However, some redundancies and nonuniform use of terms inevitably appear. Therefore, the book can be reviewed from two sides – the quality of general layout and the quality of individual chapters.

The general layout of the book leaves a good impression. The book is essentially a collection of recipes, but each chapter is prefaced by an informative introduction, together with some background material or theory on the methodology that follows. Every chapter has two outline levels that help the reader search for the material of interest. There is an obvious attempt by the editors to smooth the transition from one chapter to the next. A comprehensive list of suppliers and a detailed index conclude the volume. The only drawback in the layout is that sedimentation methods are artificially divided into two chapters: they should be merged to avoid redundancies.

As far as the quality of the individual chapters, I generally found them well written. Chapter 1 gives excellent instructions on preparing single-cell suspensions from different tissues, evaluating cell viability in a suspension and isolating the cells from a suspension. There are a couple of dozen protocols. All of them are self-sufficient; they contain the list of required equipment, describe how to prepare and store the solutions and also list the detailed steps that are often omitted for brevity in original papers. The coverage of protocols for different cell types is impressive. However, I felt that there should be a section on isolating individual blastomeres from early embryos for at least the most popular animal model systems, such as *Xenopus* sp., *Caenorhabditis elegans* and *Brachydanio rerio*.

The second and third chapters are devoted to sedimentation methods. The sections describing the principles of these methods are weak in both chapters. There are in total five formulae: four of them contain either misprints or mistakes. Stock's equation for sedimentation velocity is given three times. In two cases, centrifugal acceleration, g (cm/s^2), is mistakenly described as centrifugal force (dyne). In the third equation, centrifugal acceleration is denoted as $\omega^2 r$ (cm/s^2), and mistakenly described as angular velocity (s^{-1}). The use of three different letters, μ, η (conventional) and N, to denote the same parameter, viscosity of the solvent, is confusing. Section 8 of the third chapter on simulating centrifugal elutriation includes excessive calculations using multiple geometrical parameters of the elutriation container. Without a figure depicting a schematic of the container, the reader

requires an extraordinary imagination to follow-up the calculations. However, when it comes to the recipes, there is less opportunity for criticism. The experimental protocols clearly describe essentially all sedimentation-based techniques, with informative schematics of equipment used.

Chapters 4, 5 and 6 are very well written, and I did not find any drawbacks that deserve mention. I especially like Chapter 5 for its very clear and detailed explanation of the principles of flow cytometry. This chapter can serve perfectly as a first reading on flow cytometry. At the same time, it provides all the information that a user of flow cytometry as a cell-separation tool might need.

The final chapter concentrates on the separation of cells using free-flow electrophoresis. This technique is relatively rarely utilized but can be useful when the cells to be separated differ in their surface charge. I think that the extensive description of the construction of particular electrophoretic instruments could be omitted. Such information does not help to understand the principles and can be easily acquired from the manufacturer's manuals.

To summarize, the book is a comprehensive collection of essentially all the contemporary approaches for cell separation. To the best of my knowledge, an analogue of this book does not exist. Therefore, I am sure that the book will find its place on the bookshelves of most laboratories using cell-separation techniques. It has already taken its place on mine.

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An ABC of tackling ABCs...

ABC Transporters: Biochemical, Cellular, and Molecular Aspects (Methods in Enzymology, Vol. 292)

edited by Suresh V. Ambudkar and Michael M. Gottesman, Academic Press, 1998. $99.95 (853 pages) ISBN 0 12 182193 5

ATP-binding cassette (ABC) proteins comprise one of the largest and most fascinating protein families. ABC proteins have been discovered in all living cells, mediating transmembrane transport of a remarkable variety of substrates, including ions, heavy metals, carbohydrates, anticancer drugs, amino acids, oligopeptides, steroids, glucocorticoids, mycotoxins, antibiotics, eye pigments, phospholipids, bile acids, organic acids, peptide pheromones and even large polypeptides. Substrate transport is fuelled by ATP consumption through their hallmark domain, the so-called ATP-binding cassette or nucleotide-binding domain (NBD), which is found in all known ABC proteins. Intriguingly, ABC proteins can also function as receptors, ion channels, regulators of channels and even membrane-bound proteases. The mechanism(s) by which structurally similar proteins can fulfil such a functional diversity represents an unsolved mystery. The fact that numerous ABC proteins are medically important (i.e. multidrug resistance development in bacteria, fungi, parasites and tumour cells) or implicated in prominent genetic diseases (cystic fibrosis, adrenoleukodystrophy and Zellweger syndrome, Dubin Johnson syndrome, intrahepatic cholestasis, diabetes and hypoglycemia of infancy, macular dystrophy) readily explains the urge for an understanding of the function/mechanism(s) of individual ABC proteins.

This book is the first comprehensive methods collection available to researchers working on ABC proteins. No doubt, it is a great book, and many of us felt that such a book was overdue. Like many other previous *Methods in Enzymology* volumes, it is a nicely put-together collection of step-by-step, easy-to-follow cookbook-style protocols, which include detailed descriptions and many valuable hints to secure experimental success. Sections cover ABC proteins from bacteria, plants, fungi and, of course, mammalian cells. Individual chapters describe heterologous expression systems, studies on membrane topology, structure–function studies, vector constructions, phosphorylation and drug-resistance assays, biochemical purification and reconstitution, just to mention a few keywords. Because some chapters deal with functional orthologues from different organisms, there is some evident methodological redundancy, although this does not harm the overall quality at all. The primary target groups for this book are graduate students and postdocs, as well as undergraduates facing the tantalizing challenge of working on a particular ABC protein. In fact, I believe the book might even help some principal investigators, who have lost touch with benchwork reality, to appreciate how tough experimental work on ABC proteins can be.

The scientific content of several chapters is somewhat biased and so is the list of chapter contributors. For instance, the book is packed with functional studies on mammalian P-glycoproteins and their role in development of multidrug resistance. However, several exciting and important members of the ABC family such as SUR, the MRPs, the TAP antigen transporter or peroxisomal ABC proteins were not allotted adequate attention or space. Most unfortunately, the retinal ABCR implicated in genetic eye diseases, the sister of P-glycoprotein, newly identified mammalian ABCs and functional studies on fungal drug efflux pumps are completely missing. Maybe these slight shortcomings reflect how fast the whole field is progressing, with new exciting discoveries occurring almost on a daily basis. In fact, much of the published information on ABC proteins available today emerged just within the past few years. At least part of this new information might not have been available to the editors at the time the book was put together. These rapid developments, however, with more than one thousand ABC genes known today, demand frequent updates and revisions to keep the book timely and attractive to a wide audience.

The major challenges in ABC protein research are still ahead – for instance, the identification of physiological substrates, and the development of drugs and therapies to cure or overcome diseases associated with mutated ABC genes or their overexpression. Although the book also nicely addresses some potential future strategies and directions, including gene therapy applications, we still fall far short of understanding the molecular mechanism of a single ABC protein. Likewise, a cure for any

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