

Preface

This is a book about methods. You may ask: Why do we need a dedicated book about methods and why should I buy it? We can offer at least two good answers.

The first answer is of a theoretical nature: the method determines the quality of the scientific finding gained in that manner. Only by understanding a method, its strengths and, more importantly, its weaknesses, is it possible to estimate the general applicability of an observation or hypothesis. The development or improvement of a method is therefore a means to expand and improve the "tentative truth" generated by experimental science. Great value has been placed on describing the material critically and illuminatingly to enable the reader to engage with the material and gain a thorough understanding. This is, in our opinion, the most important reason why methods must be offered for classroom study. However, a deep and broad knowledge of methods is just as important for ongoing experimental work as it is for understanding past experiments.

The second answer is the intent – hopefully successful – of this book to make getting to know and understand these methods clear and straightforward in order to make this book an irreplaceable tool for both students and teachers. Our intent results from our conviction, backed by our experience, that today every individual, whether student, teacher, or scientist, is hopelessly overwhelmed by the large number of different techniques currently in use in biological sciences. At the same time, using these techniques is imperative.

We proudly undertook this intellectual enterprise to describe these techniques as completely as possible in an up-to-date manner. To the best of our knowledge, no English language textbook exists that is dedicated to these same goals and with the same level of coverage.

One might wonder why the most apparent reason to publish this book has not been mentioned: namely, using this book to learn, or hope to learn, methods that are needed directly for ongoing experimental work. We wish to make two things clear: This is not a "cook book". This means that after digesting a chapter the reader will not be able to go to his or her laboratory bench and apply what has just been read like a recipe – for that to be possible it will be first necessary for the reader to work through the

literature relevant to the topic covered. The reader should be in a position – at least this is our goal and wish – to optimize his approach through the overview and insights acquired. As for the second point of clarification: This book does not see itself as competition for existing laboratory manuals for diverse techniques, such as protein determination or PCR. The intent is much more to use carefully coordinated and complete descriptions of the methods, using frequent cross-referencing of other chapters, either in the text or in a flanking box, to illustrate the connections between apparently unrelated techniques and to show their mutual dependencies. We believe that the reader will profit from these lessons by gaining a sense of orientation and will understand the relationship between different techniques better, or possibly appreciate them for the first time. We do not wish to conceal the fact that for us, the editors, certain methodical relationships only became clear in the course of working through some of the manuscripts. As such, this book intends to provide coverage at a higher level, more than any single method manual or a simple collection of methods could.

What is the actual content of this book? The book is titled *Bioanalytics*, which indicates that it is about analytical methods in biological sciences. This must be qualified. What are the biological sciences? Is it biochemistry or also molecular genetics, or cell and developmental biology, or even medicine? In any case, molecular biology would be included. This matter gets more complicated when one considers that modern medicine or cell biology are unimaginable without molecular biology.

This book cannot satisfy all the needs of these sciences. In addition, not all analytical methods are contained within it, instead only those that involve biological macromolecules and their modifications. Macromolecules are most often proteins, but also include carbohydrates, lipids, and nucleic acids like DNA and RNA. Special methods for the analysis of small molecular metabolites are also not included. On occasion, we have crossed over the boundaries we have set for ourselves. For example, methods for the preparation of DNA and RNA are presented, simply because they are so closely and necessarily associated with the subsequent analytical techniques. In addition, many techniques, such as electrophoresis or chromatography, can be used at

both analytical and preparative scales. For other techniques it is not easy to distinguish between preparation and analysis if one does not wish to follow the traditional division between the two based solely on the amount of material involved. Is the identification of interaction partners using the two-hybrid system an analytic method, when the final step is based on the labor-intensive construction of the corresponding clones, that is, to say based on a method that, at first, does not have anything to do with investigating the interaction? Similar is the case of site-specific mutation of genes for the investigation of gene function, which first requires the construction (and not the analysis) of the mutated sequences *in vitro*. On the other hand, we intentionally omitted the description of a few techniques that are clearly preparative. The synthesis of oligonucleotides – a clearly preparative technique – and the cloning of DNA were omitted. The latter is, despite being a requirement or goal of a large number of analytical methods, not an analytical method itself. In this case our decision was easy since there are already numerous good introductions and manuals about cloning DNA. In summary, the book describes the analytical methods of protein and nucleic acid (bio)chemistry, molecular biology, and, to a certain degree, modern cytogenetics. In this context, “molecular biology” means those parts of molecular genetics and biochemistry that involve the structure and function of nucleic acids. Methods of (classical) genetics, as well as traditional cell biology, are therefore rarely, if ever, included.

We wish to emphasize that chapters that directly relate to the function of proteins and nucleic acids have been collected into a special section of the book, the “Systematic Analysis of Function.” We have gone along with the shift in paradigm from traditional bioanalytics to holistic analysis approaches. In this section many topics are addressed – even though they are sometimes not entirely mature – which are on the cutting edge of science. We are aware of the fact that this area is subject to rapid change and a few aspects could in the near future, perhaps, appear to be too optimistic or pessimistic. However, we believe that discussion of the most modern techniques and strategies at this point in time covers fascinating aspects and hopefully proves to be inspiring. The increasing availability of DNA and protein sequences of many organisms is, on the one hand, the critical fundament for this systematic function analysis and, on the other hand, makes high-throughput analysis and analysis of the data increasingly important. Information gained from the genome, proteome, and metabolome is compared with *in silico* analysis, which factors in the localization and interaction between biomolecules and unites everything into complex networks. The long-term goal of completely understanding the system can surely only be reached by the incorporation of further areas of expertise that are not yet an accepted component of bioanalytics. Bioanalysts must become, and are becoming, a kind of systems biologists, more interdisciplinary, and more successful in close cooperation together with experts in the fields of informatics, system theory, biotechnology, and cell biology.

Who is this book addressed to? What has already been said provides a hint: Primarily biologists, chemists, pharmacists, physicians, and biophysicists. For some (biologists, chemists) the book will be interesting because it describes methods of their own discipline. For the second group (e.g., pharmacists, physicians, and biophysicists) the book is relevant because they can

find the background and fundamentals for much of the knowledge, which they find in their own discipline. Beyond these groups, this book is dedicated to interested readers who would like to know more about the subject matter.

The material covered presumes that the user has taken at least an introductory course in the fundamentals of biochemistry or molecular genetics/gene technology, ideally both, or is in the process of doing so. We can imagine that this book would be an ideal supplement to such a course. It can and should especially be consulted when involved in experimental activities. This book is intended to be of equal value to students, teachers, and workers in these fields of science.

The organization of the material proved to be one of the most difficult aspects of putting this book together. It is almost impossible to treat the techniques used in such complex fields in the two dimensions paper offers accurately without simultaneously compromising the didactic intentions of the book. We had a choice of two approaches: a more theoretical and intellectually stringent approach or a more practically oriented approach. The theoretical approach would have been to divide the methods exclusively according to type, for example chromatography, electrophoresis, centrifugation, and so on. Under each type of method its use would be divided according to objective and by the differing types of starting materials. This approach is more logical, but harder to comprehend and unrelated to actual practice. The more practical presentation begins with the concrete problem or question and describes the method that answers the question best. This is more intuitive, but inevitably leads to redundancies. A complete deep, “multidimensional” understanding of the material is only possible after the entire book has been absorbed. The approach in this book, for the most part, follows the second, practically oriented, approach. When possible, such as in the section “Protein analysis”, the methods were grouped and presented according to the topic addressed. This includes the fundamentals of instrumental techniques, which is knowledge required for the complete understanding of other sections. We approached the problem of redundancy by cross-referencing the first instance in which a method is described. Sometimes we left redundancies in place for didactic reasons. We leave it to our readers to determine if our choices represent the optimal solution to the problem of structuring the subject matter.

An overview of the presented methods and their relationships can be found on the inside back cover. This flowchart should – particularly for readers new to the topic – illustrate how one can employ the analytical approaches, from splitting open the cells down to the molecular dimensions. In the diagram, the natural turbulences of the flow are deliberately sacrificed for the sake of clarity. Hopefully, the expert reader will forgive us!

At this point we would like to explain a convention in this book, which is not in general use: the use of the terms *in vitro* and *in vivo*. To avoid misunderstandings, we explain here that we use these terms as molecular biologists usually understand them, which means using *in vitro* for “cell free” and *in vivo* for “in living cells” (*in situ* translates literally into “in place” and is used and understood as such). In contrast, pharmacologists and physicians often use the term *in vivo* to refer to experiments in animals and lack suitable terminology to distinguish between experiments conducted in cell culture and those done in test tubes. In cases

