Analysing Gene Expression

A Handbook of Methods: Possibilities and Pitfalls

Edited by
Stefan Lorkowski and Paul Cullen

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Cover Illustration: The molecular structure of a hydrogel-coated surface plasmon resonance protein biochip. A covalently-attached polysaccharide monolayer serves as an immobilisation matrix for receptor molecules and reduces the possibility of non-specific interactions with the surface of the biochip.
The binding of ligands is detected in real-time via modulations in the intensity of a reflected laser beam (Reprinted with kind permission from XanTec bioanalytics GmbH, Münster, Germany.
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Foreword

In the past century, humankind has made truly remarkable progress in understanding the mystery of life, from the discovery of DNA as a basic form of genetic material, the revelation of the chemical and structural nature of genes, and the establishment of a genetic central dogma, to the complete sequencing of the human genome. Although the central genetic dogma applied to a majority of life forms is defined as genetic information flow from DNA, to messenger RNA, then to protein, the complete sequencing of the three billion-letter human genome has shed little light on how precisely such unidirectional information flow of tens of thousands of genes is programmed. If the achievement of complete sequencing of the one-dimensional linear genetic code of the human genome can be compared with man landing on the moon, then the complexity and difficulty in interpreting the genomic instruction into the form of gene expression in a four-dimensional biological context, such as during development and disease, will prove to be a much more challenging and daunting task than that of getting man back from the moon to the earth.

Although classical genetics has been powerful in dissecting molecular diseases affected by the loss of function of a protein encoded by a single gene, such an approach has proved to be less fruitful for the understanding of phenotypes or diseases that are controlled by multiple genes, such as cancer, type 2 diabetes and heart disease. In fact, many of these genes themselves are signalling molecules, each of which controls the expression of a subset of downstream genes. Thus, analysing differential gene expression, or RNA genetics, a concept put forth by the late Ruth Sager, has become one of the most widely practiced strategies for studying the more complicated biological systems. Perhaps one of the earliest success stories of this approach was the discovery of the p53 tumour-suppressor protein in late 1970s as a protein over-expressed when normal cells were infected by DNA tumour viruses. Two-dimensional protein gel electrophoresis later was developed to provide a more complete picture of cellular protein expression. Methodologies that focused on mRNA expression, such as differential screening and subtractive hybridisation, were invented in the early 1980s, which proved to be more comprehensive, sensitive and informative in gene identification than two-dimensional protein gels. The discovery of T-cell receptors by Mark Davis and colleagues, when they compared the differences in mRNA expression between T and B-cells using such strategies,

provided the most beautiful example of gene discovery through the analysis of gene expression. The success of T-cell receptor discovery fuelled a great flood of biomedical research using gene expression analysis as a basic strategy in understanding a wide spectrum of biological systems. Several modern and more sophisticated molecular biological tools for the global analysis of gene expression at mRNA levels were invented in the 1990s. These methods, including differential display (DD), serial analysis of gene expression (SAGE) and DNA microarrays, have led to an explosion in the amount of research in gene expression analysis. The combined Medline hits of DD, SAGE and DNA microarrays have exceeded 6,000! Thereafter, numerous modifications of these technologies, as well as new approaches focusing on the analysis of gene expression at various levels both *in vivo* and *in vitro*, recently have been described.

This timely book entitled "Analysing Gene Expression" edited by the German scientist Dr. Stefan Lorkowski and the Irish physician Dr. Paul Cullen, and published by Wiley-VCH, took years to complete and represents a collective effort of over 200 researchers in the world, many of whom are leaders in the field of gene expression analysis. Unlike many previous books that focus on a single technology, "Analysing Gene Expression" provides a comprehensive description of nearly every technique and methodology ever invented for the analysis of gene expression, making it truly an encyclopaedia on this emerging subject in modern biology. The book includes not only basic background knowledge of gene expression at the levels of transcription, post-transcription, translation and post-translation, but also step-by-step protocols with a balanced and unbiased treatment of each methodology. Thus, whether you are a novice or a veteran in the field, this book will guide you through the jungle of old and new methodologies for gene expression analysis, and allow you to make informed choices as to which method(s) may best fit the type of biological problem under investigation.

For those who have never been there before, it cannot be emphasised enough that no methodology in gene expression analysis is foolproof, and finding a truly differentially expressed gene is only the first important step of a long journey. Undoubtedly, this book has everything you need to know to make that right first step. Ultimately, it will be the functional characterisations of each gene by genetic, cell biological and biochemical methods, that likely will provide the real proof (or disproof) of the relevance of a gene to the biological system under investigation. In the preface to a methodology book on protein purification, Dr. Arthur Kornberg once quoted an admonition of Efraim Racker, who said, "Don't waste clean thinking on dirty enzymes" to illustrate the importance of the good biochemical practices that are at the core of enzymology. A similar doctrine, "Don't waste clear thinking on dirty data", certainly will continue to produce a better quality of science in the field of gene expression analysis in the new millennium.

Peng Liang
Nashville, September 2002

Preface

In February 2001, the draft sequence of the human genome was published. While at the time of writing many of the details still remain to be worked out, broad consensus now exists on the architecture of our genetic makeup. Understandably, therefore, the focus of cutting-edge research is now on functional genomics, *i.e.* the study of gene expression, and of its regulation, at the mRNA and the protein level.

Techniques for studying gene expression have burgeoned. However, as far as we are aware there does not yet exist a single comprehensive work devoted to this topic. This is what inspired us to compile and co-author this book. Our aim was two-fold. First, to provide a compendium of current methods of analysing gene expression with sufficient detail to allow the novice to decide what technique is most suitable for a particular application. Second, to put these different methods into perspective with relation to each other and to highlight the relative advantages and disadvantages associated with each.

We have divided this book into seven chapters, not because of the biblical charm of the number seven, but because we found this to be the most logical way to organise the content. Chapter 1 describes the fundamental biology of gene expression and chapter 2 outlines the tools needed to prepare samples and carry out gene expression analysis. Chapter 3 describes methods of mRNA expression analysis that can be implemented in the normal research setting, while chapter 4 is devoted to high-throughput methods more suitable to the industrial environment. Chapter 5 describes methods for analysing protein expression, chapter 6 is devoted to methods for analysing gene expression *in situ* and in the living organism at the mRNA and protein levels. Finally, chapter 7 rounds off the book by describing currently available bioinformatics approaches and internet databases.

To our knowledge, this work represents the most complete text currently available devoted solely to the topic of analysing gene expression. We have done our best to make it as up-to-date as possible. Most contributions represent the state of the art in March/April 2002. Although the two volumes of this book run to nearly 1,000 pages, it is still not possible for them to provide all the experimental detail needed for the researcher to implement the protocols in his or her laboratory. For that reason, we have made a special effort to comprehensively cite relevant literature, and to ensure that the papers and books cited are as recent as possible.

This is reflected in the large number of citations from 2001 and 2002. Despite our best efforts, it is possible that this book has overlooked important techniques or approaches. We apologize for any egregious gaps, responsibility for which is entirely our own. Wherever possible, we have tried to eliminate redundancy in the text. However, in a compendium of this nature, a degree of repetition is unavoidable, and we ask readers to overlook any particularly irritating examples that still remain.

No scholarly endeavour can today ignore the impact of the internet. In deference to this fact, we have included as much information as possible on currently available internet resources. One of the drawbacks of the world wide web is its ephemerality, and we hope that readers will excuse us if some of the links cited no longer function, or if their content is not as described.

This book would not have been possible without the encouragement and unflagging support of our publishers at Wiley-VCH Verlag. We are particularly grateful to Dr. Hans-Joachim Kraus for hours of helpful discussion and advice with regard to content and scope, and to Hans-Jochen Schmitt for expert advice with regard to the layout of the text. We thank Professor Gerd Assmann of the Institute of Arteriosclerosis Research for providing us with the facilities to carry out this work. Furthermore, we are grateful to our partners, Bernadette Biermann and Susanne Cullen for their support at all stages of the project. We also thank Bernadette Biermann for countless hours of correction and proof-reading. Parts of the work were made possible by a grant from the European Union to Dr. Paul Cullen (grant no. QLG1, 1999-01007). Limitations of space prevent us from naming the funding sources of all contributors, for which we would like to sincerely express our gratitude and that of the respective authors.

Throughout the text, we have referred to researchers in the masculine form only. This is purely a convention to save space and is in no way intended as a slight on our female colleagues.

Finally, we would like to thank all the contributors who made this work possible. We hope that it proves useful to our fellow-scientists in their attempt to navigate the choppy seas of the post-genomic era.

Stefan Lorkowski and Paul Cullen
Münster, September 2002

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