

# Gene Expression Profiling

Methods and Protocols

Second Edition

Edited by

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## Preface

It was with great pleasure that I accepted the invitation to be Editor for this volume of *Methods in Molecular Biology*. This book collates chapters by experts on a wide range of topics relevant to gene expression profiling.

Understanding gene expression and how it changes under normal and pathological conditions is essential to our understanding of the fundamentals of cell biology through to the targeted treatment of disease. This book compiles protocols, written by experts in their respective fields, for a broad range of techniques, currently available and being further developed, for the analysis of gene expression at the DNA, RNA, and protein levels.

In summary, the topics addressed in this volume of *Methods in Molecular Biology* span the field of gene expression from basic to advanced methodologies, including step-by-step protocols which the reader can easily follow, as well as trouble-shooting tips and tricks to help ensure the success of their application. The chapter by Gurvich and Skoblov brings us through the fundamentals of polymerase chain reaction (PCR), extending to multiplex approaches. Rani and O'Driscoll advance on this strong basis to the application of PCR for the analysis of specimens where very limited amounts of starting material exist, i.e., in the extracellular environment. Considering the importance of being able to globally co-analyze all mRNAs transcribed, Mehta outlines microarray methods. Following microarray analysis, researchers often find progression to the stage of being able to correctly analyze their data to be a substantial challenge. For this reason, Mehta and Rani proceed to explain software and tools for the analysis of such datasets. Friel, Crown, and O'Driscoll detail gene expression analysis specifically in relation to cancer cells and circulating tumor cells. As formalin-fixed paraffin-embedded tissue is quite extensively available for gene expression studies, relative to the availability of fresh/frozen tissue, April and Fan have described methods for such studies, using the whole genome DASL assay. Moving into the world of microRNAs (miRNAs), Hennessy and O'Driscoll outline methods for their determination, including procedures which are applicable to the assessment of both intracellular and extracellular miRNAs. The final steps in our journey through profiling of gene expression involve analysis at the protein level. Here, initially considering extracted proteins, Germano and O'Driscoll describe basic, but essential, methods for assessing proteins by Western blotting, while Meleady advances this to more global protein studies that involve 2D gel electrophoresis and mass spectrometry technologies. A powerful advancement for in situ protein evaluation is our ability to construct "user-friendly" arrays representative of cell lines and/or tissues of interest. Experts in this field, Gately, Kerr, and O'Byrne, detail their optimized methods for design, construction, and analysis of such cell/tissue arrays. Basic and advanced immunohistochemistry and immunofluorescence techniques are clearly described by Katiakreddy and O'Sullivan, while Hanrahan, Harris, and Egan detail laser scanning confocal microscopy in such a way as to make it less daunting to consider, when analysis and co-analysis of proteins in situ is desired. Rani, O'Brien, Kelleher, Corcoran, Germano, Radomski, Crown, and O'Driscoll describe procedures for the isolation of exosomes and their subsequent gene expression analysis as potential representative of the cells from which they have been secreted.

Finally, Buckley, Davies, and Ehrhardt take us through step-by-step approaches to the successful application of atomic force microscopy and high-content analysis for analyzing expression of particular genes of interest.

I hope that this collection of clearly described and illustrated chapters will be helpful to researchers in academia, in hospitals, and in industry who are interested in applying techniques, whether they be very basic or very advanced, for the analysis of gene expression. I also hope this will be of use to those who want to review progress in this very exciting, evolving field. Mostly I hope it will contribute, in some way, to a better understanding of gene expression changes that occur under normal and pathological conditions which can advance progress toward better treatments for those suffering from disease.

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