Next-Generation MicroRNA Expression Profiling Technology

Methods and Protocols

Edited by

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Preface

The rapid pace of microRNA (miRNA) research continues to drive the advances of techniques for miRNA expression profiling. However, several unique attributes of miRNAs, including their small size, which limits the choice of probe selection, huge dynamic range of expression level, lack of polyadenylated tails, significant sequence homology among family members, and tendency to cross-hybridize to their mRNA targets with imperfect sequence homology, have made them challenging to quantify. To meet all these challenges, innovative technologies that are more sensitive, specific, quantitative, and that are compatible with a wide range of biospecimens have been developed during the past few years.

In this volume of the Methods in Molecular Biology series, we assembled a broad spectrum of methods and protocols that cover most of the next-generation miRNA expression profiling technologies. Two introductory chapters serve as references for the context of the more specialized chapters that follow. Chapter 1 (Gommans and Berezikov) provides a general overview to the miRNA field, such as miRNA biogenesis and regulation in human development and diseases, and Chapter 2 (Aldridge and Hadfield) provides a summary of most of the widely used miRNA assay technologies as well as insightful cross-platform comparisons.

We also include comprehensive coverage of methodologies that have been developed for miRNA profiling, including (1) quantitative PCR using either a stem-loop RT-PCR method (Chapter 3: Hurley et al.) or a Poly(T) adaptor RT-PCR approach (Chapter 4: Shi et al.), (2) in situ hybridization (Chapter 5: Nielsen), and (3) microarray analysis with a variety of microarray platforms, such as Agilent (Chapter 6: Andrade and Fulmer-Smentek), Illumina (Chapter 7: Chen et al.), Affymetrix (Chapter 8: Dee and Getts), Luminex (Chapter 9: Sorensen), Febit (Chapter 10: Beier et al.), and LC Sciences (Chapter 11: Zhou et al.). All these techniques allow high-throughput measurement of well-annotated miRNAs.

The past few years have witnessed the rapid development of next-generation sequencing technology and fast adoption of the technology for miRNA research. This area is covered by several excellent chapters that involve the Illumina miRNA-Seq platform (Chapter 12: Luo), Roche 454 GS FLX technology (Chapter 13: Soares et al.), Life Technologies SOLiD platform (Chapter 14: Linsen and Cuppen), and Helicos single-molecule sequencing technology (Chapter 15: Kapranov et al.). The sequencing approach provides several technical advantages over microarrays. It offers (1) more comprehensive coverage and de novo discovery potential, (2) single base specificity, and (3) better detection sensitivity and dynamic range.

In addition to hands-on “wet-lab” protocols, this volume also covers the use of miRNA databases, which deal with the annotation and discovery of miRNAs and other noncoding RNAs (Chapter 16: Yang and Qu), and the function of miRNAs in human diseases and biological processes (Chapter 17: Ruepp et al.), as well as data normalization methods (Chapter 18: D’haene et al.), next-generation sequencing data analysis (Chapter 19: Gunaratne et al.), and integrated miRNA expression analysis and target prediction (Chapter 20: Ritchie and Rasko).
Furthermore, specialized applications, such as cancer studies (Chapter 21: Zhong et al.) and miRNA-based noninvasive biomarker development (Chapter 22: Debey-Pascher et al.), are also addressed.

The content within this book is intended for students, researchers, and scientists in the field, at both the beginner and advanced levels, and contains sufficiently detailed protocols, particularly pointers, that will assist with troubleshooting. While each of the methods has some technical limitations, many of them have been used successfully in broad scientific researches. The choice of the method mainly depends on the users' desired application.

Finally, I would like to thank all the authors for their outstanding contributions to this timely developed protocol book. I would also like to thank Dr. Craig April for his assistance with the preparation of the chapters, as well as Professor John Walker, the Methods in Molecular Biology series editor, and David Casey at Humana Press. I truly hope this book will help accelerate the expression analysis of miRNA and expand our understanding of the biological functions of miRNA in different species and human diseases.

San Diego, CA, USA

Jian-Bing Fan
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