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METHODS IN MOLECULAR BIOLOGY™

Microarrays

Volume 1: Synthesis Methods

SECOND EDITION

Edited by

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Dedication

To my parents and gurus.

Preface

To meet the emerging needs of genomics, proteomics, and the other omics, microarrays have become unique and important tools for high-throughput analysis of biomolecules. Microarray technology provides a highly sensitive and precise technique for obtaining information from biological samples. It can simultaneously handle a large number of analytes that may be processed rapidly. Scientists are applying microarray technology to understand gene expression, to analyze mutations and single-nucleotide polymorphisms, to sequence genes, and to study antibody–antigen interactions, aptamers, carbohydrates, and cell functions, among many other research subjects.

The objective of *Microarrays* is to enable the researcher to design and fabricate arrays and binding studies with biological analytes. An additional goal is to provide the reader with a broader description of microarray technology tools and their potential applications. In this edition, *Microarrays* is divided in two parts: *Volume 1* deals with methods for preparation of microarrays, and *Volume 2* with applications and data analysis. Various methods and applications of microarrays are described and accompanied by exemplary protocols. *Volume 2* also covers topics related to bioinformatics, an important aspect of microarray technologies because of the enormous amount of data coming out of microarray experiments. Together, the two volumes provide useful information to the novice and expert alike.

From this point onward, I will discuss the contents of *Volume 1: Synthesis Methods*. For readers just entering the array technology field, as well as those who are well versed, the history of microarray technology from its conception is covered in the first chapter. Surface activation chemistries and various types of matrices involved in the synthesis of microarrays are summarized in Chapters 2 and 3. As the major objective of this volume is to provide detailed synthesis methods for constructing microarrays, so the emphasis of the remaining chapters is on methods and protocols. I tried to include various types of protocols. Some may look very similar, but in fact each protocol has a unique utility based on the research problem or individual interests. Chapter 4 details array optimization processes based on numerous factors, for example, the printing quality, spot morphology, and quantification of hybridized target. Chapter 5 presents array-based comparative genomic hybridization (array CGH) and includes procedures for making bacterial artificial chromosome

DNA arrays. Chapter 6 describes the 60-mer oligonucleotide probes immobilized on coated glass slides to study the effect of target concentration, retention, signal linearity, and properties of fluophores in quantitative gene expression measurements. The array production method using premodified DNA can be directly applied to construction of oligo or cDNA arrays. Such arrays can be used for detection of chromosomal abnormalities in complex genomes. Chapter 7 highlights the use of unique bifunctional reagents, NTMTA and NTPAC for building glass and plastic biochips. Chapter 8 explains the use of sensitive reagents for the determination of the functional group density in the microarray system by spectrophotometric methods. Chapter 9 illustrates the synthesis of high-density arrays using a digital microarray synthesis platform. The use of long optimized oligonucleotide probes (150-mers) for high and specific signal intensity for the measurement of gene expression is described in Chapter 10. In addition to sequence and probe length, the importance of other parameters, such as the surface of the glass slide, linkers/spacers, and the conditions for hybridization are also highlighted in Chapter 10. Chapter 11 deals with *in situ* synthesized oligoarrays using the Southern Array Maker (SAM) synthesizer and standard phosphoramidite chemistry. The array probes, including cystic fibrosis, were synthesized onto the flat surface of aminated polypropylene. The printing and use of the synthetic oligonucleotide probes for the detection of multiplex ligation-dependent probe amplification products is explained in Chapter 12. The synthesis and the use of grafted pyrrole oligonucleotide probes are demonstrated in Chapter 13. Chapter 14 deals with the optimization of hybridization conditions for *in situ* synthesized oligoarrays on plastic. Chapters 15 and 16 are devoted for the synthesis of peptide arrays. Creation of protein microarrays in microplate is described in Chapter 17. Arrays of the captured monoclonal antibodies corresponding to specific interleukins are printed down onto the bottom of the wells. A Biomek[®] 2000 workstation equipped with a high-density replicating tool is used for printing the low-density arrays. For higher density arrays, a microarrayer system (BioDot, Inc.) is employed. Printing the protein arrays onto specially polymer-coated glass slide while maintaining the activity and structure of the protein is described in Chapter 18. Chapter 19 focuses on the printing of cell microarrays for the functional exploration of genomes. Chapter 20 is related to suspension arrays. The oligo-coated microparticles are hybridized with the target molecule. The protocol for quantification of oligohybridization complex is analyzed by a europium (111) detection system. Glyco-bead array for calculating the sugar-binding lectins is described in Chapter 21. As we all are aware that array technology is moving forward from micro to nano, Chapter 22 describes this emerging technology.

The chapter highlights the utility of nanoarrays, particularly the analysis of nanoarrays by using label-free nucleic acids and proteins and others.

I believe this volume, *Synthesis Methods*, will provide valuable information to scientists at all levels, from the novice to those intimately familiar with array technology. I would like to thank all the contributing authors for providing manuscripts. My thanks are also due to colleagues for their help in completing this work. I thank John Walker for editorial guidance and the staff of Humana Press for making it possible to include large body of available microarray technologies in this volume. Finally, my thanks to my family, especially to my sweet wife Sushma Rampal, for providing all sorts of incentives to complete this project successfully.

Jang B. Rampal

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