MICROARRAYS METHODS AND APPLICATIONS

Nuts & Bolts

Edited by

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Summary of chapters

Chapter 1: Microarray Technologies-An Introduction

The chapter provides a brief introduction to the field of microarray technology. It traces the evolution of array technology, compares DNA microarrays and genechips and introduces promotor arrays. Chapter 1 is intended as a reading material for students and researchers with different scientific backgrounds who are just entering the exciting field of microarray research and applications.

Chapter 2: Laboratory Automation For Microarray Experimentation

Microarray experimentation requires automation for creating and for scanning the arrays. Both operations have two characteristics that demand automation: a large number of small features and very tight tolerances. These characteristics and the demand to make orderly arrays to be able to track features and data preclude manual array creation and data collection. The author discusses laboratory automation that can greatly aid microarray experimentation namely automated hybridization, sample preparation, and probe synthesis or preparation.

Chapter 3: DNA Microarrays and The Core Facility

Microarray fabrication, target preparation, array hybridization, data acquisition and data analysis require expensive instrumentation and reagents, and a highly skilled team of individuals who are experts in the various components of the overall procedure. Microarray technology in many institutes is made accessible to investigators through common CORE facilities, which in addition to providing conventional microarray service, provide essential platforms for advancing new technologies. In chapter 3 the authors outline the various issues commonly encountered by researchers when setting up a microarray facility for centralized research and development where higher throughput is required. There are three main components to the process, microarray fabrication, fluorescent target preparation and hybridization and finally data collection, management and analysis. The authors discuss the implementation of standardized working protocols for expression analysis in the core facility setting.

Chapter 4: Resonance Light Scattering Nano Particles as Labels for Ultra-sensitive Detection of DNA, Protein, Tissue and other Types of Microarrays

The need for ultra-sensitive reporter labels has been exacerbated by the development of microarray technology where the amount of material per microspot is even lower than in standard formats due to the small microspot size (typically less than 250 micron diameter). In chapter 4 the authors present a new ultra-sensitive method of detection which uses nanosized resonance light scattering (RLS) particles as light scattering labels with the ability to detect very small analyte quantities. The sensitivity of this signal generation and detection technology derives from the high light scattering power of individual gold and silver particles, which produce signals equivalent to that of about 500,000 fluorescein molecules (for 60 nm diameter gold particles). The technology is further characterized by its ease of use. Thus, the spots in a microarray that have been treated with RLS Particles can be detected by eye using a small magnifier and a simple filament lamp illuminator.

Chapter 5: How To Evaluate A Microarray Scanner

This chapter examines how the researcher can obtain the most reliable performance from a microarray scanner. After conducting extensive studies on a variety of commercial scanners a technology for calibration was developed by the authors, which uses a scanner calibration and validation slide. These tools can be used to assist in the selection and evaluation of a microarray scanner.

Chapter 6: Genome-Scale mRNA Profiling in Agricultural Genomics

Genome-wide RNA profiling is a very powerful and booming technology in plant genomics. The availability of the plant genome sequences, Arabidopsis and rice, and large EST databases from various model plants and crops has quickly transformed array-based transcriptome characterization into an important tool for agricultural genomics and biotechnology. In chapter 6 the author reviews recent advances in this field.

Chapter 7: Microarray Technologies in Target Validation and Drug Discovery

The drug discovery process is labor intensive, costly, and more often than not results in failure. It is currently estimated that only one in five chemical entities that progress as far as human clinical testing actually make it to market. The pharmaceutical industry is constantly looking towards emerging tech-

nologies that can improve the efficiency and success rate of the drug discovery process. Genetic techniques, including microarray technologies have provided a means by which geneticists, biologists, and pharmacologists can bridge the gap between gene sequence and function. In chapter 7 the author explores these approaches in the context of drug discovery, focusing mainly on their impact upon target validation.

Chapter 8: Protein Arrays and Biochips: Applications in Proteomics

DNA microarrays are powerful tools for gene expression profiling on a genomic scale. However, they are limited to the detection of gene transcription and often mRNA abundance in a cell correlates poorly with the amount of protein synthesized. The challenge in recent years has been to develop high-throughput approaches to facilitate systematic protein analysis in biological samples, to map functional interactions between proteins on a global scale, and place them in a biological context. In chapter 8 the authors review the development of protein array platforms from low-density patches on filter membranes to high grid densities on miniaturized chips.

Chapter 9: Spanscript™: A Method for Rapidly Deriving Non-Redundant 3′ cDNA Libraries Microarray Production

Central to the utility of gene expression profiling on the DNA microarray platform is the availability of gene-specific probes for microarray production. Gene specific oligonucleotide-probe design relies on the availability of sequence data, thus limiting oligonucleotide microarrays to organisms where significant genomic sequencing resources have been employed and the transcriptome is well characterized. Alternatively, cDNA-probes can be synthesized (amplified) from cloned genes (mRNAs) or cDNAs to be employed as microarray probes, even when little or no genomic sequence information is available. A cDNA probe set derived using this approach can be expected to be highly redundant in that highly expressed genes (mRNAs) can represent >30% of the entire probe set. In chapter 9 the authors describe the Spanscript™ method which they have developed to derive a non-redundant cDNA library (probe set) for creating cell-, tissue-, and disease-specific cDNA microarrays in any eukaryotic organism.

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Chapter 10: A Comprehensive Microarray Data Management Approach

A microarray data set may include expression levels of hundreds of thousands genes across hundreds of conditions. As more biologists start to utilize microarray technology, the amount of data generated increases exponentially. Therefore, researchers need reliable bioinformatics tools to archive large sets of gene expression data within an individual laboratory, to transform this data into meaningful biological results, and to share expression data/results with other scientists. The objective of this chapter is to outline the strategy of managing microarray data with the database GeneDirector(tm). This database is a comprehensive data management system that covers all stages of the microarray process.

Chapter 11: Identifying differentially expressed genes from microarray data with The Limit Fold Change (LFC) Model.

One of the first and most important and critical steps in a microarray experiment is to determine those genes which are significantly and differentially regulated in response to the experimental condition being studied. In many studies, selection of differential gene expression is performed through a simple fold change cut-off, typically between 1.8 and 3.0. However, there is an inherent problem with this selection criterion, as genes that are lowly expressed have a greater inherent error (i.e. variation) in their measured levels. These genes will then tend to numerically meet a fold change of 2 even if the gene is not truly differentially expressed; thereby producing false positives. In contrast, highly expressed genes, while having less error in their measured levels, may not meet an arbitrary fold-change cut-off of 2 even when they are truly differentially expressed; thereby leading an increased number of false negatives. In chapter 11 the authors describe a model that considers both expression levels and fold changes for the identification of significant differentially expressed genes.

Chapter 12: Analysis of Gene Expression in Caenorhabditis elegans using Microarrays of Covalently Attached 50mer Oligodeoxyribonucleotides

In chapter 12, the authors describe in detail how to fabricate an oligonucleotide-based microarray. They tested the validity of these microarrays with a series of experiments, which analyze gene expression in the nematode Caenorhabditis elegans. A total RNA based labeling scheme was developed which requires much less material than standard mRNA-based methods.

Chapter 13: A Microarray-Based Method for Detecting Metabolic (P450) mRNA Induction in Rat: Implications for Xenobiotic Screening and Drug Development.

In developing novel therapeutics, candidate compounds are prioritized prior to toxicological assessment with short dosing studies in a rat model to determine how the vertebrate liver responds. An increase in the level of specific oxidative enzymes (P450s) in the rat liver following exposure to a given compound raises concerns of potential hepatotoxicity, Consequently P450-specific enzyme assays are performed on rat hepatic microsomal samples to screen for xenobiotic-mediated induction and inhibition of specific P450 enzymes in the rat liver. In chapter 13 the authors demonstrate the value of screening mRNA levels in addition to enzyme activity assays. A custom DNA microarray focused on hepatic and metabolic gene products (171 genes total) is described.

Chapter 14: Development and Application of an Oligonucleotide Microarray for Mutational Analysis

Many efforts have been made to identify genetic alterations in the biological research field. Many cancer-causing genes have been identified and mutational analysis is now widely performed. This has accelerated our understanding of disease-related mechanisms, in terms of basic knowledge and clinical practice. However, each gene has different mutation characteristics and a different size. Most of the APC, BRCA1, and BRCA2 mutations found in cancers are protein truncating nonsense or frameshift mutations. However, missense mutations dominate in the RET proto-oncogene responsible for MEN2 (Multiple Endocrine Neoplasia type 2) syndromes in hot-spot areas. Thus, mutational analysis techniques need to be selected according to the characteristics of each gene. In chapter 14 the authors describe address this problem and describe the development and application of an oligonucleotide microarray the detection of the RET gene mutation.

Chapter 15: Microarray Technology Comparison, Statistical Analysis, and Experimental Design

Microarray experiments have multiple sources of variation and experimental design should ensure that the effects of interest are not confounded with ancillary effects. This chapter presents a brief overview of four separate microarray platforms with sample data, an examination of differences in spot morphology, quality control methodology, and a procedure for determining the technical variability and corresponding resolution of expression microarrays.

Editor

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Gary Hardiman is the Director of BIOGEM (an organized research unit and core facility) specializing in high throughput genomic approaches, including DNA microarray technology and bioinformatics, at The University of California San Diego. He teaches a course "From Gene to Biological Function: An Overview", an elective course in the University of California San Diego Extension (Bioscience) Drug Discovery and Development Professional Certificate and coordinated the UCSD extension Microarray Technologies meeting March 13-15, 2002. His Ph.D is in microbiology from the National University of Ireland (Galway) and his interests are in genomic approaches to understand the molecular mechanisms of disease.

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Cover Artist

The cover design of this book contains an image of “Exploring Molecular Worlds” by Dr. Hunter O’Reilly. This work is mixed media; paint on plexiglass and X-Ray films detecting DNA, RNA, and protein. It is a center of triptych, 930x30 in. The Design Director of DNA Press decided to incorporate it because this book is about “exploring molecular worlds”.

Both an internationally shown artist and an experienced geneticist, Hunter O’Reilly reinterprets science as art through abstractions, digital art and installations. Her artwork has been shown in galleries in New York, San Francisco, England, Italy, Japan and the Czech Republic. She holds a Ph.D. and Masters Degree in Genetics from the University of Wisconsin-Madison, and a bachelors of science from the University of California-Berkeley. O’Reilly teaches both biology and art at the University of Wisconsin-Milwaukee.

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Preface

Gene expression profiling using microarrays has become a popular molecular biological approach in the last few years. The availability of complete genome sequence information along with improved technological advances has created a situation where these techniques can be applied in a broad manner, thereby facilitating profiling of large sections of the transcriptosome. I remember being fascinated the first time I saw an actual microarray. This was in 1995 when Synteni gave a presentation on this subject at DNAX in Palo Alto, where I was a post doc. Later in 1998 as a scientist working in biotech I had the opportunity to become a part of this exciting field. Since then DNA microarrays have matured considerably, and the technology for fabrication and methods for analysis have become more sophisticated. The genesis of this publication was a course I helped organize at 'The University of California San Diego Bioscience Extension' called 'Microarray Technologies - An Overview' in March 2002. This provided an important forum for reviewing recent advances and future trends in this field. This course provided a detailed overview of microarray and gene-chip technologies and examined evolving technology standards for microarray research, micro-fluidic technologies and instrumentation, printing and detection methods, and the tools, kits and reagents required for successful gene expression and SNP analysis. This is set to become a yearly event with the recent conclusion of the 2003 meeting.

This book contains chapters from top scientists, academicians, programmers and engineers many of whom were involved with this course, and I am grateful to those who have generously contributed material. As the editor of this compilation, it is a privilege to have been associated with this publication. Many people have contributed along the way to making my involvement in this project possible. Thanks go out to my parents Joe and Maureen, family members David, Aidan and Nono for their encouragement, Professor Frank Gannon my graduate advisor for introducing me to the wonder of molecular biology, Drs. Sharon Wampler and Derry Connolly and colleagues at the UCSD Extension Department of Bioscience, Elizabeth Hickman for a fantastic job in putting the course together, Dr. Alexander Kuklin for offering me this editorship, Alex Nartea for his patience and dedication in the formatting of this manuscript for publication, Professors Scott Emr, Bill McGinnis, Geoff Rosenfeld and Chris Glass for their continued interest and support of microarray technologies at UCSD, the members of the BIOGEM laboratory Katrine Verdun, Richard Rouse, Jackie Vignes, and Ivan Wick, my colleagues and friends for their constant support and my wife Patricia for all her love and affection.

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