Microarrays for the Neurosciences
An Essential Guide
Microarrays for the Neurosciences
Cellular and Molecular Neuroscience
Charles F. Stevens, editor

*Drive: Neurobiological and Molecular Mechanisms of Sexual Motivation*
Donald W. Pfaff, 2000

*Neural Transplantation: An Introduction*
William J. Freed, 2000

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*Culturing Nerve Cells*
Gary Banker and Kimberly Goslin (eds.), 1991

*Microarrays for the Neurosciences: An Essential Guide*
Daniel H. Geschwind and Jeffrey P. Gregg (eds.), 2002
This book is dedicated to the memory of a great father and scientist, Stanley Geschwind. I thank my wife, Sandy, and three children, Eli, Maya, and Jonah, for their patience and love.

— DG
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We are just entering a dramatically new period in the biological sciences, the postgenomic era. As additional complete—or nearly complete—genomes become available, we have the possibility of determining which genes are active, and how active, in a variety of experimental circumstances and disease states in different organisms. This new potential for examining the role for expression of many genes in any context depends on knowing the sequences of all of the genes, the output of the genome projects, and on being able to detect gene expression, the job of the gene chips described in this book.

Although we all believe that DNA microarrays will revolutionize neurobiology, we do not have much idea of exactly how; at the outset of any new era, the course of the future is largely a delightful surprise. Many of us used to believe that the complexity of the brain arose from the “fact” that the majority of our genes are brain-specific. We now know that humans have many fewer genes than expected, and that brain complexity will have to arise out of patterns of gene expression rather than from the use of many brain-specific genes. We can be thus sure that many neurobiology laboratories will need to use gene chip analysis, and this book describes how to do it.

Charles F. Stevens
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Over the past several years there has been an explosion in biological information. This has been fueled by the exhaustive efforts of the Human Genome Project and private industry to sequence the human genome, the mouse genome, and the *Drosophila* genome, as well as the genomes of a number of model organisms and bacteria (Celniker, 2000; Collins and Mansoura, 2001). This unprecedented undertaking has generated a new discipline termed “functional genomics,” or the study of the relationship of the genetic code and its biological potential. This staggering and complex amount of information and its associated biological relevance is quite unlike the traditional simplified analysis encompassing the study of one gene and one gene function. We now have the opportunity to observe the function of the predicted 30,000 human genes and determine their biological function—not on an individual basis, but globally (Lander et al., 2001; Venter et al., 2001). The ability to monitor the human genome (and other genomes) is due not only to our decoding of it, but also has been influenced by the development of sophisticated equipment and technology.

One of the first core technologies that has been utilized in genomics (after the automated sequencer) is DNA microarray technology for the study of gene expression (Schena, 1996; Lockhart and Winzeler, 2000). DNA microarrays represent one of the many areas where molecular genetics and genomics are revolutionizing neuroscience. Why all the excitement? Rather than looking at one gene at a time, DNA microarray technology offers the unprecedented ability to monitor the expression patterns of large numbers of genes simultaneously; this makes it relevant to almost every aspect of neuroscience research, including anatomy and physiology.
Classically, neuroscientists have often been divided into systems neurobiologists and molecular neurobiologists—two groups with little in common. One exciting aspect of the microarray revolution is that it offers the potential to elevate molecular genetic approaches for studying the nervous system to the systems level.

Using DNA microarrays to monitor the expression of thousands of genes simultaneously requires a more macro or large-scale view of the system being studied. As microarray studies become more common and technical details become less pressing concerns, methods for data reduction and modeling will become the prominent themes in the application of this technology. These are areas where systems neuroscientists and physiologists tend to be stronger than the molecular neurobiologists.

To us, the most interesting phenomenon is the intersection, or clash, of the genomics paradigm with neuroscience. Typically, neuroscience has looked askance at “fishing expeditions,” while genetics has thrived on “screening.” The most state-of-the-art modern neuroscience has been focused on detailed functional studies of single molecules, and usually has not been descriptive. Despite each of their biases, both the genetics and neuroscience paradigms have been remarkably successful. Now in an era where almost every human gene has been identified, the application of genetic screening methods to the problems in which neuroscientists are most interested will likely reap great rewards. One recent example of this is the study of brain tissue from deceased schizophrenic patients to identify a potentially unifying molecular theme in this disease (Hakak et al., 2001; Mirnics et al., 2000).

In this book we begin to explore DNA microarrays, emphasizing topics of relevance to the neuroscientist and tackling the unique aspects and challenges that the nervous system poses for this new discipline. We believe this is the first book on microarrays written with a central nervous system (CNS) theme.

The genesis of this book stems from a short course on microarrays in which we were involved at the Society for Neuroscience meeting in Miami, Florida, in 1999. Prior to the meeting, one of us
had published a paper on looking at gene copy number by using microarrays (Geschwind et al., 1998a) and had one of the few abstracts on the use of microarrays at the meeting (Geschwind et al., 1998b). Based on this small body of work, he was asked by the society’s Education Committee to organize a short course on the topic, focused on the concerns of the neuroscientist. Although other fields, particularly cancer biology, had rapidly adopted arrays, neuroscience was more hesitant. We do not think that this was an inappropriate oversight or demonstrated ignorance of a powerful new technology, but rather was a reflection of the caution of investigators who appreciated the complexity of the nervous system relative to other organs or model systems.

In contrast to the study of the CNS, the disciplines of yeast, cell, and cancer biology have the luxury of having biological systems that are more easily adaptable to gene expression studies. These systems are much less complex, usually involve one cell type, and obtaining abundant appropriate material for studying gene expression is relatively simple and inexpensive. For example, many cancers are believed to be clonal expansions of a single cell type that has undergone an incipient genetic mutation. Therefore, the comparison of the parental cell (normal) with the expanded clonal neoplastic population is built on the fact that the two populations are more alike than they are different; a single (or perhaps a few) genetic insult has caused a catastrophic change in the cell behavior, but the initial genetic complexity of the system is relatively small.

The CNS has the most cellular heterogeneity and tissue complexity, and it is believed that most of the predicted 30,000 human genes will be expressed at some level in the CNS, whereas only a small subset will be expressed in any particular somatic tissue. However, the CNS poses more difficult challenges than just number of genes and amount of data. The number of cell types, the architecture, the developmental program, and the importance of environmental factors in CNS development and functioning are unprecedented in their contribution to CNS tissue complexity. Do
changes in gene expression reflect alterations in tissue composition, rather than changes in gene expression by a particular cell type (e.g., Eberwine et al., 1992)? Furthermore, the presence of many different types of cells in a single small piece of tissue may limit the microarray detection of low-abundance RNAs that are of interest (Geschwind, 2000). In addition, on a very practical level, the CNS network being studied may involve small anatomical regions with only a few thousands of cells, and the ability to obtain these samples poses additional challenges (Eberwine et al., 1992; Emmert-Buck et al., 1996).

Cell lines, which are the staple of other disciplines, are often considered too simple, and not representative of the true in vivo behavior of neurons and glia. Processes discovered or studied in neuronal or glial cell lines usually have to be confirmed in vivo or in tissue culture so as to be most convincing. All of these issues pose significant challenges for the study of comprehensive gene expression in the brain and nervous system.

This book focuses on the neuroscientist and includes specific chapters that address these prominent obstacles. We have assembled a group of individuals who have each approached their area of expertise with care and considerable thought.

We begin with a basic technical introduction to the key aspects of the methodology. In the first chapter, Dr. Gregg gives a broad overview of the technology platforms for gene expression studies, including a discussion of equipment and resources. In the next chapter, Dr. Pickett and colleagues provide a detailed discussion of array scanning and image acquisition, followed by Drs. Shah and Shams, who consider the complex issues of image and data analysis. Dr. Nadon and colleagues then give a broad but elegant discussion of the statistical methods needed for array analysis.

The next group of chapters discusses specific applications of gene expression studies in the CNS, each with its unique perspective and technology. Dr. Becker and colleagues discuss the benefits of using membrane arrays to study gene expression and provide detailed descriptions of the methods this group has used to profile
gene expression in neurologic disease. Rather than using the typical two-color fluorescent hybridization, they use radioactively labeled probes, enabling the use of small starting RNA quantities with high sensitivity of detection. Dr. Becker therefore demonstrates that the membrane arrays provide a tried-and-true platform that one can adapt without new equipment and large expense.

Dr. Chiang and colleagues then take us through a strategy of informatically and experimentally selecting genes enriched in the nervous system for inclusion onto arrays, rather than blindly purchasing a commercial array simply because it is available. This ensures that genes of interest will be studied. With proper design, the information content of each array can be very high and contain very few irrelevant data points. Dr. Chiang also uses radioactive probes on membrane arrays, further demonstrating the utility of this approach described by Dr. Becker. Protocols and websites with updates are provided to enable the readers to adapt this technology in their laboratories.

In the next chapter by Dr. Awad and colleagues, the focus changes to the GeneChip, an oligonucleotide platform developed by Affymetrix. This chapter provides a complete overview of oligonucleotide technology. In addition, several applications of this technology are discussed, including the study of regional and strain-specific variation in gene expression in the mouse brain. This kind of basic information on strain and regional differences will be an important foundation from which future experimentation will benefit greatly. It highlights the need for centralized repositories of expression data so that other investigators can make maximal use of this important resource (Becker, 2001; Geschwind, 2001). But, despite its clear benefits, controversy over data sharing remains (Miles, 2001; Mirnics, 2001).

Postmortem human tissue is an important source of material for the study of human neuropsychiatric disease. Its use raises many issues, including the need to control for genetic heterogeneity and postmortem artifacts. The chapter by Dr. Van Deerlin and colleagues provides a detailed summary of the issues and strategies to
be considered when using human postmortem tissue. It also discusses RNA amplification from small samples and is an invaluable resource in this regard. Dr. Potier and colleagues describe one powerful approach to limiting cellular heterogeneity by discussing different strategies for microdissection and isolating single cells for further gene expression analysis. By isolating a defined region or cell type that has been studied electrophysiologically, they systematically decrease the complexity and minimize the extraneous “noise” other cell types may contribute. This further allows elegant genotype–phenotype functional correlation on a single-cell level.

The applications portion of the book finishes with a chapter by Drs. Geschwind and Nelson, describing the strategy of using cDNA subtraction coupled to the microarray to identify differentially expressed genes. This strategy offers the ability to quickly collate groups of differentially expressed genes that include novel or previously unsuspected genes. This novel strategy benefits from letting the biology select the important genes, rather than relying on theoretically predefined clone sets or arrays. It also allows for novel gene discovery in addition to gene expression analysis. This chapter also emphasizes the need to follow up on some of these array screening experiments with expression studies such as \textit{in situ} hybridization.

In the future, analytic approaches will gain center stage as the technology becomes more widely used and more data are generated. The field of microarray data analysis is in its infancy, and as an example of its potential, we conclude with a chapter by Dr. Fuhrman and colleagues that describes novel methods using microarray data to develop hypotheses about regulatory networks.

Where possible, websites are included to permit more in-depth exploration of the methods and resources available. We have also included our current protocols where possible so as to facilitate any of these experiments in the reader’s laboratory. It is our hope that this book gives individuals that have never performed an array experiment the excitement and energy to apply this technology to their own studies. For the more experienced microarray enthusiast,
we hope that we have provided the tools to help perform a better microarray experiment. We urge our fellow neuroscientists to adopt this technology, and have a chip on their bench, rather than on their shoulder.

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