Microarray Technology

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Citation

M Abhilash, H Makari, R Patil. *Microarray Technology*. The Internet Journal of Medical Informatics. 2008 Volume 4 Number 2.

Abstract

A DNA microarray is a high-throughput technology used in molecular biology and in medicine. It consists of an arrayed series of thousands of microscopic spots of DNA oligonucleotides, called features, each containing picomoles of a specific DNA sequence. The proper and harmonious expression of a large number of genes is a critical component of normal growth and development and the maintenance of proper health. Disruptions or changes in gene expression are responsible for many diseases.Microarray is a valuable tool used for gene expression studies

INTRODUCTION

A microarray is a tool for analyzing gene expression that consists of a small membrane or glass slide .With only a few exceptions; every cell of the body contains a full set of chromosomes and identical genes. Only a fraction of these genes are turned on, however, and it is the subset that is “expressed” that confers unique properties to each cell type. “Gene expression” is the term used to describe the transcription of the information contained within the DNA, the repository of genetic information, into messenger RNA (mRNA) molecules that are then translated into the proteins that perform most of the critical functions of cells. Scientists study the kinds and amounts of mRNA produced by a cell to learn which genes are expressed, which in turn provides insights into how the cell responds to its changing needs. Gene expression is a highly complex and tightly regulated process that allows a cell to respond dynamically both to environmental stimuli and to its own changing needs. This mechanism acts as both an “on/off” switch to control which genes are expressed in a cell as well as a “volume control” that increases or decreases the level of expression of particular genes as necessary. The use of a collection of distinct DNAs in arrays for expression profiling was first described in 1987, and the arrayed DNAs were used to identify genes whose expression is modulated by interferon. The use of miniaturized microarrays for gene expression profiling was first reported in 1995. and a complete eukaryotic genome (Saccharomyces cerevisiae) on a microarray was published in 1997.

IMPORTANCE OF MICROARRAY

Microarrays are a significant advance both because they may contain a very large number of genes and because of their small size. Microarrays are therefore useful when one wants to survey a large number of genes quickly or when the sample to be studied is small. Microarrays may be used to assay gene expression within a single sample or to compare gene expression in two different cell types or tissue samples, such as in healthy and diseased tissue. Because a microarray can be used to examine the expression of hundreds or thousands of genes at once, it promises to revolutionize the way scientists examine gene expression. This technology is still considered to be in its infancy; therefore, many initial studies using microarrays have represented simple surveys of gene expression profiles in a variety of cell types. Nevertheless, these studies represent an important and necessary first step in our understanding and cataloging of the human genome.As more information accumulates, scientists will be able to use microarray to ask increasingly complex questions and perform more intricate experiments. With new advances, researchers will be able to infer probable functions of new genes based on similarities in expression patterns with those of known genes. Ultimately, these studies promise to expand the size of existing gene families, reveal new patterns of coordinated gene expression across gene families, and uncover entirely new categories of genes. Furthermore, because the product of any one gene usually interacts with those of many others, our understanding of how these genes coordinate will become clearer through such analyses, and precise knowledge of these inter-relationships will emerge. The use of micro

arrays may also speed the identification of genes involved in the development of various diseases by enabling scientists to examine a much larger number of genes. This technology will also aid the examination of the integration of gene expression and function at the cellular level, revealing how multiple gene products work together to produce physical and chemical responses to both static and changing cellular needs. A basic difference between microarray data analysis and much traditional biomedical research is the dimensionality of the data. A large clinical study might collect 100 data items per patient for thousands of patients. A medium-size microarray study will obtain many thousands of numbers per sample for perhaps a hundred samples. Many analysis techniques treat each sample as a single point in a space with thousands of dimensions, then attempt by various techniques to reduce the dimensionality of the data to something humans can visualize

DNA MICROARRAYS: THE TECHNICAL FOUNDATIONS

Two recent complementary advances, one in knowledge and one in technology, are greatly facilitating the study of gene expression and the discovery of the roles played by specific genes in the development of disease. As a result of the Human Genome Project, there has been an explosion in the amount of information available about the DNA sequence of the human genome. Consequently, researchers have identified a large number of novel genes within these previously unknown sequences. The challenge currently facing scientists is to find a way to organize and catalog this vast amount of information into a usable form. Only after the functions of the new genes are discovered will the full impact of the Human Genome Project be realized. The second advance may facilitate the identification and classification of this DNA sequence information and the assignment of functions to these new genes: the emergence of DNA microarray technology. A microarray works by exploiting the ability of a given mRNA molecule to bind specifically to, or hybridize to, the DNA template from which it originated. By using an array containing many DNA samples, scientists can determine, in a single experiment, the expression levels of hundreds or thousands of genes within a cell by measuring the amount of mRNA bound to each site on the array. With the aid of a computer, the amount of mRNA bound to the spots on the microarray is precisely measured, generating a profile of gene expression in the cell.

WHAT EXACTLY IS A DNA MICROARRAY

DNA Microarrays are small, solid supports onto which the sequences from thousands of different genes are immobilized, or attached, at fixed locations. The supports themselves are usually glass microscope slides, the size of two side-by-side pinky fingers, but can also be silicon chips or nylon membranes. The DNA is printed, spotted, or actually synthesized directly onto the support.The American Heritage Dictionary defines “array” as “to place in an orderly arrangement”. It is important that the gene sequences in a microarray are attached to their support in an orderly or fixed way, because a researcher uses the location of each spot in the array to identify a particular gene sequence. The spots themselves can be DNA, cDNA, or oligonucleotides.

BASIC STEPS INVOLVED IN MICROARRAY EXPERIMENT

One might ask, how does a scientist extract information about a disease condition from a dime-sized glass or silicon chip containing thousands of individual gene sequences? The whole process is based on hybridization probing, a technique that uses fluorescently labeled nucleic acid molecules as “mobile probes” to identify complementary molecules, and sequences that are able to base-pair with one another. Each single-stranded DNA fragment is made up of four different nucleotides, adenine (A), thymine (T), guanine (G), and cytosine (C) that are linked end to end. Adenine is the complement of, or will always pair with, thymine, and guanine is the complement of cytosine. Therefore, the complementary sequence to G-T-C-C-T-A will be C-A-G-G-A-T. When two complementary sequences find each other, such as the immobilized target DNA and the mobile probe DNA, cDNA, or mRNA, they will lock together, or hybridize.

Now, consider two cells: cell type 1, a healthy cell, and cell type 2, a diseased cell. Both contain an identical set of four genes, A, B, C, and D. Scientists are interested in determining the expression profile of these four genes in the two cell types.

To do this, scientists isolate mRNA from each cell type and use this mRNA as templates to generate cDNA with a “fluorescent tag” attached. Different tags (red and green) are used so that the samples can be differentiated in subsequent steps. The two labeled samples are then mixed and incubated with a microarray containing the immobilized genes A, B, C, and D. The labeled molecules bind to the sites on the array corresponding to the genes expressed in each cell. After this hybridization step is complete, a researcher will place the microarray in a “reader” or “scanner” that consists of some

lasers, a special microscope, and a camera. The fluorescent tags are excited by the laser, and the microscope and camera work together to create a digital image of the array. These data are then stored in a computer, and a special program is used either to calculate the red-to-green fluorescence ratio or to subtract out background data for each microarray spot by analyzing the digital image of the array. If calculating ratios, the program then creates a table that contains the ratios of the intensity of red-to-green fluorescence for every spot on the array. For example, using the scenario outlined above, the computer may conclude that both cell types express gene A at the same level, that cell 1 expresses more of gene B, that cell 2 expresses more of gene C, and that neither cell expresses gene D. But remember, this is a simple example used to demonstrate key points in experimental design. Some microarray experiments can contain up to 30,000 target spots. Therefore, the data generated from a single array can mount up quickly.

A DNA MICROARRAY EXPERIMENT

- Prepare your DNA chip using your chosen target DNAs.
- Generate a hybridization solution containing a mixture of fluorescently labeled cDNAs.
- Incubate your hybridization mixture containing fluorescently labeled cDNAs with your DNA chip.
- Detect bound cDNA using laser technology and store data in a computer.
- Analyze data using computational methods.

STANDARDIZATION

Microarray data is difficult to exchange due to the lack of standardization in arrays. This presents an interoperability problem in bioinformatics. Various grass-roots open-source projects are trying to ease the exchange and analysis of data produced with non-proprietary chips:

For example, the “Minimum Information About a Microarray Experiment” (MIAME) checklist helps define the level of detail that should exist and is being adopted by many journals as a requirement for the submission of papers incorporating microarray results. But MIAME does not describe the format for the information, so while many formats can support the MIAME requirements, as of 2007 no format permits verification of complete semantic compliance.

The “MicroArray Quality Control (MAQC) Project” is being conducted by the US Food and Drug Administration (FDA) to develop standards and quality control metrics which will eventually allow the use of MicroArray data in drug discovery, clinical practice and regulatory decision-making.

The MicroArray and Gene Expression Data (MGED) group is working on the standardization of the representation of gene expression data and relevant annotations.

TYPES OF MICROARRAYS

- DNA Microaary
- Spotted Microarray
- Oligonucleotide microarray
- Genotyping microarray
- cDNA Microarray

IN BRIEF: MICROARRAY APPLICATIONS

Figure 1

Microarray type	Application
CGH	Tumor classification, risk assessment, and prognosis prediction
Expression analysis	Drug development, drug response, and therapy development
Mutation/Polymorphism analysis	Drug development, therapy development, and tracking disease progression

- Gene expression studies
- Gene function for cell state change in various conditions (clustering, classification)
- Disease diagnosis (classification)
- Inferring regulatory networks
- Pathogen analysis
- Drug Discovery
- identify appropriate molecular targets for therapeutic intervention
- monitor changes in gene expression in response to drug treatments
- Targeted Drug Treatment
- Agro industries

- Water quality management
- Pharmacogenomics
- Detection of biological warfare

CONCLUSION

DNA microarray technology is revolutionizing many aspects of biological research, allowing the expression of many thousands of gene transcripts to be monitored simultaneously. This provides powerful tools for the genome-wide correlation of gene transcript levels with physiological responses and alterations in physiological states. To date, microarray analyses have been applied almost exclusively to a few model species for which the abundant gene sequence data permit the fabrication of whole-genome microarrays. However, many interesting physiological traits and responses are poorly expressed or absent in model species and may be better illustrated in no model organisms. Comparative approaches to understanding function traditionally focus on species that by virtue of their unusual adaptations, lifestyles, and phylogeny are particularly suited to address a specific biological process or problem.

ACKNOWLEDGEMENTS

We are very much thankful to Mr S.Narasa Raju, Chairman of Children's education trust,Mr. Narasimha raju, Executive director of Children's education trust ,Prof K Basavaraju, Dr.T.Krishnan Principal,Dr Kusum Paul of The oxford college of Engineering,Bangalore for their support and encouragement.

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