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Advances In : Biochips – Array of Applications



Stimulated by emerging tools and technologies, DNA microarrays have moved far beyond the laboratory. They now offer applications in areas as diverse as diagnostics, clinical profiling, and screening genetically modified organisms.

by Peter Gwynne and Gary Heebner



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This is the first of four supplements this year on biochips. The others will appear in the 6 May, 19 August, and 30 September issues of Science.

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DNA microarrays enable researchers to analyze the expression of thousands of genes in a single experiment under tightly controlled conditions. First developed in the early 1990s, they initially provided a powerful tool for scientists trying to understand the fundamental aspects of cellular function and the genetic causes of disease.

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In recent years, DNA microarrays have moved out of the research lab and into a wide variety of practical applications. “We have seen the evolution of microarrays from being primarily a gene expression tool to being used for many other types of applications,” says Siobhan Pickett, director of genomic systems for **Molecular Devices**. “We all expected that this would happen eventually, because the microarray technology is just a tool. But it’s really exciting to see how quickly and broadly that’s been happening.”

DNA microarrays, often known as chips or biochips, continue to find their most common application in studies of gene expression and detecting single nucleotide polymorphisms (SNPs). “In business as a whole, gene expression is still dominant,” says Roland Green, chief technology officer and vice president of R&D for **NimbleGen Systems**. “However, we see the bulk of the growth in new applications such as ChIP and arrayCGH.” Indeed, a profusion of new uses has emerged during the past two years. And growing numbers of users are finding that, in the words of Jochen Müller-Ibeler, product line manager for DNA microarrays at **Eppendorf**, “Microarrays are nice toys to play with.”

Explosion of Uses

What new uses have emerged? “There’s been an explosion of applications of microarrays to comparative genomic hybridization [CGH],” says Wendy Price, business area manager for DNA arrays at **Invitrogen**. In that application, explains Jeremy Clarke, global product manager of **Genomic Solutions**, “You hybridize DNA from two sources against metaphase chromosomes. The mix is then hybridized against a large panel of DNA probes to look for regions of DNA gain or loss.” Diagnostics has also benefited from microarrays. “Roche had the first approval for a diagnostic tool using microarrays,” says Müller-Ibeler. “The diagnostic market is big and important for us.” Another German company, **Greiner Bio-One**, takes a similarly optimistic view. “We are trying to push arrays into the diagnostic field, says Jörg Stappert, head of the firm’s biochip group. “We are looking at lots of arrays to analyze for a few markers.”

DNA microarraying has also started to move into treatment technologies. “Clinical profiling is coming along,” explains Sean Yu, vice president of operations at **SuperArray Bioscience**. “There are a number of clinical trials for the use of microarrays for prognosis or therapeutic guidance.” **Affymetrix**, the company responsible for the first commercial DNA microarrays, recently participated in a program that identified the first gene linked to sudden infant death syndrome. “This is just one example of how DNA analysis microarrays are accelerating discovery and bridging the gap between basic scientific research and its impact on human health,” says Affymetrix’s chairman and CEO Stephen Fodor.

In addition, pharmaceutical firms have started to use microarray data to determine the success of clinical trials of new drugs. Beyond the clinic, the technology is finding application in food science and forensics. And basic research also benefits from the technology. “We’re seeing a broad range of protein-based applications, including research on protein-protein interactions and antibody studies, that use both DNA and protein microarrays,” Pickett says. “Researchers are also using DNA microarrays to study DNA-protein interactions.”

New and Improved Microarraying

The scope of the fresh uses depends in large part on the development of new and improved tools and technologies for microarraying. “People are more aware of the need to build better controls in their microarray experiments, especially when they are applied to diagnostics,” says Price. Reusability has emerged as another key advance. “We are developing protocols to reuse microarrays three to five times,”

Green notes. “It’s a matter of finding the right buffers and stripping the microarrays so that they don’t pick up dirt. We think it will enable a new set of applications that were previously too expensive.”

Certainly reusability improves the economics of microarraying investigations, particularly with expensive items such as NimbleGen’s human genome 38-chip set. “We can use that array set five times; that makes it much more economical,” Green continues. “I predict that this will be standard practice for many labs soon.”

Vendors have also started to offer high throughput DNA microarrays. “Until now, it was one chip, one sample. Now it’s moving into one chip, multiple samples,” Stappert explains. “That will be important for drug screening. It’s why we have started development of high throughput arrays. We are trying to push arrays into the diagnostic field, which needs lots of arrays to analyze for a few markers.”

The development of so-called tiling arrays represents another advance critical to the development of new microarraying applications. “Tiling arrays use millions of DNA probes evenly spaced, or “tiled,” across the genome, including coding and noncoding regions alike,” Fodor explains. “These tiling arrays provide scientists with the only single tool available for genomewide analyses of many important biological functions, including transcription, transcription factor binding sites, sites of chromatin modification, sites of DNA methylation, and even chromosomal origins of replication.”

Another key advance involves a new type of microarray. Several companies, including Affymetrix, **Agilent Technologies**, **Applied Biosystems**, and NimbleGen, now produce DNA microarrays that contain the entire human genome on a single chip.

User-Friendliness and Low Cost

Several companies have combined appropriate materials and solutions into kits for microarray fabrication and hybridization experiments. Those supplies lower the barrier against entry into microarraying for average scientists. **GE Healthcare**, for example, has added a range of chips and supplies to its CodeLink product line. The SensiChip line developed by **Zeptosens** and marketed by **Qiagen** features microarrays, reader, software, buffers, and a hybridization station.

SuperArray, meanwhile, aims to make microarraying more attractive in two ways. “To broaden into everyday uses such as clinical applications, you have to simplify the microarray and the data analysis,” Yu says. “It’s essential to simplify use and lower cost. A lot of our users are not expert in gene expression profiling, and so can’t tell you what they are looking for. We’ll help them start their microarray analysis.”

Users of microarrays can also benefit from a choice between ready prepared and customized microarrays. “You have predefined and customized arrays, and low-density and high-density arrays,” Eppendorf’s Müller-Ibeler says. “It gives you more flexibility.” SuperArray provides customizable features with its oligo arrays. “Researchers can start with general arrays and then customize them with our help for as low as \$100 per array,” Yu says. “We can do the customizing within two weeks.”

Two Types of Preparation

Scientists can choose between two basic sources of DNA microarrays: ready-to-use versions that contain oligonucleotides synthesized directly on the chips, and more customizable forms that contain DNA spotted onto the chips.

Affymetrix uses photolithographic masks similar to those involved in making computer chips to prepare

its high-density, ready-to-use microarrays. The masks control the light-sensitive removal of protective groups from hydroxyls in unmasked regions of the substrate, allowing the altered nucleotides to react with bases in the reaction solution and grow the DNA sequence.

The company has led the way in large-scale production of DNA microarrays with a broad range of offerings from its standard GeneChip System to custom services. It recently announced a high throughput microarray prototype that contains 96 individual arrays mounted onto a single plate. “Each array contains the same genomic information as our original human genome U133 arrays, but in approximately a five times smaller surface area,” Fodor reports. “Soon each array on the 96-array plate will contain over 1.4 million probes, able to measure the expression of approximately 40,000 human transcripts.”

NimbleGen has developed maskless photolithographic technology that gives users more opportunity to adapt and reprogram their microarrays to their needs. “The Affymetrix system is good for high-volume printing runs, like making a newspaper,” explains NimbleGen’s Green. “Ours is more akin to using your laser printer to print reports that you’ve just written. The main benefit of our approach is that customers get to tailor the arrays to their experimental needs rather than vice versa – designing experiments to fit the arrays. Customers can design their arrays to answer their questions. Once they realize that, they start thinking about projects they never thought about before.”

Source for Cell Signaling

If you want to learn about signal transduction, you’ll find a good starting point in *Science*’s **STKE** (Signal Transduction Knowledge Environment) website. The site features original perspectives, reviews and protocols solicited by the site’s editors, and the Connections Map database, which contains information on signaling components and relationships among them. STKE also contains links to full-text articles on signal transduction from journals distributed by 19 publishers. Users of the site can also participate in forums and download animated teaching resources. Finally, you can personalize the site to meet your own specific needs for information on signal transduction.

<http://www.stke.org/>

Hitting the Spot

The alternative to photolithographic methodology requires vendors or users to spot complementary DNA (cDNA) – produced from messenger RNA using the reverse transcriptase polymerase chain reaction – onto chips. Scientists who want to do their own spotting must first prepare their cDNA. To help them, companies such as **Ambion**, **BD Biosciences Clontech**, and **Promega** offer reagents and kits for isolating and purifying DNA and RNA. GE Healthcare, **Mirus Bio**, and **Roche Applied Science** have kits for labeling nucleic acid samples for fluorescent detection.

Users have a choice of approaches for spotting. The most common methods involve solid or split metal pins. Dipped into wells containing the DNA samples of interest, each of a set of pins picks up a small amount of the DNA, which it drops onto the chip’s surface. “Solid pins have the advantage that, if you work with viscous substances like proteins, you don’t have to worry about blockage,” Genomic Solutions’ Clarke says. “With split pins you can do several hundred spots with the same intake of substance.” Suppliers such as GE Healthcare and **Hitachi Genetic Systems/MiraiBio** produce spotting robots for use with both types of pin. Genomic Solutions is launching a suite of products for the protein arraying market to address the requirements of these new protein printing applications.

The other main spotting technique, based on inkjet technology adapted from the printer industry, eliminates cross contamination of nucleotides by using separate print heads for each base. “There are two types of inkjet: solenoid valve and piezo-electric,” Clarke says. “Both are relatively expensive, and you

have to be very specific with your buffer set and to calibrate your surface very carefully. Solenoid technology delivers large spots, usually in the 20 nanoliter range, and is volumetrically controlled. Piezo delivers a very small spot, but you need tighter control.” Companies such as **Arrayjet**, **GenHunter**, Genomic Solutions, and **PerkinElmer Life Sciences** use inkjet technology.

Some researchers prefer to produce their own DNA chips in their laboratories. For these do-it-yourselfers, who often lack the engineering expertise required to develop their own robotic systems and software, several companies focus on user-friendliness. “We offer very comprehensive training packages,” Clarke says. “And we help our customers to develop solutions to their arraying needs even before the purchase. We also work with third parties on applications of our products.”

Labeling, Scanning, and Interpretation

The detection method that scientists use with DNA chips depends on the type of label they choose for their experiments. Fluorescence labeling, offered by Affymetrix, Genomic Solutions, Invitrogen, Molecular Devices, and other suppliers, has proved markedly more popular than the alternatives. “There have been radioactive tags, but I don’t see any significant switch away from fluorescent labeling,” says Pickett of Molecular Devices. However, Eppendorf will soon introduce a colorimetric method. “It’s cheaper and easy to use,” Müller-Ibeler explains. “You can incorporate it into your lab as a full system.”

To detect fluorescent labels, researchers use confocal laser scanners tailored for use with DNA microarrays. “We make continuing gradual improvements to all aspects of our family of four GenePix scanners and GenePix Pro and Acuity software,” Pickett says. “Changes in the level of automation and the precision of spot handling have made automated analysis possible and robust.” Genomic Solutions supplies high-resolution, auto-focusing semi-confocal array readers that allow researchers to read arrays on uneven surfaces without having to worry about the best parameters to choose.

Invitrogen provides kits that help scientists handle fluorescence scanning from soup to nuts. “We have worked hard on improving reproducibility and accuracy in sample labeling to introduce more standardization in this portion of the workflow,” Price says. Adds group leader Kate Rhodes: “Our SuperScript Plus kits have our superscript enzyme, very streamlined and simple protocols, including low elution volume purification, and very well matched fluor dyes to generate more true positives with greater accuracy.”

The need for automated analysis stems from the huge volumes of data created by DNA microarrays with thousands of samples or spots. To avoid bottlenecks in storing and analyzing the data, some researchers start out by performing array experiments with the Affymetrix-style comprehensive chips and then downsize their efforts to focus on a specific family of genes. Suppliers such as Affymetrix, **Lion Biosciences**, Molecular Devices, **Spotfire**, and **Silicon Genetics** produce software packages for analyzing and interpreting data from DNA microarrays. Invitrogen offers its Vector Xpression software package for microarray analysis. “It probably has more complete ability to do statistical analysis,” Price says. “We’ll probably focus less on it as a stand-alone effort and use it more as a component of our platform technologies, built into web based solutions.”

Abundance of Applications

New applications of DNA microarrays abound. Perhaps the most far reaching involve medical sleuthing.

Two decades of research has shown an etiological relationship between certain human papillomaviruses (HPVs) and many cases of cervical cancer. Greiner Bio-One will introduce its PapilloCheck DNA

microarray that types 24 HPVs. “It has much greater resolution than the present test systems,” Stappert says. “With the current tests’, you can only prove high risk or low risk. With our genotyping, you can get the details.” The company plans to launch the system in Europe in summer or fall, and in the United States once the **U.S. Food and Drug Administration** approves it.

Affymetrix has contributed to an effort to discover a mutation that had eluded researchers for decades. Scientists at the **Translational Genomics Research Center** and the **Clinic for Special Children** used the company’s mapping 10K arrays to discover the first gene linked to a form of sudden infant death syndrome. The research team used the arrays, each of which genotypes 10,000 single nucleotide polymorphisms, to analyze the DNA of just four infants and their family members. “Within five days,” Fodor says, “the group identified the mutation that had so tragically affected certain Amish families.”

Microarraying has also emerged in clinical trials. In a recent phase 3 trial, expression profiles helped researchers at **Novartis Pharmaceuticals** to predict that the company’s Gleevec drug had a low probability of success in treating chronic myelogenous leukemia. And in a phase 2 trial, researchers at the **Dana-Farber Cancer Research Institute** applying Affymetrix’s GeneChip arrays to myeloma patients treated with the **Millennium Pharmaceuticals** drug Velcade discovered a pattern of 30 genes that correlates with response or lack of response to the therapy.

From Cancer to Food Safety

Eppendorf offers gene expression arrays, which it calls DualChips, for several conditions, including cancer, aging, and apoptosis. “And an inflammation array will come on the market this year,” Müller-Ibeler says. In a collaboration with European Union institutes, the company is also developing a microarray system for diagnosing the safety of foods, most notably genetically modified organisms (GMOs). “It will come onto the market in the first half of this year,” Müller-Ibeler says. “It will contain the most important features of the GMOs accepted in the European Union.”

Applications in medicine and food safety represent only a start for microarray technology. The future plainly holds more advances in the design, function, utility, and additional applications of DNA microarrays.



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**Dana-Farber Cancer Research
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hospital and nonprofit research
organization

<http://www.dana-farber.org/>

Eppendorf AG

DNA microarrays

<http://www.eppendorf.com/>

GE Healthcare

DNA microarrays and systems

<http://www.amershambiosciences.com/>

GenHunter Corporation

inkjet-based microarray spotters

<http://www.genhunter.com/>

Genomic Solutions

DNA microarrays, fabrication
instruments

<http://www.genomicsolutions.com/>

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Hitachi Genetic Systems/MiraiBio

microarray spotters

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Invitrogen Corporation

DNA microarrays

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LION Bioscience AG

bioinformatics software

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Millennium Pharmaceuticals, Inc.

pharmaceutical company

<http://www.mlnm.com/>

Mirus Bio Corporation

nucleic acid labeling kits and reagents

<http://www.mirusbio.com/>

**Molecular Devices (formerly Axon
Instruments)**

image detection systems

<http://www.axon.com/>

NimbleGen Systems, Inc.

DNA microarrays and systems

<http://www.nimblegen.com/>

Novartis Pharmaceuticals

pharmaceutical company

<http://www.novartis.com/>

PerkinElmer Life and Analytical Sciences

inkjet-based microarray spotters

<http://las.perkinelmer.com/>

Promega Corporation

nucleic acid purification kits and reagents

<http://www.promega.com/>

Qiagen GmbH

DNA microarrays and systems

<http://www.qiagen.com/>

Roche Applied Science

DNA microarray-based diagnostic kits

<http://www.biochem.roche.com/>

Science's STKE (Signal Transduction Knowledge Environment)

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<http://www.stke.org/>

Silicon Genetics

bioinformatics software

<http://www.silicongenetics.com/>

Spotfire, Inc.

bioinformatics software

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SuperArray Bioscience Corporation

DNA microarrays

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Translational Genomics Research Institute (TGen)

nonprofit research organization

<http://www.tgen.org/>

U.S. Food and Drug Administration (FDA)

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