Microarrays Demystified

Scientists are continually searching for new, better, and faster ways to determine which chemicals found in the environment cause adverse health effects, and how they do so. At the same time, the development of new drug therapies depends upon a clearer understanding of how environmental agents cause genetic changes that lead to disease. Since 1997, the NIEHS Microarray Group has been using microarrays to analyze changing patterns of gene expression across the entire genome, studying thousands of affected genes at a time and revolutionizing the way that toxicologic problems are investigated. Today, microarray "chips" allow researchers to complete within a day or so an experiment that would once have taken months using traditional assays focusing on just one gene at a time.

One goal of toxicogenomics is to use gene expression as a highly sensitive and informative marker for toxicity. Using microarrays, researchers can quickly and accurately screen for large numbers of gene expression responses to toxic substances (alone or in mixtures), determine if toxic effects occur at low-dose exposures, highlight vulnerable tissue or cell types, and begin to extrapolate effects from one species to another. Over time, researchers hope to identify genes associated with the development of environmentally caused diseases, including immune dysfunction and cancer, as well as pulmonary, liver, and neurologic diseases.

The Equipment

To make the type of microarray chips used by the NIEHS group, up to 20,000 complementary DNAs (cDNAs) or oligonucleotides are spotted onto a small glass substrate using high-speed robotics and mechanical contact-printing pens. To test a particular toxicant, RNA from treated and control samples is reverse-transcribed into cDNAs while incorporating fluorescent tags. If a red fluorescent tag is used for the treated sample, then a green fluorescent tag will be used for the control sample (or vice versa).

The two fluorescent-labeled cDNA groups are combined onto a microarray chip that contains spots of gene fragments complementary to the labeled cDNAs. An overnight hybridization step allows the labeled cDNAs to find and bind to their complementary gene fragments. Laser scanners detect the red and/or green fluorescent signals of the spotted gene fragments to reveal the relative abundance of the treated and control cDNAs, and hence the relative abundance of the original RNA transcripts. The resulting patterns of color form a gene expression profile, or signature, that points out a possible toxic condition.

According to center director Richard Paules, the first human "ToxChip" microarrays created in the lab contained only the genes believed to be critically involved in toxicity—about 2,000 in all. By the year 2000, researchers were using larger chips with 7,000 clones of rat DNA. "Our goal was to continue to increase our coverage to represent [a] whole genome on a chip," Paules explains. Now chips containing 20,000 elements are available for human, mouse, and rat DNA. Paules says the group has processed more than 12,000 chips since its inception.

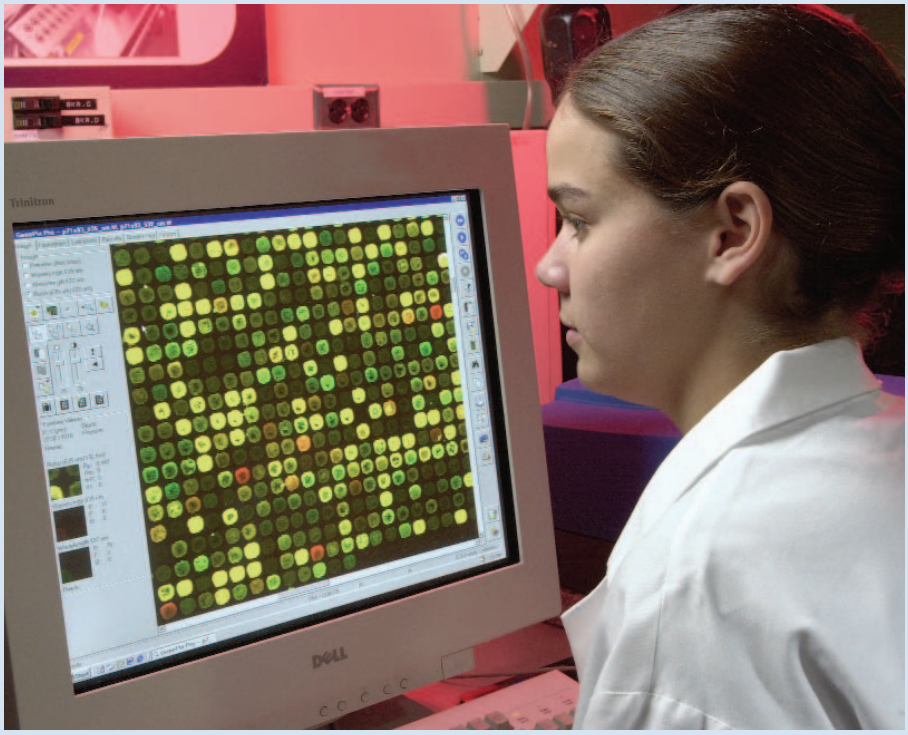
The group's laboratory sports a phalanx of sophisticated, often custom-made equipment for fabricating microarrays, scanning the images they produce, and analyzing and archiving the resulting data. A high-speed robotic arrayer prints 96 chips at once, dipping a printer head with 32 pins into a 384-well plate, with each well containing the genetic material that is deposited onto the slide. "We adjusted humidity and temperature controls and introduced liquid-handling robotics to make the spots consistent and uniform," explains microarray lab manager Jeff Tucker, whose background is in biomedical engineering.

Though the group first used its own custom-made chips exclusively, it now for the most part uses chips made by Agilent Technologies, based in Palo Alto, California. Agilent's 20,000-clone human chips are precisely uniform with perfectly circular spots, Tucker says, giving more gene coverage and higher-quality data overall.

As the chips have become more advanced, so has the technology necessary to interpret the data they produce. Three laser scanners collect images produced by the fluorescent dyes used to label RNA on the microarrays. The newest scanner, produced by Agilent Technologies, can scan 48 slides in one carousel run with a time of 7 minutes per slide. The Agilent scanner complements another scanner, manufactured by Axon Instruments of Union City, California, which scans one slide in 15 minutes. (By comparison, the lab's original laser scanner took two hours to scan one slide.) Once scanned, the images are statistically analyzed and combined with clone identification information using commercial software.

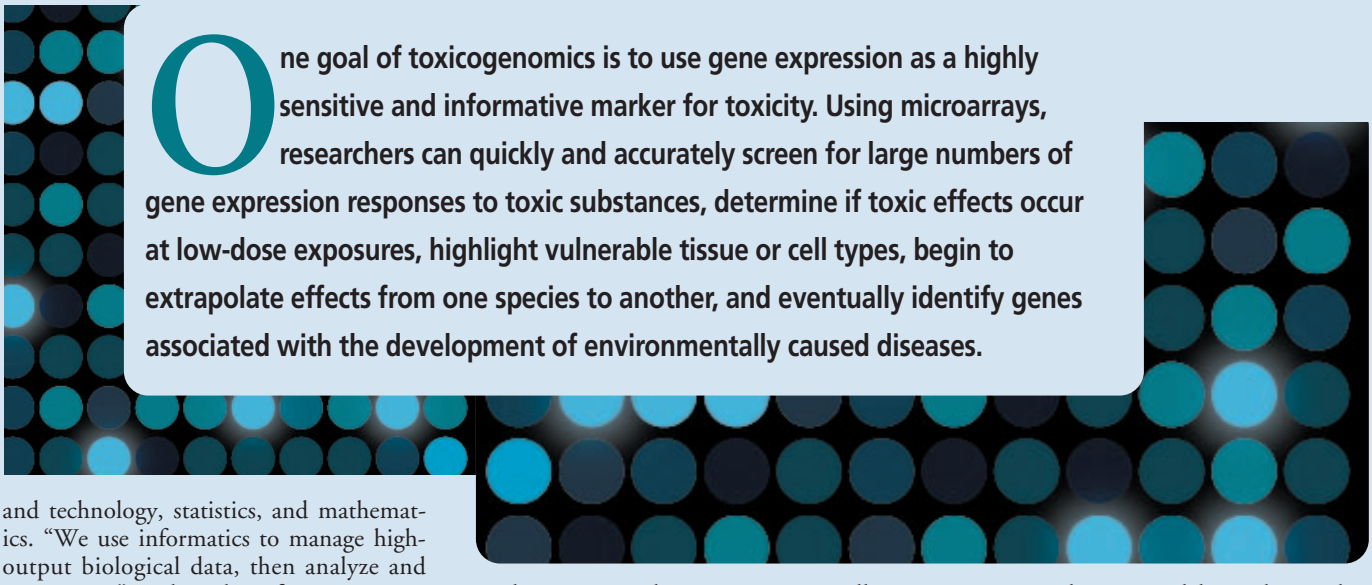
The Experts

Analyzing massive amounts of data and converting them to useful information requires professionals skilled in bioinformatics, a multidisciplinary field that combines biology, genetics, information science



Connecting the dots. Since 1997, scientists in the NIEHS Microarray Group have processed more than 12,000 microarray "chips" in the search for better knowledge of how environmental factors affect gene expression.

Arnold Greenwell/EHP



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and technology, statistics, and mathematics. "We use informatics to manage high-output biological data, then analyze and interpret it," explains bioinformatics manager Pierre Bushel, a molecular biologist and informatics expert.

Even before a microarray is produced, Bushel's staff creates a database cataloguing all the genes that will be deposited onto the chip, along with each gene's precise location within the 384-well plates. This effort alone produces thousands of records and several fields, all for just one microarray. The next step is image processing of each chip to generate pixel intensity and ratio measurements of each spot and eventually to determine any significant gene expression changes.

"Once data are structured and managed using a professional database, we verify data reliability and consistency," explains Bushel. He and his staff also develop their own custom computational programs, as well as novel algorithms and statistical methods for identifying patterns in gene expression data. "Our challenge is to make the data accessible and interpretable to scientists," he says. "We build web application environments and database portals so that scientists from any location, at any time of day, can retrieve the data they need and analyze it." The daily interaction between bioinformatics specialists and scientists "has been very fruitful," he adds. "We deal with problems so complex that we need a team-oriented approach."

Paules agrees. "We're really a collaborative program that is unique [within] the NIEHS. Working together, we form a coalition across multiple types of expertise," he says. That expertise includes veterinary pathologists, toxicologists, biochemists, and specialists in microarray technology, informatics, proteomics, and pharmacokinetics. The group also collaborates with pharmaceutical consortia, biotechnology groups, academicians, and statisticians, as well as government agencies such as the Food and Drug Administration (including its National Center for Toxicology Research) and the Environmental Protection Agency.

One of the group's original mandates, besides establishing a state-of-the-art gene expression analysis research program and doing proof-of-concept toxicogenomics studies, was to serve the NIEHS's intramural research program. In keeping with that purpose, the Microarray Group has collaborated so far on more than 100 intramural research projects. It also collaborates on National Toxicology Program projects and conducts its own studies as part of the institute-based National Center for Toxicogenomics.

Taking On Toxicants

So far, most of the group's experiments have focused on hepatotoxicity. "We've adopted a careful approach to look at several hepatotoxicants that are well known in terms of how they affect the liver," Paules explains. "Our goal is to identify adverse changes early, before irreversible damage occurs," thereby improving treatment methodologies and boosting chances for prevention of liver disease. Microarray studies may one day help scientists identify a person's genetic predisposition to developing liver disease. So far, the group has evaluated approximately 10 different compounds designated as possible liver toxicants by the National Toxicology Program, as well as compounds that humans may be exposed to specifically via drugs as well as elsewhere in the environment.

One such project, headed by postdoctoral researcher Alexandra Heinloth, focuses on the toxic effects of acetaminophen on the liver. Known to cause liver failure in excessive amounts, acetaminophen is the active ingredient in widely used over-the-counter pain relievers such as Tylenol. It also shows up in many over-the-counter cold remedies, as well as in some prescription painkillers. Alcohol exacerbates and intensifies the drug's effect on the liver because it induces the activity of one of the enzymes that metabolizes acetaminophen into its toxic metabolite, explains Heinloth's colleague, postdoctoral fellow Todd Auman.

Currently, the research team is using differential gene expression profiling to understand how acetaminophen damages liver cells. Using microarrays, the team has performed numerous experiments using different dosing levels and durations, which can determine whether liver damage is permanent or temporary. Searching for clues, the researchers study multiple doses at multiple time points and look for repeating patterns that might signal a genetic change or identify a gene involved in a metabolic pathway that had not been noted before.

Taking things a step further, the researchers are now using microarrays to find clues as to whether gene activity that takes place is occurring at a functional level. To do this, researchers scrutinize cellular RNA changes, then test for different protein or metabolite levels.

The researchers are moving away from cDNA arrays to oligonucleotide arrays, which Auman says "are coming close to more complete coverage of the genome." However, the greater number of genes that are being analyzed produces more data to sift through and interpret. "It makes the work more challenging," Auman admits. "It's great for discovery; it opens more avenues to research. But at the same time, it's harder to determine the story that is unfolding." **–Jennifer Medlin**