Chips Ahoy

Microchips covered with DNA emerge as powerful research tools

By JOHN TRAVIS

Good things often come in small packages. The latest proof of that familiar saying lies in the thumbnail-sized chips of glass or silicon that have recently become the talk of the molecular biology community.

Resembling the microprocessors that revolutionized the computer industry, the chips carry checkerboardlike arrays of DNA rather than intricate electronic circuits. These devices, often called DNA chips, promise their own revolution, biologists have been saying for a decade.

A surge of journal articles in the last few months may finally herald the realization of that promise. Academic investigators and a few companies are designing or have developed DNA chips that quickly screen tissue samples for disease-producing microorganisms or cancer-causing gene mutations.

Scientists already envision the day when they can use a small number of DNA chips, or perhaps just one, to examine the action of every human gene, all 100,000 or so, in a group of cells.

"The molecular biology of the '70s, '80s, and first part of the '90s has mostly focused on studying one gene or one protein at a time. Now, we're moving into systems analysis, where we can analyze entire genomes at a time," says Leroy Hood of the University of Washington in Seattle.

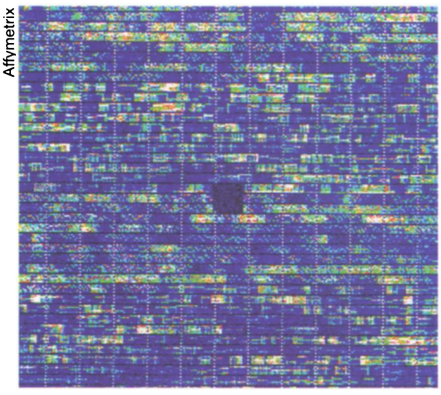
The key to DNA chips is a phenomenon known as hybridization, the zipperlike joining of two strands of genetic material. A gene normally exists as two strings of nucleotides entwined in a helical shape resembling a spiral staircase. There are four DNA nucleotides—A, C, G, and T—and the sequence of these nucleotides in a gene encodes the information a cell uses to build a specific protein.

In hybridization, one chain of nucleotides binds to another. Since A binds only to T and G only to C, the nucleotide sequence ATTCG will only hybridize to TAAGC, its complementary sequence. This specificity, which provides the foundation for DNA's ability to copy itself, is also the key to the DNA chip's effectiveness.

To create such a chip, researchers divide a surface, typically glass or silicon, into hundreds, thousands, or even millions of sites called features. At each feature, they firmly attach millions of copies of a single DNA segment, or probe, its length ranging from several nucleotides to millions.

They then label the genetic material to be tested with a fluorescent marker and apply this sample to the chip. The marked DNA will stick to the device only where it hybridizes to a DNA probe whose sequence is complementary to all or much of its own.

Affymetrix



Data from a DNA chip. This detailed pattern of colors compares the DNA sample being tested to the DNA sequence of the cancer gene p53.

By carefully designing and arraying DNA probes, investigators can create chips that represent an entire gene's nucleotide sequence. Such chips can reveal visually—in a fluorescent glow, when viewed with a microscope—whether a tested sample's DNA differs by even a single nucleotide from the standard version. For example, a gene might normally contain the nucleotide A at one point in its lengthy sequence and so hybridize to a probe sequence with a T there. If the DNA being tested hybridizes instead to features where the DNA probes contain a G at that spot, it would signify that the tested sample had a C instead of an A there, representing a single mutation.

DNA chips vary greatly in how the probes are attached to a surface. Hood makes use of ink jet printer technology to shoot droplets containing the DNA probes onto a chip. Another strategy immobilizes the DNA probes in a gel.

"There are lots of approaches. Internally, we've used at least 10, I believe," says Stephen P.A. Fodor, president of Affymetrix, a firm in Santa Clara, Calif., that has been at the forefront of DNA chip manufacturing and research.

Affymetrix normally relies on photolithography, a manufacturing technique critical to the semiconductor industry. Photolithography uses ultraviolet light and stencil-like masks to activate microscopic regions of a glass surface. With photolithography, Affymetrix attaches selected nucleotide sequences to specific sites on the chip.

One of the most dramatic developments in DNA chip technology has been Affymetrix's continuing ability to reduce the size of the features where the DNA probes reside. The firm can easily make chips with features measuring 20 by 20 micrometers, and much smaller features are on the horizon. Consequently, researchers can pack many more DNA probes onto a chip and derive even more information from a single experiment.

"When I first started working here, we were working with 256 probes. Now, we have the resolution where we're typically making chips with 260,000 probes. We've even made chips with up to a million probes," says Mark S. Chee of Affymetrix.

The DNA chip's most obvious commercial use may be in diagnosing diseases. Affymetrix produces a chip that can analyze whether samples of HIV have drug-resistant versions of the genes for protease and reverse transcriptase, the two viral enzymes that the most effective AIDS drugs target. The chip should help researchers understand how quickly HIV can evolve resistance to the antiviral drugs and how many strains of the virus are naturally resistant.

DNA chips may also gain widespread use in detecting mutations in the growing list of genes linked to human illnesses. Scientists have learned, to their dismay, that most disease genes can undergo a bewildering array of mutations, notes Joseph G. Hacia of the National Human Genome Research Institute (NHGRI) in Bethesda, Md. That variety can complicate the development of a genetic test. In many cases, scientists may have to sequence the entire gene for a person to guarantee

they've detected all possible mutations. That's a job perfectly suited to a DNA chip, says Hacia.

Working with Affymetrix, Hacia and his NHGRI colleagues have recently begun using DNA chips to detect mutations in a gene called *BRCA1*. Inherited alterations in this gene appear in many of the families whose members have been plagued by breast and ovarian cancer (SN: 9/24/94, p. 197).

In the December 1996 NATURE GENETICS, Hacia and his coworkers report on the effectiveness of a DNA chip designed to spot almost all mutations in a large part of the *BRCA1* gene. Two sources of DNA—the patient's *BRCA1* and a normal, reference copy of the gene—are labeled with fluorescent markers of different colors; investigators then hybridize both genes to the DNA chip at the same time. "We're looking for differences between the two samples," says Hacia.

With this technique, the researchers accurately diagnosed 14 out of 15 patients with known *BRCA1* mutations, and they had no false positives when examining 20 samples free of mutations. "This is encouraging for a pilot study. What we're now pursuing is a chip that looks at the entire gene," says Hacia.

In collaboration with OnCorMed, a company in Gaithersburg, Md., Affymetrix plans to market a chip that screens for mutations in *p53*, a gene mutated in more than half of all cancers. The *p53* chip contains DNA probes that cover the entire normal sequence of the gene, as well as probes specific to several hundred known *p53* mutations. "Since you have the real estate on the chip, you might as well make good use of it," says David Mack of Affymetrix.

Nanogen, a biotech firm in San Diego, is also betting its future on DNA chip technology. Nanogen plans to develop diagnostic tests for infectious diseases, says company president Tina Nova. A DNA chip might, for example, contain an array of genetic sequences that can identify bacteria which cause pneumonia or strep infections.

That same chip might even reveal whether the bacteria in a tested sample, which could come from a drop of a patient's blood, have evolved resistance to various antibiotics.

According to Nova, Nanogen's primary advantage is a proprietary technique called electronic hybridization, in which an electric current draws the DNA being tested to the probe sequences on a chip. This greatly speeds analysis of samples, she contends.

While companies like Affymetrix and Nanogen envision clinical uses of DNA chips, it may be basic biological research that benefits most from the technology. Take yeast, one of just a few organisms for which scientists have identified and sequenced every single gene (SN: 5/4/96, p. 278).

"There are around 6,000 yeast genes, and we have absolutely no idea what more than half of them do. One of the simplest ways to find out what a gene does is to knock it out and monitor the fitness of that mutant yeast cell under a bunch of different selection conditions," observes Daniel D. Shoemaker.

Pursuing that strategy, Shoemaker, Ronald W. Davis, and their colleagues at Stanford University Medical Center have joined an international consortium that plans to spend the next year or two creating a massive library of yeast strains that have specific genes deleted. Exposing the strains to a specific environmental condition—low temperature, lots of salt, a particular drug, and so on—can then provide clues to the roles of the missing genes.

For example, if one strain of yeast grows poorly when exposed to ultraviolet radiation or any other DNA-damaging agent, its missing gene may be involved in the repair of DNA.

"You put every one in a single test tube, perform the selection, and ask which strains didn't grow," explains Shoemaker.

That's where a DNA chip enters the picture. Whenever the researchers delete a gene to create a mutant strain of yeast, they add a unique identifying DNA sequence, says Shoemaker, who likens these genetic tags to the bar codes used by stores.

An Affymetrix DNA chip that recognizes those tags then acts as the bar code reader, revealing how well the various yeast strains have survived a particular experiment. In the December 1996 NATURE GENETICS, Shoemaker, Davis, and their colleagues described a successful pilot study that tested 11 yeast strains with different genes deleted.

The sequencing of the complete yeast genome has also opened the way to using DNA chips in studies of the organism's genetic activity. When a gene is active, the cell reads the information in the gene's DNA and creates strands of messenger RNA, a nucleic acid similar to DNA. These strands instruct the cell's machinery to string together specific amino acids to form a protein.

Much like samples of DNA, messenger RNA can be made to hybridize to the DNA sequences that spawn it. Consequently, DNA chips with probes characteristic of specific genes can examine how much messenger RNA from those genes is present, the traditional measure of a gene's activity, or expression.

Researchers at Affymetrix and elsewhere are nearing completion of DNA chips that monitor the expression of all 6,000 or so yeast genes. With such a tool, investigators expect to gain unprecedented insight into the normal pattern of genetic activity inside yeast cells, which in many ways resemble human cells.

The same chips can also reveal how yeast cells respond genetically to various stimuli. "When you make the yeast do something, you can ask, How does the expression of every single gene change?" says Hood.

Other studies have already shown the power of DNA chip technology to detect gene expression. Last year, researchers used a DNA chip to demonstrate how the pattern of genetic activity in a plant's root differs from the pattern in a leaf. In the December 1996 NATURE GENETICS, another team described how a chip monitoring the activity of several hundred human genes provided information about what makes a cell cancerous. Affymetrix has its own "cancer chip," which provides data on some 250 genes implicated in the formation of tumors. The company is also creating a chip to monitor the activity of nearly 50,000 human genes, many of which still have no known function.

DNA chips will speed the examination of genetic diversity among people, predicts Fodor. In the Oct. 25, 1996 SCIENCE, for example, Chee and his coworkers describe a mitochondrial DNA (mtDNA) chip.

Mitochondria, which are inherited only from the mother, are the microscopic power stations of cells. Each contains its own bit of DNA distinct from the genome inside a cell's nucleus. Biologists examine populations for variations in small regions of this mtDNA and use the data to examine human origins and evolution (SN: 2/22/92, p. 123). The new chip lets investigators quickly compare a person's complete mtDNA sequence to a standard sequence. The device may also prove useful to researchers probing possible links between mtDNA and various diseases, says Chee.

Two other projects seek to extend the potential of DNA chips even further. Hood is creating chips whose probes are double-stranded DNA rather than the usual single chain of nucleotides. With such arrays, he says, investigators can look for transcription factors, which turn genes on and off, and for other proteins that interact with DNA.

Finally, Edward Southern of the University of Oxford in England, one of the first scientists to describe the potential of DNA chips, back in the late 1980s, has used the devices to look for nucleotide sequences, called antisense, that interfere with cellular production of specific proteins and may have uses in agriculture and medicine (SN: 2/16/91, p. 108).

As the growing list of applications attests, DNA chips stand poised to make their presence felt in almost every aspect of biological research and eventually to become as commonplace in laboratories as today's electronic chips. □