DNA chips, which identify DNA by binding it to samples on a substrate, let researchers tune in to the symphony of gene expression. They look set to revolutionize drug discovery and diagnostics, too

Microchip Arrays Put DNA on the Spot

Last January, a new kind of microchip saved Patrick Baeuerle from going down a multimillion-dollar, dead-end street. Then the head of drug discovery at a South San Francisco–based biotechnology company called Tularik, Baeuerle and his colleagues had just synthesized a new drug compound that, in cell cultures, drastically reduced levels of low density lipoprotein, which has been linked to hardening of the arteries. The next step was to learn how the compound worked, a puzzle that can take years to unravel.

FUTURE CHIPS

Researchers are finding new uses for microchip technology. Soon, DNA sequencers, chemical plants, and satellite propulsion systems will all come in credit-card–sized packages.

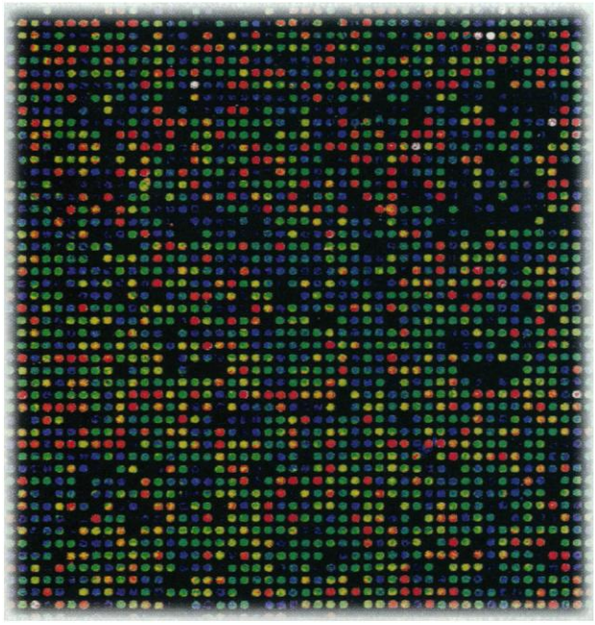
▶ DNA ANALYSIS LABS ON A CHIP MICROMACHINES

Looking for a shortcut, Baeuerle opted to try and find which genes a cell switches on in response to the compound. He and his team turned to researchers at Synteni, another Bay Area start-up firm, which makes DNA chips. These chips carry arrays of different snippets of DNA that serve as probes for detecting DNA fragments with a complementary nucleotide sequence.

When Synteni researchers used their chips on fluorescent-labeled DNA from cells exposed to either the new Tularik drug or a related drug already on the market, the pattern of fluorescence showed that the new drug had caused a completely different cellular response. "It dramatically changed the profile of gene expression," says Baeuerle, who just left Tularik to head up research at a biotech start-up in his native Germany.

Unfortunately, the change wasn't for the better. The pattern of genes turned on by the new drug candidate strongly resembled that from a completely different class of compounds that had also looked promising but proved to be toxic. "It killed the prospects for [our] compound," says Baeuerle. Although the result was a disappointment, the DNA tests likely saved Tularik millions of dollars by helping it weed out an unsuitable drug candidate early on, rather than later in animal or human tests.

Such experiences underscore the promise of what many are now calling the microchip of the 21st century. These 2- or 3-centimeter-wide slices of either silicon or glass, bearing anything from hundreds to hundreds of thousands of immobilized snippets of DNA, have the unique ability to track the expression of many (if not all) of a cell's genes at once, allowing researchers to witness for the first time the behavior of thousands of genes in concert. Moreover, tracking cells' responses to drugs is far from the only application of these chips. Genetic diagnostics companies are turning to DNA arrays hoping that unique gene-expression patterns can pinpoint the onset of diseases from cancer and Alzheimer's to osteoporosis and heart disease.



Light show. In a computer simulation of a DNA chip, colored spots reveal levels of expression in hundreds of genes.

CREDIT: INCYTE PHARMACEUTICALS

Elsewhere, researchers hope that arrays will help them gauge the success of HIV drug treatment, tailor medications to patients with specific genetic makeups, and sequence genes. And that's just for starters. The drug companies and biotech firms pursuing the technology are hoping that DNA chips will prove to be a primary research tool in a genetic-medicine revolution. They expect that understanding the genes active in disease will spawn a new generation of therapeutic drugs that treat underlying causes rather than symptoms.

"In the past, we compared the activity of single genes," says Wei Zhang, an oncologist at the M. D. Anderson Cancer Center in Houston, Texas. "With the new technology, we can analyze a huge number of genes at the same time. That provides hope for a new era of diagnostics and therapeutics." Jeffrey Trent, who heads DNA array research at the National Human Genome Research Institute (NHGRI) in Bethesda, Maryland, agrees. "It's a remarkably different approach to genetics," he says. "[It] allows us to track pathways instead of individual genes." Because of that advantage, the use of the new arrays "is just exploding in all kinds of directions," says Francis Collins, NHGRI director. "The limits will not be found anytime soon."

That promise has touched off a race to capitalize on DNA chips. Surveys by brokerage houses and market research firms indicate a nearly immediate annual market for the chips of about $1 billion, with plenty of room to grow. Not surprisingly, in recent years, about a dozen companies have jumped into the DNA chip–making business, each vying to become the Intel of genomics. Affymetrix of Santa Clara, California, an array pioneer, netted nearly $100 million in its June 1996 initial public stock sale. Even after the market's recent downturn, its outstanding stock is now worth more than $575 million, although

Will Patent Fights Hold DNA Chips Hostage?

The pioneering technology of DNA chips, which can identify genes by getting them to bind onto a large array of sample sequences fixed to a surface, is sparking a modern-day gold rush, with companies big and small competing frantically to stake their claims (see main text). But legal scuffles could bring that rush to a halt. The disputes—over patents on both the "hardware" (the chip technologies themselves) and the "software" (the actual genes that dot the arrays)—haven't dampened enthusiasm yet. But just about everyone involved expects the legal situation to heat up if and when companies begin to earn profits. "It's really at a critical juncture right now and has the potential to limit access and availability of the technology," says Jeffrey Trent, who heads a DNA array project at the National Human Genome Research Institute in Bethesda, Maryland.

On the hardware side, leading chip producer Affymetrix has filed suit against two companies, Incyte Pharmaceuticals Inc. and Synteni Inc., both of Palo Alto, California, and Affymetrix is trading lawsuits with another chipmaker, Hyseq Inc., of Sunnyvale. Affymetrix claims that Synteni, which Incyte recently acquired, is infringing on an Affymetrix technology for making dense arrays, containing more than 1000 gene fragments per square centimeter. Synteni/Incyte officials counter that the suit has no merit, as they use their own proprietary technology to immobilize much larger gene fragments than the short oligonucleotides arrayed by Affymetrix.

Hyseq, meanwhile, maintains that Affymetrix is infringing on its array technology known as sequencing by hybridization, which uses DNA arrays in combination with separate oligonucleotide probes whose sequence is known either to identify or completely sequence genes of interest. Last month, Affymetrix countered these claims with a suit of its own. But all these companies could soon be feeling some heat from University of Oxford molecular biologist Edwin Southern, who in December was awarded a broad-ranging U.S. patent on a basic technology for laying down short snippets of DNA in arrays. Although Southern says he has yet to try to enforce his patent with companies such as Hyseq and Affymetrix, he adds that "We will be talking to them."

Indeed, just about every conceivable wrinkle in array technology, including the schemes for synthesizing the arrays, attaching fluorescent tags to nucleotides, and detecting the fluorescence when the tags bind, is tangled in patents. "It's complicated. Everyone will step on somebody's turf," says Uwe Müller, who heads technology development for Vysis Inc., an arraymaking company in Downers Grove, Illinois. "As soon as you go out the door, you're slapped with three lawsuits." Adds Jay Flatley, chief executive of Molecular Dynamics, another arraymaker: "I think it's going to be quite a number of years before all this is worked out."

The picture is even more complicated on the "software" side. Patent offices around the globe already allow the patenting of newly discovered genes, as long as a known function can be ascribed. Once genes are patented, DNA chip companies may be forced to obtain licenses before using portions of them on their arrays. "If people have legitimate claims, then we will respect them," says Affymetrix vice president Rob Lipshutz.

The scale of the licensing problem can be overwhelming, however. The array on a 2.5-centimeter chip can contain some 40,000 sequences. "If each spot on the array involves a gene that's patented, they have to get licenses for each spot," says Trent. It's a "nightmare" of a problem, says Müller. "If everyone wants a percentage, you're going to run out of profits [really] fast." In addition, patent holders could conceivably withhold licenses in the hope of capitalizing on commercial possibilities themselves or offer an exclusive license to one chip company, which would effectively freeze others out of the business.

So why is anybody still in the game? Müller and others believe that a number of forces will conspire to head off the worst logjams. First, Müller notes that much DNA sequence is now in publicly available databases, giving chip companies a source of sequences for which they won't have to pay fees. Also, a report on biochips last year by the brokerage firm Lehman Brothers noted that the distribution of patents among many companies means that "no one company has enough of a patent position to completely block its competitors." Hyseq President Lewis Gruber—a patent attorney himself—adds that this form of parity has brought about cooperation in other research-intensive industries such as microelectronics, where companies regularly agree to swap access to one another's technologies. As for the current squabbles within the array community, Gruber says, "It seems daunting, but that sort of problem has been worked out before." –R.F.S.

the company has yet to make a profit. Other array companies are reporting similarly brisk business.

Despite this flurry of interest, only a handful of actual products exists. Development has been hampered by a host of technical challenges, such as difficulties in distinguishing sometimes weak fluorescent signals from background noise. But the darkest cloud looming over this burgeoning business, according to chip company officials, is the threat of patent battles over key aspects of the technology and over the genes that make up the arrays (see sidebar). "There's still a lot of confusion about who owns what pieces of array technology," says Michael Albin, the vice president of science and technology with Perkin-Elmer's Applied Biosystems Division in Palo Alto, California. Still, Albin and others are confident that if and when these battles are worked out, gene chips will take the drug discovery and diagnostics markets by storm.

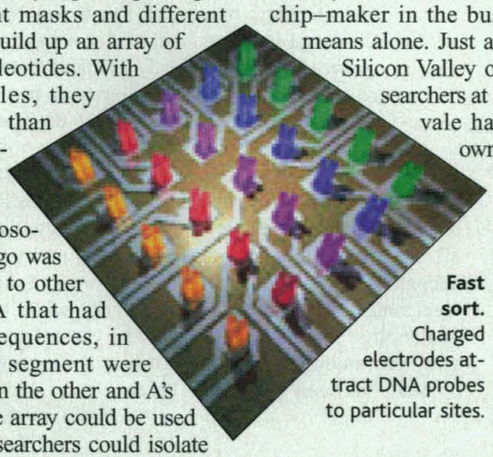
Array of options

DNA arrays owe much of their current research and financial promise to the international Human Genome Project. Although sequencing the entire 3 billion nucleotides that make up a person's 23 pairs of chromosomes is a huge task, it is only the first step to making use of the genome. Equally important is linking each gene to its role in the cell—a field of research dubbed functional genomics. This task is daunting, too. In a typical cell, tens of thousands of genes wink

on and off to help the cell churn out proteins involved in everything from metabolism to defense. Researchers can track the behavior of genes either alone or in small handfuls, but they have had no way to watch the dance of all the genes at once.

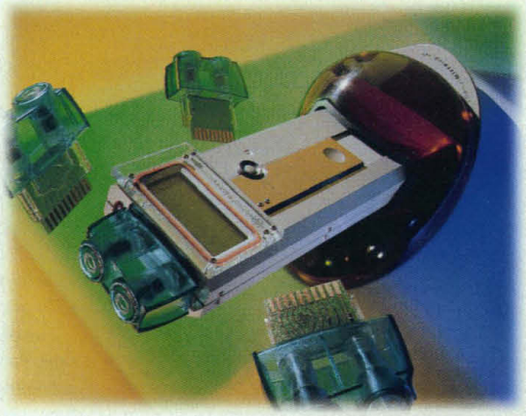
A key breakthrough came in a 1991 *Science* paper by Stephen Fodor and colleagues at a drug-discovery company called Affymax (*Science*, 15 February 1991, p. 767). Fodor's team came up with a scheme to use the same lithographic production techniques employed in computer-chip manufacturing to synthesize a checkerboard array of either short protein fragments called peptides or short DNA fragments called oligonucleotides—each of which ended up with a unique chemical signature. The researchers were looking for a way to generate a large number of compounds quickly; these could then be tested either as drugs, in the case of peptides, or for gene identification, with oligos.

To make their oligo arrays, for example, they started with a silicon surface coated with linker molecules that bind the four DNA building blocks, adenine (A), cytosine (C), guanine (G), and thymidine (T). Initially, the linkers are capped with a "blocking" compound that is removed by exposure to light. The researchers shone light onto the chip through a mask so that only certain areas of the chip became exposed. They then incubated the chip with one of the four bases, binding it to the exposed areas, then reapplied the block. By repeating this process with different masks and different bases, they could build up an array of different oligonucleotides. With just 32 such cycles, they could create more than 65,000 different oligos, each eight base-pairs long.



Fast sort. Charged electrodes attract DNA probes to particular sites.

Just like chromosomal DNA, each oligo was capable of binding to other stretches of DNA that had complementary sequences, in which G's on one segment were matched with C's on the other and A's with T's. Hence, the array could be used as a sensor: The researchers could isolate the RNA molecules that signal gene expression from tissues, chemically convert them to DNA, and label them with a fluorescent tag. After floating these tagged strands across an array of oligos, allowing complementary sequences to bind, and washing away the unbound strands, they could detect the strands that had bound by exciting the fluorescent tags with a laser. And because they knew the sequence of each oligo on their chip, the position of the fluorescent spot told them the sequence of the gene fragment that had bound there. In 1993, Affymax spun the idea into a new company—Affymetrix—and gene chips were born.



Quick read. Electronic DNA-detection scheme could pave the way for rapid diagnostics.

Today, Fodor and his Affymetrix colleagues have developed more than 20 different DNA arrays for research purposes. They also offer commercial arrays where the oligos fastened to the chip are chosen specifically to scan for mutations in the HIV genome and the *p53* tumor-suppressor gene, which has been implicated in up to half of all human cancers. A third chip, called Cytochrome P450, looks for variations in a set of genes involved in the metabolism of important therapeutic drugs such as beta blockers, prescribed for heart disease, and certain antidepressants.

Affymetrix remains the best known DNA chip–maker in the business but is by no means alone. Just a couple of miles up Silicon Valley on Highway 101, researchers at Hyseq Inc. in Sunnyvale have developed their own oligo-based scheme for sequencing genes. The Hyseq scheme does not involve labeling the unknown DNA with a fluorescent tag; instead, it is mixed with a tagged oligo of known sequence and washed over the array. Where you get an array oligo and the tagged oligo binding side by side to the unknown DNA, you get a fluorescent spot—and you know part of the unknown sequence. This process is repeated with different labeled oligos, and finally a computer works out what the sequence of the DNA must be to account for all the partial-sequence information. Last year, Hyseq teamed up with gene-sequencing powerhouse Perkin-Elmer to market its chips. The first is expected to be available to researchers within months.

Synteni was bought out last winter by Incyte Pharmaceuticals, a genomics company, which now offers glass chips that lay out an array of gene fragments 500 to 5000 base-pairs long. To use them, researchers isolate messenger RNA from normal tissue as well as tissue affected by disease or exposed to a drug. These two sets of RNAs are labeled with different-colored fluorescent tags and applied to the chip simultaneously. Scanning for the two colors then gives researchers an instant snapshot of how gene expression differs between normal cells and those affected by diseases or drugs.

Meanwhile, researchers at San Diego–based Nanogen are putting the finishing touches on chips that apply a controlled electric field to maneuver the DNA fragments around on the chip, looking for a match. The upshot, says Nanogen's Michael Heller, is that fragments find their complementary oligo more quickly, and detection takes just minutes—rather than the hours needed with ordinary chips, which let the DNA fragments diffuse randomly. And an altogether different approach is being taken by the 2-year-old start-up Clinical Micro Sensors (CMS), of Pasadena, California. Researchers there have designed a unique approach that uses electrical signals, rather than fluorescence patterns, to indicate the position of DNA binding to oligos on the array. CMS builds its arrays on a grid of electrodes rather than a passive chip; when DNA binds to its matching oligos, a separate probe molecule, carrying iron, also binds to the complex—an addition that can be detected by the electrodes.

Data flood

Just where all this is going depends on whom you talk to. CMS President Jon Kayyem argues that the big market will be in diagnostics, and not just for diseases that can't be diagnosed today. To take one of his favorite examples, when parents bring in a child with a sore throat, doctors typically are limited to taking a throat swab and sending the culture to a lab for testing. The culture can take days, so the doctor often winds up prescribing antibiotics or other drugs without knowing if they will do any good. An instant diagnostic scan that reveals not only the type of infection but the precise strains would be a vast improvement.

But Patrick Klyne, director of genomics at Millennium Predictive Medicine (MPM) in Cambridge, Massachusetts, says such tests have a long way to go before hitting the

CREDITS: (TOP) HENRY BLACKHAM / CLINICAL MICROSENSORS; (BOTTOM) NANOGEN

market. Not only would they have to wind their way through clinical trials and regulatory approval, they would have to come down in cost, too. Affymetrix chips can run anywhere between $45 and $850, not to mention the scanners and fluidics stations that go with them, which can cost more than $100,000. "To be viable [for diagnostics], the cost needs to come down to about $5," says Stanley Abromowitz of the National Institute of Standards and Technology. CMS's Kayyem says that his company's electronic detection scheme has a shot at making low-cost readers. But for now, the company has only a prototype device.

That's why Klyne and others argue that the initial breakthrough market will consist of genomics and pharmaceutical companies, which will use DNA arrays as a research tool to sift through the complex patterns of gene expression in cells and pinpoint particular genes that are turned on in disease. That's the approach being taken by MPM and its rival, diaDexus of Santa Clara, a joint venture between the big drug firm SmithKline Beecham and Incyte. DiaDexus, for example, has already used its arrays to show that prostate cancer cells crank out a protein called PLA2, while the same gene remains dormant in healthy cells. MPM researchers, meanwhile, have shown that melanoma cancer cells turn up production of a protein called melastatin. Both companies hope to turn these insights into new and improved diagnostic screens that would rely not on arrays themselves but on conventional and cheap techniques such as enzyme assays.

Other basic research with arrays is also beginning to pay off. In 1996, Collins and colleagues at NHGRI used Affymetrix chips to detect mutations in the familial breast cancer gene *BRCA1* in subjects at risk for the disease. Upstairs from Collins's lab, Jeffrey Trent and his colleagues are gauging, with their own array system, how radiation treatment affects gene expression in cancer cells.

What's certain is that these studies are just a taste of what is to come. Already, researchers with access to DNA arrays find themselves with an enviable problem: too much information. "We are drowning in cool data here," says Stanford array pioneer Pat Brown, whose team has made more than 7 million measurements of the expression of individual genes under different conditions. "More than 99% of the data we have is unpublished. It's so easy to think of an interesting experiment to do using this approach [that] we haven't been able to find the time to publish it all." **–ROBERT F. SERVICE**

FUTURE CHIPS
▶LABS ON A CHIP

Coming Soon: The Pocket DNA Sequencer

Microfluidics, chips that process tiny volumes of fluids rather than electronic signals, aim to put a whole lab in the palm of your hand

In May, a new private venture declared its aim to sequence nearly the entire human genome in 3 years for as little as $300 million. The plan beat the U.S. government's timetable by 4 years, at a tenth of the cost, and encouraged the government to move up its own genome deadlines. Leaders of the new venture, headed by genomics pioneer Craig Venter and funded by instrument maker Perkin-Elmer, hailed miniaturization—small, automated DNA sequencers—as the key. But the miniaturization behind this project is only a first step in the downsizing of the analytical laboratory.

The Venter project will replace conventional manually controlled DNA sequencers with machines that perform the same task nonstop, inside hair-thin capillaries the length of a knitting needle. But researchers at a handful of universities and companies hope to shrink sequencing equipment much further—all the way down to postage stamp–sized microchips etched with a maze of tiny channels and reaction chambers. Because these chips can be mass-produced with a technology similar to that used for silicon-based computer chips, they stand to push down drastically the price of DNA sequencing and, if used in quantity, speed up such sequencing too. And DNA sequencers are just one of the labs on a chip now in gestation.

A new breed of chipmaking companies is working to shrink to pocket size all types of chemistry equipment, including high-pressure liquid chromatography assays, high-throughput drug-screening systems, portable environmental screening equipment, biological weapons detectors, and even chemical production plants (see sidebar on next page). Harking back to the microelectronics revolution, researchers refer to these chips as "microfluidics" and expect them to have some of the same impact as the earlier development. "What will happen to laboratory equipment in the future is the same thing that happened to mainframe computers," says Wally Parce, research director for Caliper Technologies, a microfluidics company in Palo Alto, California.

Just as electronics miniaturization has led to computer-controlled home appliances and children's toys, Parce and others believe that the miniaturization of chemical equipment will lead to a host of as-yet-undreamed-of applications. "I even have folks at [NASA's Jet Propulsion Laboratory] who want to send [our microsystems] to Mars," says Rolfe Anderson of the biochip start-up Affymetrix in Santa Clara, California.

At present, however, these labs on a chip owe most of their appeal to their potential for doing the same job as existing equipment at a much lower cost. Current DNA sequencing, for example, requires a half-dozen tabletop machines to separate DNA from a tissue sample, select the desired fragment for analysis, amplify it, and sequence its component nucleotides—all at a high cost in technicians' salaries and in reagents. With microfluidics, both of those costs come down considerably. "The entire budget just falls

Microlab. Micrometer scale piping steers reagents together to synthesize drug compounds.

CREDIT: ORCHID BIOCOMPUTER