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Mini revolutions in DNA technology began with the structure of the double helix. Several major advances over the last 45 years include restriction enzyme mapping, DNA sequencing, and PCR. Each advance generated much excitement, and its importance was recognized by the Nobel Prize. And now, once again, DNA technology has made a quantum leap.

Even a few years ago it would have seemed preposterous to suggest that DNA analysis could be carried out on a thumbnail-sized glass chip. But that is exactly what is happening in several laboratories and biotechnology companies around the country. The brainchild of Stephen Fodor (President and Chief Executive Officer of Affymetrix with headquarters in Santa Clara, CA), the GeneChip, introduces the idea of parallel genomics—the marriage of two disciplines: biotechnology and semiconductor manufacturing. As excitement in the wonders of this technology mounts, an increasing number of university laboratories and several biotechnology companies are anticipating the needs of the experimenter by preparing to offer competing technology. Hopefully, this will allay the fears many scientists have of finding this technology inaccessible and ultimately unaffordable.

The Technology and its Cost

At Affymetrix, an independent company spun out of Affymax in 1991, the technique of photolithography as a method for synthesizing peptides on a solid support was adapted for nucleic acids (see Box 1). Currently, Affymetrix markets two probe arrays for clinical research: one for the protease and reverse transcriptase of HIV-1 and the other to resequence the p53 antioncogene, including the >400 mutations that have been associated in tumors, as well as a series of research expression chips for human, mouse, and yeast genes.

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To completely comprehend gene expression and regulation, it is important to have arrays where a battery of genes, not oligonucleotides, can be simultaneously probed (see Box 1). Patrick Brown's laboratory at Stanford University does just that. A high-density array of PCR products consisting of all the open reading frames of the yeast genome is spotted onto glass slides using an arrayer manufactured by the group (Fig. 1). Hybridization is then carried out with fluorescently labeled mRNA prepared under different growth conditions. Joseph DeRisi, a graduate student, has thus determined that in a time course of fermentation, a change in metabolism is visualized as a change in the expression of 1740 genes of which 50% have not thus far been characterized.

The power of this technology has enticed several other biotechnology companies to enter the field. Also exploring this technology, Molecular Dynamics has joined hands with Amersham Pharmacia Biotech to develop an integrated microarray platform, including hardware, software, and chemistry (Fig. 2). Intending to give the experimenter greater flexibility, their technology will permit the user to custom make one's own array using a ''spotter.'' Like the arrayer used by Patrick Brown's group, this will allow one to make arrays using cDNAs obtained from, for example, PCR reactions. David Barker, of Molecular Dynamics, is quick to point out that their technology is quite complementary to that being marketed by Affymetrix. Once changes in gene expression are discovered using cDNA microarrays, one can then go to the Affymetrix probe arrays, which use oligonucleotides, and zoom in on the specific genes one wants to follow. Companies like Synteni and Hyseq have joined the group in using competing methods to make arrays with DNA on glass slides or filter paper. Incyte Pharmaceuticals is planning on adapting the technology from ink-jet printers to make arrays (see Box 1).

Results—But at What Cost?

Meaningful scientific questions are already being answered using this technology. David Lockhart and colleagues at Affymetrix have used oligonucleotide arrays to measure changes in gene expression (Lockhart et al. 1996). RNA transcripts present in the cell at a frequency of 1:300,00 have been measured, and an expression chip containing 50,000 expressed sequence tags from the public databases is being developed. Three other reports also exemplify the kind of work that serve as forerunners to the experiments that will be seen at a greater frequency. The group of Francis Collins from Human Genome Research, in collaboration with Affymetrix, has screened individuals for mutations in the breast cancer gene, *BRCA1* (Hacia et al. 1996). Ron Davis and colleagues at Stanford University, again in collaboration with Affymetrix, have side-stepped the more laborious classification methodologies for understanding function by gene disruption in the yeast *Saccharomyces cerevisiae* (Shoemaker et al. 1996). Using 11 auxotrophic mutants and 20-mer oligonucleotides that serve as ''tags,'' the gene-chip technology facilitated the identification of the ''molecular barcode'' for each deletion strain and provided an example of how the experimenter can easily follow the growth patterns of numerous bar-coded deletion strains under a variety of growth conditions. Finally, the groups of Patrick Brown at Stanford University and Jeffrey Trent of the National Center for Human Genome Research at The National Institutes of Health (NIH) have analyzed changes in the expression of 900 genes in a human melanoma cell line after suppressing its tumorigenic phenotype, showing 1.7% of the genes were down-regulated while 7% were up-regulated.

The potential for this technology in clinical applications is quite apparent. The HIV chip, marketed by Affymetrix for $100, can detect genetic variants of

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Box 1 Methods for Creating a DNA Array

Photolithography: The first step is manufacturing a GeneChip probe array. Affymetrix's gene chip assembles a series of DNA probe arrays of known sequence on a glass wafer using repeated cycles of photolithography. A combinatorial array of known DNA sequences is thus manufactured at specific sites on the chip. In the next step a fluidics station automates the hybridization of the array with the target DNA marked with a fluorescence tag. The times, temperatures, and stringency of hybridization are controlled in much the same way an experimenter does in a Northern or Southern blot hybridization. Using an argon-ion laser, a scanner excites the fluorescent tag, and the amount of emitted light is proportional to the amount of target DNA at each position of the array generating a quantitative two-dimensional image of hybridization intensity. Image processing software converts the fluorescent intensities from the probe array to generate genetic information.

Fragment-Based DNA Printing: An important difference between this technique and photolithography and the ink-jet methodology is that this method uses DNA fragments, generated, for example, from cDNAs, BACs, and other biological products, and binds them onto an array rather than building synthetic products onto an array. The basic method, currently in use by both the Brown Laboratory and Molecular Dynamics, is essentially a simple chromatographic procedure. A glass slide is coated evenly with a substance that gives the slide an even binding surface that is positively charged, such as amino silane (Molecular Dynamics) or polylysine (Brown Laboratory). DNA fragments are suspended in a denaturing liquid and spotted onto the prepared slide by the arrayer by lowering the printing tips until they just barely touch the surface of the slide. The denatured DNA is favored to react with the positively charged glass slide and binds immediately. These arrays are then hybridized with cDNAs from two separate sources (e.g., similar samples treated under different conditions or samples from different tissue types or organisms). The cDNA from each source is labeled with a different fluorescent marker and hybridized to the array. The scanner, working under similar conditions to those described above, allows one to detect the relative expression of the genes of interest, providing a snapshot of the overall expression levels of genes of interest. [The Brown laboratory website (http://cmgm.stanford.edu/pbrown) provides a complete protocol for their printing methodology.]

Ink-Jet Method: This method, being used for example, by Incyte Pharmaceuticals and Rosetta Biosystems, Inc., is uniquely adapted from the technology currently used in ink-jet printers. In fact, Rosetta Biosystems directly uses only marginally modified ink-jet cartridges supplied by Epson printers. Ink-jet technology works through the piezoelectric effect, whereby a narrow tube containing a liquid of interest (olgonucleotide synthesis reagents for the arrayer) is encircled by an adapter. An electric charge sent across the adapter causes the adapter to expand at a different rate than the tube and forces a small drop of liquid containing phosphoramidite chemistry reagents from the tube onto a coated slide. This methodology allows one, drop by drop, to precisely synthesize an oligonucleotide directly on the slide. Analysis of the array from there proceeds very much along the lines of the previous methods.

the virus infecting the individual. Although it is bemoaned that each test can cost $400, there is actually an immense cost effectiveness in not prescribing drugs that are likely to have no effect in patients who have developed resistance. The drug regimen can thus be designed to suit an individual in question. The impact in the area of drug management could therefore be enormous, as insurance companies will save millions of dollars and patients will be left with only those drugs in the cocktail that are likely to produce results. Putting things in perspective, it should also be remembered that not so long ago individuals were charged as much as $150–$200 per ELISA or PCR-based test.

The p53 chip, also marketed by Affymetrix, can be used to determine whether individuals are carrying p53 mutations that predispose them to cancers. The therapeutic value of this is immense in that early diagnosis allows for early intervention therapy that could add years to a patient's life. No doubt, there will be custom-designed BRCA1 chips and, possibly, chips for every gene associated with a cancer phenotype. Currently, the p53 chip is available from Affymetrix for $125. It should be noted that custom-designed chips will, however, cost much more. Affymetrix, more than anyone, contends that ''market availability will be enhanced by manufacturing cost-effectively custom-designed chips.''

The cost of this technology can, however, become mind boggling considering that one may have to use several chips per experiment, and that, for Affymetrix, the instrumentation alone comes with a $135,000 price tag. Molecular Dynamics will be providing a fully integrated system, including the arrayer, the scanner, the computer, the software (operating and bioinformatics), and optimized reagents, at somewhere near $250,000 when they start to market their high-end genomics/pharmaceutical technology.

Such pricing must overwhelm the experimenter—forever contending with shrinking NIH budgets. The exasperation, evident at meetings where cost questions are always raised, is matched, however, by equal exasperation from researchers such as David Botstein of Stanford University who remembers the advent of restriction enzyme technology and how inaccessible that once seemed—then available only to those who had the ability and technology to purify enzymes themselves; today freezers in laboratories are dedicated to them. But even with current costs, surely Howard Hughes laboratories around the country could have financial access to chip technology—and then there are core facilities instituted at universities for precisely that purpose. When one thinks that an ABI DNA sequencer costs as much as $120,000, there should be less reason to moan and groan.

For those interested in gene expression, function, and regulation, Joseph DeRisi ascertains that one can build an arrayer for under $25,000. This covers all of the robotics stages and the table but does not include the price of the PCR primers to PCR genes of interest into an array on the glass slide nor the cost of the scanner, which will depend heavily on the type of scanner. Commercial

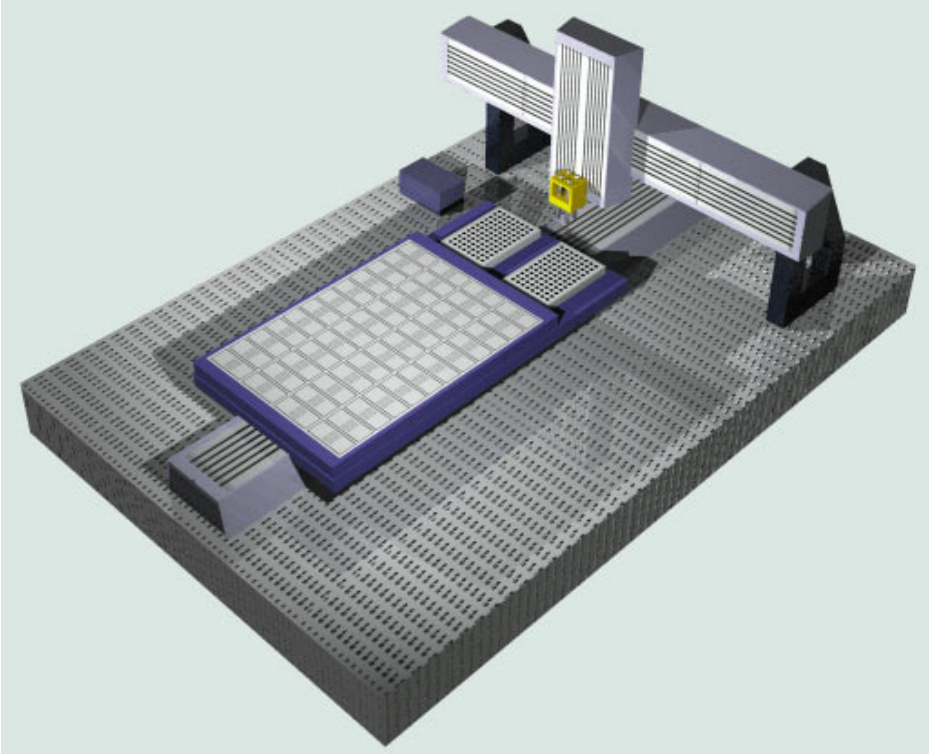

Figure 1 Schematic of the arrayer designed in Patrick Brown's laboratory. Shown is the basic system. Three servo-motor powered linear rail tables (Daedal Series 500000) are mounted on an anti-vibration table (Newport). Inside the PC, a Galil DMC-630 controller card (Galil Motion Control, Sunnyvale, CA) controls the system via communication through a 60 pin ribbon cable to the Galil power supply and servo driver.

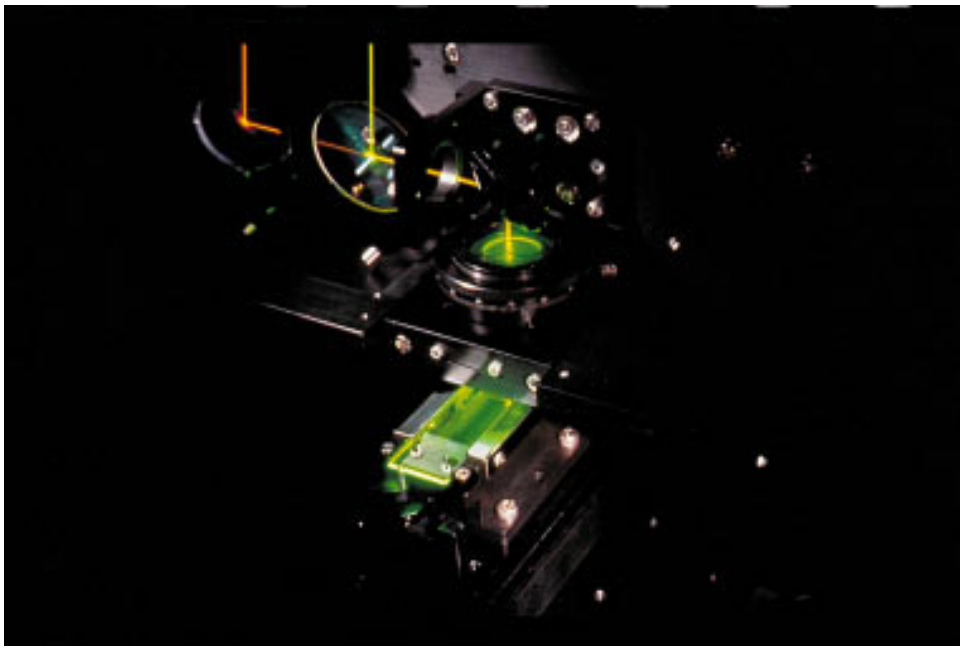

Figure 2 Microarrays from Molecular Dynamics and Amersam Pharmacia Biotech utilize confocal laser microscanner technology for widefield detection.

scanners are available but can also be built. The wherewithal to build all the neccessary equipment for the expression chips is commercially available, and the Brown laboratory happily shares all of the technological information on the Internet for no price at all (http://cmgm.stanford.edu/pbrown). To further the availability of this technology to researchers, the Brown laboratory is in the process of creating a comprehensive guide for the *nonengineer* to set up the necessary infrastructure.

However, for the more expensive equipment, there still remains a ray of hope considering that Affymetrix plans to set up centers around the country to make this technology accessible. To allay the frustration of scientists who feel this technology is unattainable, Robert Lipshutz, Vice President of Corporate Development at Affymetrix, says that Affymetrix hopes to set up as many as nine core centers in various regions around the country by the end of 1998. There is already one on site at the company's headquarters in Santa Clara, CA. The Affymetrix user centers will literary be freely accessible to scientists who competitively apply. The support for this, according to Robert Lipshutz, comes from the companys own resources and from external grant funding. The description of these will presently be available on Affymetrix's website at http://www.affymetrix.com or usercenter@affymetrix.com.

Molecular Dynamics and Amersham Pharmacia Biotech are also devising ways to make these products more accessible to the general researcher. They are currently developing a microarray technology access program that will have two distinct platforms: one aimed at high-end pharmaceutical applications and the other geared toward a lower cost research version, which is destined for academic laboratories. Additional information about the products available at Molecular Dynamics can be found at their website at http://www.mdyn.com.

The survival of all the companies now exploring microarray technology, however, will undoubtedly lie in the collaborations they set up with pharmaceutical giants. For what is variously called a ''development fee and milestone payments'' or ''technology access program,'' these agreements allow the development and testing of specific probe arrays for the detection of choice gene

sequences, mutations, or organism's genomes. Among others, Affymetrix has struck deals with Glaxo Wellcome for generating an HIV database where correlations will be made between mutations and response to antiviral drugs; with OncorMed to study *p53*, *BCRA1*, and *BCRA2*, and other genes in cancer diagnosis; with MIT for gene expression to be used in therapeutic discovery and the analysis of polymorphism; with Hoffman-LaRoche for gene expression monitoring in *Haemophilus influenzae* and *Streptococcus pneumoniae;* with Incyte Pharmaceuticals for developing a genomics database; and with Merck and Pfizer for monitoring gene expression. Molecular Dynamics and Amersham Pharmacia Biotech, with the establishment of a microarray technology access program, have already concluded agreements with SmithKline Beecham, Zeneca Limited, Sequana Therapeutics, Memorial Sloan-Kettering Cancer Center, Genos Biosciences, Huntsman Cancer Institute, the Insitute for Genomic Research, and Chiron Corporation.

These megamillion dollar collaborations, which will drive these companies from the initial loss in earnings reported to profitability, will ultimately provide prices discounted from the current ones. And to make the system more appealing to the experimenter, there are plans at Affymetrix to develop ''gene family chips,'' which would be more attractive to those studying specific systems and pathways. Other interesting developments include those presented at the recent Genome Sequencing Meeting at Hilton Head (The Ninth International Genome Sequencing and Analysis Conference. Hilton Head, SC, September 13–17, 1997) by David Cox (Stanford University in association with Affymetrix) of a strategy using gene-chip technology for determining the minimal tiling path needed to sequence a stretch of 6 Mb of DNA. Interestingly, although the number of chips required for the procedure seemed to make the experiment prohibitively expensive, after calculating the estimated cost to an average of only ~2–4 cents per base—which could bring the cost for the remaining mapping portion of the sequencing efforts way down and, in addition to providing the minimal tiling path, also allowed one to confirm BAC integrity and to check the sequence after sequencing—the use of chip technology seemed relatively cheap. Chips are also being used for mapping single nucleotide polymorphisms (SNPs). These will find eventual applications in linkage studies, loss of heterozygosity, DNA fingerprinting, and, as described by Eric Lander, also at Hilton Head, the resequencing of genomes after they have been completed. When the human genome is sequenced, ''human gene chips'' can allow for genome-wide expression analysis.

Finally, it should be stressed that this is *developing* technology and, as expected, comes with a higher cost at this stage. It should likewise be anticipated that the cost will decrease. It is also imperative to remember that as developing technology, there remain substantial obstacles to overcome in the design and analysis of these microarrays. Problems concerning, for example, the identification of heterozygous mutations still remain, and overall quality control from array to array needs to be addressed. In addition, as indicated by Richard Johnston, Director of Advanced Research at Molecular Dynamics (who is working to uncover error sources in this technology), effort needs to be put into designing ways to maximize the information obtained from microarrays. This too, in turn, should reduce overall cost per experiment as well as produce higher quality, more reliable results.

Given the above caveats, however, DNA array technology holds powerful promise, and current concerns about cost are likely to be minimized as commercial competition grows and technological advances are made. It could even be conceivable that at some point in the future an individual's entire genome could be placed on a chip at a reasonable cost; the term ''genochipped'' to describe such an analysis may become part of everyday medical lingo. The advantages of this technology in the fields of biomedical research, genomics, and clinical diagnosis are evident. The limitations on using this technology will only be determined by the limits set by one's own imagination.

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