Innovations

One-step genome analysis
Synteni, Inc.

Ignore all claims from genome-project proponents that gene discovery is dead. Until the function of each and every gene is determined, it will be vital to rediscover genes in different contexts so they can be placed in the right part of the biological puzzle. Synteni, in Palo Alto, California, is promoting one of the more recently devised methods of gene discovery and rediscovery, which uses arrays of complementary DNA (cDNA) clones on chips. The chips can assess the relative expression levels of thousands of genes simultaneously, information that can be used in drug-discovery projects, diagnosis, toxicology and basic research. Genes whose expression increases or decreases under a particular condition can be pulled out and studied as potential regulators or effectors of that condition.

The technology

In 1992 Patrick Brown of Stanford University was contemplating a forbidding task — a complex hybridization involving many probes and many targets that would help him in a linkage-mapping experiment. It occurred to Brown that hybridization technology could be pushed a great deal further with some intelligent engineering, and to take on this project he enlisted Dari Shalon, Mark Schena and Ron Davis. Shalon, now President and CEO of Synteni, was a doctoral student in Brown's laboratory at the time.

The results of their labors were published in *Science* in 1995. 'The principles are very simple,' says Brown, 'and it's very robust'. A robotic device dips tiny capillaries into the wells of a standard microwell plate containing solutions of individual cDNAs. Loading of the solution into the capillaries is driven by surface tension, and the solutions are 'printed' onto a glass slide with a gentle tap. The robot offsets the next set of samples slightly so clones can be deposited with as little as 100 μm between them. The DNA is linked to the slide by various chemistries, heat-denatured, and probed with mixtures of other cDNAs that have fluorescent markers attached. If the fluorescent probes stick, the hybridization is detected by a laser confocal scanner (see Fig. 1).

Why is this useful?

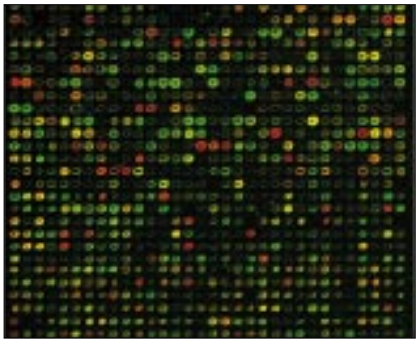
The choice of glass chips and fluorescent detection is not random. Fluorescent signals do not disperse and so can be used at high density. More importantly, several fluorescent detectors can be used at once. This allows the expression levels of large numbers of genes to be compared simultaneously. In the original *Science* paper, for example, the authors probed a panel of plant cDNAs with total cDNA obtained from root and leaf mRNA. A clone that hybridized with the leaf probe more strongly than with the root probe would obviously be of interest to a researcher studying leaf development or physiology. Since then, the Stanford and Synteni groups have analyzed samples collected both before and after heat shock, addition of phorbol esters (activators of growth-promoting signal-transduction pathways) and addition of a chromosome 6 fragment that suppresses the tumorigenic properties of a human melanoma cell line.

Synteni now has a long list of clients, including a number of the largest pharmaceutical companies. The company does not disclose the nature of these projects, but Shalon says they fall into several classes. The most exciting type of project involves the direct identification of a drug target. The target gene is identified on the basis of its increased expression in a cell where a target pathway (such as an inflammatory response) is known to be activated. In theory, Synteni should be able to evaluate entire pathways, identifying, for example, all genes whose expression is regulated by a particular transcription factor. A second type of project uses smaller chips displaying a few well characterized clones to provide an initial toxicity screen for drug candidates. Using this screen, Synteni can detect whether cells treated with a compound increase the expression of damage-induced genes; if so, animal testing is less likely to be warranted. Finally, the chips can be used for the diagnosis of certain disease states that have characteristic patterns of gene expression.

The competition – cDNA analysis

In an area with so many applications it is not surprising that Synteni is not

Figure 1



A sample experiment using a Synteni chip, in which 1056 clones randomly selected from a peripheral lymphocyte library were printed onto a 1 cm^2 chip. Two probe sets (Jurkat cell total cDNA (green) and bone marrow total cDNA (red)) were then hybridized to the array. The intensity of the signal gives an indication of the expression level. The greener the spot, the more that gene is expressed in Jurkat cells; the redder the spot, the more that gene is expressed in bone marrow. Yellow spots indicate equal expression of the gene in the two tissues. The colors are for visualization purposes only; expression levels are normally quantified in numerical terms.

Innovations is a new monthly column that surveys the science done at biotechnology companies.

Table 1

Synteni and cDNA arrays vs. Affymetrix and oligonucleotide arrays – claim and counterclaim.

Synteni claims that their chips...	Affymetrix counters that...
Can be used to discover unknown genes.	This is soon to be irrelevant with the pace of genome projects.
Can use arrays with less features (1 spot = 1 gene).	True, although the greater density of oligonucleotides partially offsets this. Also, no gene is repeated in Affymetrix arrays, whereas cDNAs are often repeated in libraries.
Show less contradiction in hybridization results, as longer probes give less false positives.	Affymetrix's mismatch controls detect false positives. Any conflicting information is processed by a fully automated algorithm.
Can differentiate between genes that are up to 70% homologous because of long probes.	Affymetrix chips have better resolution, and can differentiate between closely related homologs and splice variants.
Have good quality control. Much of the variability that occurs in chip construction does not affect the results, as what matters is the ratio of results not the absolute values.	If the oligonucleotide arrays fail it is usually at the level of the whole chip, and this is easily detected. For the cDNA arrays each spot is a separate quality control problem.
Are built with simpler technology.	Building Affymetrix chips only requires sequence information, not cDNA libraries.

alone. Hyseq, of Sunnyvale, California, uses two techniques originally developed for DNA sequencing to provide much the same types of information that Synteni does. Radoje Drmanac, Senior Vice-President for Research, explains that, in the first technique, Hyseq arrays random clones from cDNA libraries on membranes, then hybridizes a series of oligonucleotides to the clones (usually 300 distinct hybridizations, each with a different 7-mer). Clones that show identical hybridization patterns are deduced to be identical, and their frequency in an array gives a measure of their expression level. 'It's a more cumbersome approach," says Shalon, pointing out that each sample must be hybridized many times and that the number of cDNA clones that must be arrayed is very large. Drmanac counters with the assertions that Hyseq has the ability to array enough clones, and that any approach using cDNAs should attempt such coverage in any case. "This holistic approach is critical,' he says. Synteni uses similar arrays of random cDNAs in some cases, primarily for gene discovery.

Testing known genes

Some of the applications mentioned above do not require gene discovery, using instead arrays of known genes. As genome projects advance, arrays of known genes or expressed sequence tags (ESTs) will expand, and probably replace the redundant random cDNA arrays even in target discovery projects. The Synteni technique can be directly adapted to this approach, and ~50 % of their chips already contain known cDNAs. Hyseq have developed a second technique to monitor known genes, in which they spot arrays of oligonucleotides, hybridize cDNAs, and then probe with a second set of oligonucleotides. The double-hit hybridization process means that they only need two probe sites per gene. Their largest array can monitor the expression of 100 000 genes in 384 cm^2.

The competition – oligonucleotides

The closest competition in oligonucleotide-chip technology is, however, Affymetrix Inc. of Palo Alto, California. Affymetrix, founded in 1992 as a daughter company of Affymax N.V.*, forms its arrays of oligonucleotides by direct synthesis on the chip instead of 'printing' spots of pre-made cDNAs. Affymetrix has been using its chips for DNA sequencing, but have recently entered the area of expression-level analysis.

Affymetrix's technology uses a photolithographic mask to allow photolabile groups to be deprotected selectively according to their precise position on the chip, and builds up oligonucleotides by repeated cycles of deprotection and coupling. Up to 400 000 different oligonucleotides are routinely synthesized on a ~1.6-cm^2 chip. A gene can be represented by 20 different oligonucleotides plus 20 mismatch oligonucleotides as controls, so the expression levels of up to 10 000 genes can be measured on a single chip. This gene density doesn't yet reach that of a Synteni chip, although these densities are constantly evolving. The two companies have a well-developed debate about which approach works best (Table 1). On one of the most important issues, specificity, both sides claim superiority for different reasons.

Does more complicated technology always win? Shalon doesn't think so. 'So far we haven't lost a single customer to Affymetrix,' he says. For now it seems that, despite all the claims of great speed by both companies, there is more than enough work to go around.

* see 'Variety is the spice of life', *Chemistry & Biology* 4 79–80

William A. Wells, Biotext Ltd
211 Hugo Street, San Francisco,
CA 94122–2603, USA; wells@biotext.com.