

Technology Focus: DNA Microarrays

Introduction

The sequencing of the human genome provided us with the elemental code that determines our genetic make-up. The race to decipher that code and translate how the myriads of genes and their splice variants interact in the context of health and disease spawned a new species of ultrahigh-throughput technology for genetic analysis: DNA microarrays. Although there are a variety of DNA microarray systems/technologies available, they are all based on the same simple set-up: a solid support onto which sequences of nucleic acids representing many thousands of different genes have been immobilised in a high-density array, which is then hybridised with labelled nucleic acids extracted from a sample of interest, and analysed by detecting the amount of labelled nucleic acid that has bound to the array using laser scanning devices.

The main DNA microarrays in use are **Affymetrix's** GeneChip™ arrays and a variety of cDNA microarrays. The key difference between them is the way the genes are represented on the arrays, and how the relative abundances of the transcripts are calculated, the two aspects being integrally linked. On GeneChips, individual genes are represented by a series of short 20-mer oligonucleotides (oligos) representing a significant proportion of the gene. Each matched-sequence 20-mer oligo is accompanied by a mismatched sequence that accounts for non-specific hybridisation, and the relative abundance of a given gene in a sample is calculated based on the hybridisation intensity of all the matched and mismatched oligos for that gene. In general, GeneChip experiments involve a single sample hybridised to a single chip, and differences in expression levels between samples on separate chips are calculated following software-based normalisation to account for minor variations in reaction conditions and sample preparation. For cDNA microarrays, each gene is represented by a single sequence of 60 nucleic acids or more, and lacks an internal control for non-specific hybridisation. However, cDNA microarray experiments generally involve the simultaneous hybridisation of two samples (e.g. infected and uninfected) to a single array, thus providing some kind of control. The two platforms both have advantages and disadvantages but are now well established with a wealth of quality-control data to verify their robustness.

Into the Market-Place

Although hailed as a technical success, the advent of DNA microarray technology was initially only felt in the research community, where the prohibitively high costs of using the GeneChip system saw many academic institutions develop their own in-house cDNA microarrays and share these with other not-for-profit investigators. This situation changed as the potential application of DNA microarrays in the pharmaceutical industry was realised, and production costs were driven down. In addition, many companies offered deals to academic researchers in order to promote their products and gain credibility. A new niche market was soon created, now estimated to be worth as much as US\$1 B, and set to see substantial growth over the next few years as the technology continues to expand and new applications are found.

Aside from the revenues generated from standard sales of DNA microarrays and associated capital equipment, the glowing health of this sector is reflected in the sizeable increase in the number of deals that have been made over the last decade (*Figure 1*). As might be predicted, the market leader for DNA microarrays is Affymetrix, which has made a total of 110 deals concerning the technology since 1997 (to June 2006). Other DNA microarray companies that have been active in deal making over the same period include **CombiMatrix** (of **Acacia Research**) (36 deals) and **Agilent Technologies** (19 deals). One of the reasons that Affymetrix has been so prolific in its deal making is its 'Powered by Affymetrix' (PBA) programme; this facilitates the full integration of GeneChip technology into a company's ongoing development programme and provides a full support service as well as the materials necessary to complete the research. Companies that have entered into agreements as part of the PBA programme include **bioMérieux Vitek** (Deal no. 00925), **Vita Genomics** (Deal no. 22995) and **PathWork Informatics** (Deal no. 22194).

Research and Development

DNA microarrays have enjoyed great success in almost all areas of biomedical research but, in general, experimentation falls into one of three main categories (*Text Box*), with companies offering prefabricated whole-genome arrays that are tailor-made for each application area. In addition, a number of companies provide custom-made arrays representing only genes pertinent to the research in hand. Outsourcing of DNA microarray experimentation as a bespoke service has also developed, with companies able to send away their raw sample materials for a quality-controlled DNA microarray analysis, thus allowing smaller biotechs who do not have the resources to optimise the technology in-house to integrate DNA microarray analysis into their research programmes.

In general, it has been the universities and academic institutes that have used DNA microarrays purely for fundamental research. However, it is worth noting that a number of global pharmas were quick to gain access to Affymetrix's GeneChip arrays, with **Merck & Co.** and **Roche** entering technology access agreements as early as 1996 (Deal nos. 00469 and 00648), and **GlaxoWellcome** doing the same in 1997 (Deal no. 01074). Similarly, **Chiron** entered a collaborative R&D agreement for the continued development of **Molecular Dynamics'** and **Amersham International's** DNA microarray system in 1997 (Deal no. 01534).

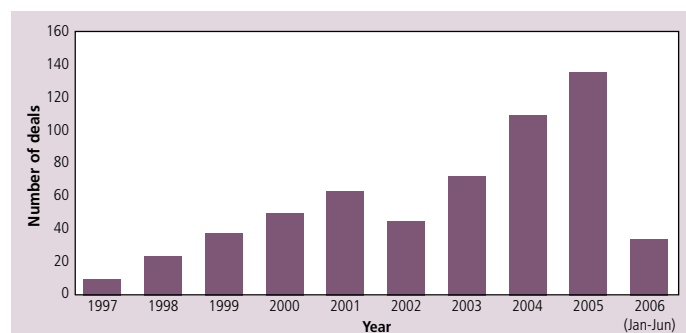


Figure 1 – Total number of DNA microarray deals from January 1997 to June 2006 (Source: PharmaDeals® Agreements).

A landmark piece of research that highlighted the value of DNA microarrays was published in 2003, in which gene expression profiles (GEPs) were used to identify subtle differences between subtypes of diffuse large B-cell lymphomas that directly related to patients' survival prognosis.¹ This showed that DNA microarrays could be used to reveal patterns of gene expression that could be targeted for therapeutic intervention, and further paved the way for DNA microarray exploitation in drug discovery and diagnostics.

Drug Discovery

Two key areas in which DNA microarrays have influenced the drug discovery process are target identification and toxicogenomics. This type of research is illustrated by the recent (March 2006) deal between **EiRx Therapeutics** and **Almac Diagnostics** to model the transformation events associated with apoptosis in the early stages of colorectal cancer (Deal no. 24271). Here, the key genes identified by the collaboration will act as targets for the design of new drugs to selectively induce apoptosis in colorectal cancer cells.

Toxicogenomics is the combination of toxicology and genomics using DNA microarrays. By establishing GEPs for a wide range of toxic compounds, it has been possible to create a database resource that allows the toxicology of a novel compound to be predicted, based on the GEP that it induces. This technology allows pharmaceutical companies to make substantial savings by cancelling those compounds that have high toxic potential early in the development process. Once again, major pharmas were among the earliest to show interest, with **Wyeth-Ayerst Laboratories** (now **Wyeth**) forming an alliance with **Gene Logic** to develop a toxicogenomic database in 1998 (Deal no. 02920). In 2001 **Pfizer** also entered into an agreement with Gene Logic, this time to develop custom DNA microarrays using Affymetrix's technology for mechanistic toxicology studies (Deal no. 07314). More recently, in February 2006, Affymetrix signed a collaborative R&D agreement with **Iconix Pharmaceuticals** to develop new solutions for toxicological studies using its proprietary DrugMatrix® 640 compound reference database in combination with Affymetrix GeneChip-derived data (Deal no. 23335).

Diagnostics

The potential for the expansion of DNA microarray technology into the diagnostics market was realised from the beginning and, since 1997, almost a quarter of deals involving DNA microarrays related to the development of diagnostic products (134 out of 580), with cancer being the focus of nearly three times as many deals as any other interest area.² bioMérieux was one of the first companies to begin developing DNA microarray diagnostics using Affymetrix's GeneChip technology (Deal no. 00925). The collaboration initially aimed to develop arrays for bacterial identification and antibiotic resistance, but the focus later shifted to include HIV genotyping arrays and sepsis diagnosis, and finally to the development of an *in vitro* diagnostic (IVD) test for breast cancer. PathWork Informatics has also been working on an IVD test for cancer, aiming to combine its proprietary oncology diagnostics with Affymetrix's GeneChip platform (Deal no. 22194). The drive for DNA microarray-based cancer diagnostics is to enable patient-specific treatments to be generated based on the GEPs of the tumour cells in question. However, DNA microarray-based tests for this type of 'personalised medicine' go further than just cancer diagnostics. Vita Genomics has been developing Affymetrix arrays to test for

The major DNA microarray applications

Gene expression profiling (GEP)

Analysis of the level of gene expression in a sample of interest relative to a reference sample, e.g. infected and uninfected cells, by hybridising the labelled transcriptome to DNA microarrays representing the individual genes of the entire human genome

Comparative genomic hybridisation (CGH)

Measurement of gains and losses in the copy number of chromosomal regions by hybridising genomic DNA from control and diseased tissues, e.g. normal and cancerous, to DNA microarrays representing the different chromosomal regions of the human genome.

Single nucleotide polymorphism (SNP) analysis

Detection of single nucleotide mutations or polymorphisms in gene sequences by hybridising genomic DNA from normal tissues to DNA microarrays representing more than 100,000 different SNP sequences, to provide an indication of disease susceptibility based on the prevalence of known disease markers.

interferon alpha treatment responsiveness in patients with HBV and HCV (Deal no. 22995), while the **US Air Force Research Laboratory** has been working with CombiMatrix to develop a personal health monitoring system that comprises a biomarker-based DNA microarray (Deal no. 23510).

Roche Diganostics has had a long-running collaboration with Affymetrix that resulted in the launch of a GeneChip-based diagnostic test (AmpliChip™ CYP450) in the EU in September 2004. To develop this, Roche licensed the GeneChip technology from Affymetrix, and the rights to a patent on polymorphisms in the promoter region of the cytochrome P450 gene CYP2D6 from **EPIDAUROS Biotechnologie** (Deal nos. 12171 and 19159). The test detects genetic variations in two CYP450 genes and provides the associated predictive phenotype that relates to the patient's ability to metabolise drugs via the enzymes that the genes encode. This test is of particular note, as there are huge numbers of drugs that are metabolised via these genes, which extends the application of the test not only into the general clinic but also into screening potential candidates for clinical trials. One of the key problems that DNA microarrays have faced in the transition to use as a clinical diagnostic is the extensive requirements that are essential for use in a clinical setting. This is why many DNA microarray manufacturers have left this type of development to others.

The single biggest issue facing the use of DNA microarray-based diagnostics is cost. For patients willing to pay the price of private care, these tests are likely to provide a valuable part of the healthcare system. However, for the wider masses, it is likely to be some time before members of the general public have their genomes scanned and their medicines 'personalised' on a routine basis, despite the obvious benefits that recent diagnostics tests, such as that for HER2 positive breast cancer, have been shown to provide.

¹ Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Alizadeh, A.A. *et al.* Nature (2000) 403(6769):503–11.

² Source: PharmaDeals® Agreements.