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An Introduction to DNA Chips

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The GeneChip, which is gaining popularity throughout the field of molecular biology and biotechnology, is a recently developed technique which provides efficient access to genetic information using miniaturized, high-density arrays of DNA or oligonucleotide probes. Both fundamental and applied research benefit greatly from the specific advantages of this method. Initially developed to enhance genome sequencing projects, the technology is rapidly evolving and has been adapted to a large set of applications. DNA chips are powerful tools to study the molecular basis of interactions on a scale that would be impossible using conventional analysis. The recent development of the microarray technology has greatly accelerated the investigation of gene regulation. Arrays are mostly used to identify which genes are turned on or off in a cell or tissue, and also to evaluate the extent of a gene's expression under various conditions. Indeed, this technology has been successfully applied to investigate simultaneous expression of many thousands of genes and to the detection of mutations or polymorphisms, as well as for their mapping and sequencing. In this chapter, we discuss the format of DNA chips, the technology used to fabricate and read them, and their applications.

Introduction

The occurrence of DNA chips in the second half of 1990s became a new biotechnical revolution equivalent in importance to the decoding of DNA structures in the 1950s, research of the fundamental laws of molecular genetics, such as genetic code, and the major dogma of molecular biology in the 1960s, discovery of

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reverse transcription, the creation of first recombinant constructions in the 1970s and research of enzyme methods manipulations with genetic material *in vitro*, like amplification technologies in the 1980s. This technology has come a long way since the first official meeting of scientists working in this field, which took place in Moscow in 1991, comprising about 50 scientists from Europe and the USA. At that time, the initial aim was the development of a technology for high-throughput sequencing of the human genome, named sequencing by hybridization. The field quickly branched out, however, in its methodology and application. Today, DNA chips seem to be a common commodity in biological sciences.

DNA chips have been greatly successful in a variety of genomic analyses (Debouck and Goodfellow 1999), ranging from detecting SNPs (Wang *et al.* 1998) to functional genomics (Lockhart *et al.* 1996). There are different names for the chips, such as DNA/RNA Chips, BioChips, GeneChips or DNA arrays. The array, described as a macroarray or microarray, can be defined as an ordered collection of macrospots or microspots, respectively, each spot containing single defined species of a nucleic acid. Large sets of these nucleic acid probe sequences are immobilized in defined, addressable locations on the surface of a substrate capable of accessing large amounts of genetic information from biological samples in a single hybridization assay. Each spot represents the equivalent of a conventional analysis in a test tube. The matrix of spots applied to the biochip reproduces each of these analyses several times – up to a total of three or four – and thus increases the reliability of the test results.

The ‘key and lock principle’ of complementary hybridization is utilized in biochip analysis. In a typical application, DNA or RNA target sequences of interest are isolated from a biological sample using standard molecular biology protocols. The sequences are fragmented and labelled with reporter molecules for detection, and the mixture of labelled sequences (‘key’) is applied to the array, under controlled conditions, for hybridization with the surface probes (‘lock’). Sequence complementarity leads to the hybridization between two single-stranded nucleic acid molecules, one of which is immobilized on a matrix (Southern *et al.* 1999). The array is then imaged with a reader to locate and measure the binding of target sequences from the sample to complementary probe on the array, and software reconstructs the sequence data and presents it in a format determined by the application. In this way highly parallel DNA hybridization assays are possible on miniaturized flat substrates or ‘chips’.

There exist two variants of the chips, in terms of the property of arrayed nucleic acid sequence with known identity: Format I – cDNA microarrays and Format II – oligonucleotide arrays (Lockhart *et al.* 1996). Although both the cDNA and oligonucleotide chips can be used to analyse patterns of gene expression, fundamental differences exist between these methods. The microarrays are subsequently exposed to a labelled sample, hybridized and the identity/abundance of complementary sequences are determined.

Moreover, there are two basic approachs to GeneChip creation. Historically the first was to deposit on the chip previously synthesized oligonucleotides or cDNA fragments. The second approach is *in situ* synthesis using photolithography or piezoelectric printing that uses inkjet printer-like technology.

The chapter is organized as follows. First, the basic structure and design of DNA chips, including their production process and functioning is presented. Requirements for DNA chip handling, pointing out advantages and disadvantages of the technique, are then described. Finally, showing prospectives and directions of the future research, practical values and possible fields of its application are suggested.

DNA chip structure and operating principles

Integrated circuits technology

An integrated circuit (IC) called a chip or microchip in electronic technology is an electronic circuit built on a semiconductor substrate, usually one of a single crystal of silicon. The circuit is packaged in a hermetically sealed case or a non-hermetic plastic capsule, with leads extending from it for input, output and power-supply connections, and for other connections that may be necessary when the device is put to use. There is also a different scale of integration of such circuits. In the case of very large-scale integration IC can contain more than 1000 transistors. Intel founder Gordon Moore observed that the number of transistors per semiconductor chip about doubles every 18–24 months. The present microprocessor chips (used in personal computers) contain more than 100 million transistors per several square centimetre. Such huge integration scale which has almost reached manufacturing technology limits, helps to make modern computers very fast, compact and relatively inexpensive.

A comparable phenomenon is observed in molecular biology. The miniaturization of certain tools is suitable for the construction of a smart and portable device – the spotted array system, which offers the pharmaceutical, biotechnology and agriculture industries more efficient and economical solutions. DNA microarrays built using photolithography have been on a similar pathway as integrated circuits. In 1998, an Affymetrix array contained less than 1000 genes; by 2000, it boasted 12 000.

In the process of manufacturing BioChips, semiconducting materials are widely used. A semiconductor is a solid material whose electrical conductivity at room temperature is between that of a conductor and an insulator. At high temperatures its conductivity approaches that of a metal, and at low temperatures it acts as an insulator. The substances first used for semiconductors were the elements germanium, silicon and grey tin. Although other materials can be

used, chips are usually fabricated on wafers of single-crystal silicon, that is, silicon in which the orientation of all of the crystal is the same. The major fabricating steps include film formation, impurity doping, photolithography and packaging. Silicon oxide, also called silica, is grown on the surface of the silicon during the film-formation stage. Photolithographic methods are used selectively to remove the oxide from areas of the silicon. A layer of photoresist is added to the oxide layer and then exposed to ultraviolet light through a mask. After exposure, the silicon wafer is developed, and an etching process removes the unwanted areas of the oxide. Impurity doping adds charge carriers to the silicon; this process produces the unique electronic properties of semiconductors. Additional layers of silicon are deposited to create for instance bipolar transistors.

DNA chip design

There are several steps in the design and implementation of a DNA chip technology: probe creation, chip fabrication, sample preparation, assay, readout, and software (Table 7.1). Many strategies have been investigated for each of these steps.

Table 7.1 Steps in the design and implementation of a DNA chip experiment

1. Probe creation	2. Chip fabrication	3. Target (labelled sample preparation)	4. Assay	5. Readout	6. Informatics
capture probe choice: • digonucleotides • PCR from genomic DNA and from cDNA	putting probes on the chip: • spotting (contact printing with needles; non-contact printing with piezo elements) • on-chip *in situ* synthesis (mask controlled synthesis; maskless synthesis)	• RNA extraction & purification => total RNA and mRNA • RNA amplification => cDNA double strand synthesis and *in vitro* transcription	• pre-hybridisation • sample hybridisation	• optical detection transmission (phosphoimager, fluorescence reader) • electrical detection (electrical readout by redox-recycling)	• data output, analysis, interpretation and visualisation

Probe creation

Production of microarrays begins with the selection of probes to be printed on the array. In most cases, these are chosen directly from databases including GeneBank (Benson *et al.* 1997) and UniGene (Schuler *et al.* 1996).

Two commonly used types of chips, Format I and Format II, differ in the size of the arrayed nucleic acids. In Format I, cDNA probes, which are relatively long DNA molecules (500–5000 bases long), are immobilized to a solid surface such as membranes, glass or silicon chips. The probes are often single-stranded DNA fragments processed with the help of polymerase chain reaction (PCR). These PCR products amplified with gene-specific primers are generated using chromosomal DNA as a template, and subsequently purified by precipitation or gel-filtration, or both. Spotting cDNAs or PCR products representing specific genes onto a matrix produces DNA arrays. The deposition of a few nano-litres of purified material usually at $100-500\,\mu g/ml$ generates each array dot. The printing is carried out either by a high-precision robotic system, i.e. by high-speed robot spotting (arrayer) or utilizing inkjet technology that spots a sample of each gene product onto a number of matrices in a serial operation or printouts, respectively. These types of arrays are used mostly for large-scale screening and expression studies.

In Format II, an array of oligonucleotide probes is fabricated either by *in situ* light-directed chemical synthesis or by conventional synthesis followed by on-chip immobilization (McGall *et al.* 1996). Those with short nucleic acids (oligonucleotides up to 25 nt) are useful for the detection of mutations and expression monitoring, gene discovery and mapping.

The membranes commonly used for nucleic acid arrays are commercially available nitrocellulose and charged nylon that are employed in standard blotting assays (Southern blot, colony and plaque blot, dot and slot blot). The disadvantages of this method are that the genetic material is non-covalently attached which may result in its loss from the support, and that only a small amount of the DNA is available for hybridization. Glass-based arrays are most often made on microscope slides. They are coated with poly-lysine, amino silanes or amino-reactive silanes, which enhance both the hydrophobicity of the slide and the adherence of the deposited DNA (Schena *et al.* 1996). In most cases, DNA is cross-linked to the matrix by ultraviolet irradiation. After fixation, residual amines on the slide surface are reacted with succinic anhydride to reduce the positive charge at the surface. As the final step, the deposited DNA is split into single strands by heat or alkali. Adapting semiconductor photolithography to synthesize oligonucleotide probes *in situ* on glass or membrane substrate produces oligonucleotide chips. These chips are designed and produced on the basis of sequence information alone, without the need for any clones, PCR products, DNA and so on. Probe arrays are manufactured by light-directed chemical synthesis, which combines

solid-phase chemical synthesis with photolithographic fabrication techniques employed in the semiconductor industry.

Silanol groups of silicate glass cannot couple oligonucleotides directly to the surface or to most plastics. It is necessary to create the surface with a group from which the growth of the oligonucleotide chain can be initiated. Such spacers also help to overcome steric interference, the ends of the probes closest to the surface being less accessible than the ends further away. Oligonucleotides on long spacers extend away from their neighbours and from the surface, and thus they allow more efficient interaction with the target. The spacer's length has a marked effect on hybridization yield. It has been shown that the optimal spacer length gives up to 150-fold increase in the yield of hybridization (Southern *et al.* 1999).

In addition to the flat-surface glass or silicon chips, supporting materials such as microscopic beads, nanochannel glass, 96-well microtitre plates, microelectrode array and phototransistor arrays are also used for depositing nucleic acid material. One of the most promising approaches is the microscopic bead-based chip technology, as it offers high sensitivity, flexibility and many replicates in one assay. These non-array methods are used efficiently to score large numbers of probes. They do not use spatial location as the key for probe identity, in contrast to flat-surface chips. Another interesting development is the use of nanochannel glass slides for array printing. Nanochannel glass materials are unique glass structures containing a regular geometric array of parallel holes or channels as small as 33 nm in diameter or as large as several micrometres in diameter (Watson *et al.* 2000). As a result, the surface area of nanochannel glass is much greater than that of regular glass, enabling larger amounts of nucleic acid material to be deposited in each spot. The hybridization kinetics are also greatly improved in this case.

DNA chip fabrication

DNA chip technology has evolved along two major paths (Table 7.2). In one method, nucleic acids (previously chemically synthesized oligonucleotides or single-stranded DNA fragments, i.e. cDNA) are immobilized on the chip surface sequentially to form capturing probes. Using either printing pins or inkjets, many copies of nucleic acid sequence can be attached to a substrate (Figure 7.1). Contact printing involves wetting a printing pin with the nucleic acid solution and tapping it on to the microarray surface. Inkjetting ejects uniform droplets of solution onto the substrate.

An alternative, known as *in situ* fabrication, builds the oligonucleotide sequence at each site one nucleotide at a time. This is done using either inkjets or photolithography (Figure 7.1). Construction of such chips begins with a substrate slide that has been chemically primed with sites ready to bind nucleotides. In inkjetting, solutions of nucleotides are ejected from the nozzle onto the substrate,

Table 7.2 BioChip basics

Method	Features and/or applications
I) accommodation on chip previously arranged nucleic acids:	
—spotting long DNA fragments (Stanford University)	• array of spotted PCR products • gene expression analysis
—array of prefabricated oligonucleotides, i.e. gel pads (Motorola) and microelectrodes (Nanogen)	• oligonnucleotides are attached to patches of activated polyacrylamide • controlled electric fields for immobilization
II) *in situ* synthesis of oligonucleotides:	
—photolitography (Affymetrix)	• light-directed oligonucleotide-synthesis on chip • adapted from semiconductor industry
—inkjet technology (Agilent)	• oligonucleotides are synthesized drop-by-drop • adapted from the technique used in ink-jet printers

and then chemically fixed to the surface. The next set of nucleotides are jetted onto the first and chemically fixed to those. The process is repeated until the desired set of nucleic acid is complete. In photolithography, a photosensitive chemical that detaches under illumination caps the sites. Light at 365 nm is shone through a patterned mask onto the chip, causing the capping chemical to break away from the areas it strikes, thus exposing the primed spots. A solution containing one of the four types of nucleotides (each molecule of which is itself attached to a capping molecule) is then washed over the chip. The nucleotides bond only to the areas that have been exposed, and add a capping layer themselves. As the procedure can be repeated with another mask and different nucleotide, a variety of DNA sequences can be built on the chip. Multiple probe arrays are synthesized simultaneously on a large wafer. This parallel process enhances reproducibility and helps achieve economies of scale (Southern *et al.* 1999). Production-scale chips can pack 400 000 probes in 20 μm patches. One weakness of the current photolithography method is that a new set of masks must be produced for every new type of array. There is a maskless technique that uses an array of micromirrors that reflect onto the appropriate spots on the chip.

The technology for spotting arrays is undoubtedly simpler than that for *in situ* fabrication. Simultaneous production of many arrays with the same set of probes makes the deposition more economical than *in situ* synthesis. Moreover, deposition is also a method of choice for long sequences, which are available as PCR products.

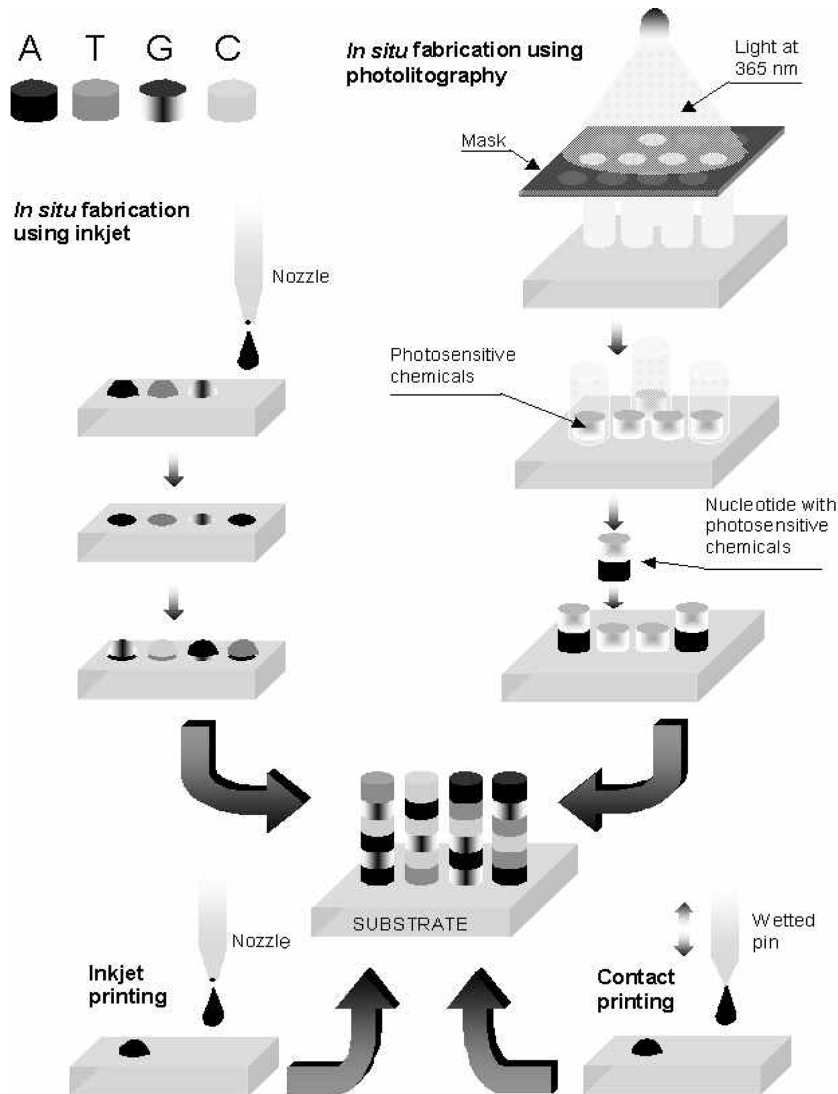

Figure 7.1 Methods for creating a DNA array

However, comparing the two types of nucleic acid arrays, arrays of prefabricated oligonucleotides or cDNAs and *in situ* (on-chip) synthesis of oligonucleotides, the latter has some advantages over deposition of pre-synthesized oligonucleotides. It is not profitable to make large arrays using pre-synthesized oligonucleotides attached to the surface. On the other hand, it is difficult to assess the quality of the oligonucleotides made on a surface. Therefore, this technique could be used for a quality control, but it is not available for most biological laboratories. In contrast, the pre-synthesized oligonucleotides can be assessed before they are attached to the surface.

Sample preparation and hybridization assay

There is still some confusion in the nomenclature of the target and probe. It is commonly accepted that the target determines the labelled material (RNA or DNA), and immobilized oligonucleotide, cDNA or PCR product is the probe. But sometimes probe is also defined as a piece of labelled RNA or DNA which is used in a hybridization assay.

The targets for arrays are usually labelled representations of cellular RNA or DNA pools. It has been found that the short targets can interact more efficiently with oligonucleotide or cDNA arrays than large ones. Ideally, target and probe should have the same length.

Lots of analyses are directed to complex targets, for example, human genomic DNA. In general, it is preferable to reduce sequence complexity to produce a good hybridization signal within a reasonable hybridization time. RNA as a target has a stable secondary structure, which can interfere with hybridization. To reduce this effect, RNA can be fragmented, preferably to a size close to that of the probes on the arrays. Secondary structure is less of a problem with DNA targets and PCR products.

The effects of different charged groups in the spacer were also examined in the hybridization process. It was shown that both positively and negatively charged groups in the spacer diminish the yield of hybridization. Additionally, the base composition and sequence of the oligonucleotides have also a large effect on duplex formation. The effect is of course due to the lower stability of A : T than G : C pairs. According to this, the oligonucleotides of the same length have correspondingly different T_m values. Adding an A : T base pair increases T_m by roughly 2°C, compared with 4°C for a G : C pair.

Readout and data analysis

The detection of the nucleic acid hybridization relies on the signal generated by the binding event. Scanning or imaging the chip surface is essential for obtaining the complete hybridization pattern. Fluorescence imaging is commonly used for such ‘reading’ of the chips (Figure 7.2). Although the predominant method for array signal detection is still based on fluorescence, many other new methods also show promise. Radioactive materials have the advantage of high incorporation efficiency, high sensitivity and low cost. They were not used in high-density arrays due to the lack of high-resolution imaging methods. Detection methods based on oxidation–reduction reaction, resonance light scattering, capacitance change after hybridization, resonance ionization mass spectrum methods and the nanoparticle-promoted silver staining detection have been also reported (Watson *et al*. 2000).

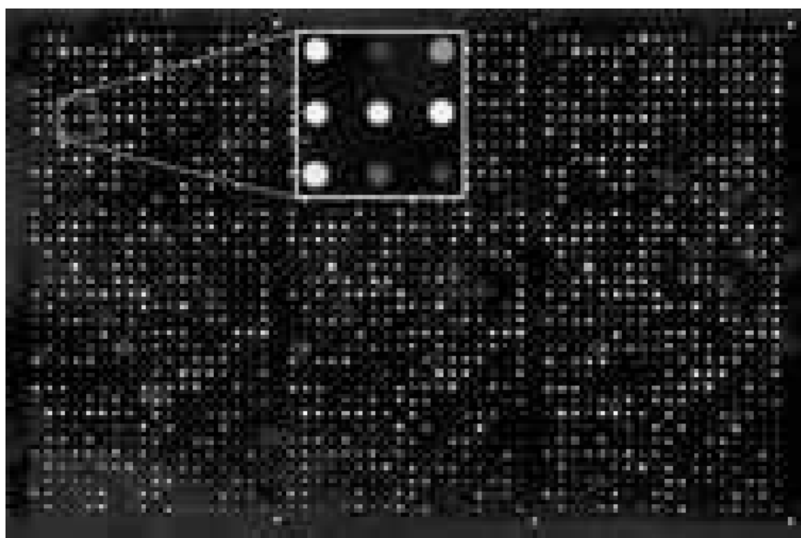

Figure 7.2 Hybridised chip with enlarged detail, ready for analysis

The use of biochip technology enables researchers to obtain experimental data using a highly parallel approach. Such experiments provide unprecedented quantities of data. Bioinformatics tools are used to relate the complexity of the data into useful information. Their interpretation should therefore be based on three main processes: statistical quality analysis, data interpretation and a presentation that makes it accessible to human thinking. Because of the shear wealth of information, only statistical procedures allow an assessment and filtering of microarray data. Data integration is essential, making connections that make subsequent interpretation feasible. This requires a modular data warehouse concept combining the storage of data such as raw signal intensities, gene annotations and their functional categories as well as experimental annotations. For later queries, the annotations should be determined by a pre-defined and catalogued vocabulary. Sophisticated computational tools for data visualization and reduction of data complexity are important to make the information accessible to a human mind, at least as long as there are no automatic expert systems.

Generally speaking, data collection and image analysis are the relatively mature part of the microarray data analysis problem. There are already many commercially available software packages or free software packages that can handle these issues reasonably well.

Achievements and future research directions

There was also a patent battle over the GeneChip (Table 7.3). This is understandable because this technology has already been applied to a diverse set of applica-

Table 7.3 The patent battle over the BioChip

Year	Event
1987	Patent filed on Sequencing By Hybridisation (SBH) R. Drmanac, Belgrade Univ. → Argonne National Laboratory → HySeq
1988–1991	Several groups published reports on SBH E. Southern, Oxford University (Oxford Gene Technology) A. Mirzabekov, Engelhard Institute, Moscow → Agronne National Laboratory S. Fodor, Affymetrix W. Bains, Bath University
1989	European patent granted to Southern on 'Oligonucleotide arrays as a testing platform'
1993	US patent on SBH granted to HySeq
1997–1998	HySeq accuses Affymetrix of patent infringement 'We are not doing sequencing, but mutation detection'
1998	Courtcase between Southern and seven chip companies (Affymetrix, HySeq, Hoffman La Roche, Abbot etc.)
1998	US patent to Incyte (Synteni) on technology to print microarrays with density higher than 100 polynucleotides per cm^2
1998–1999	Affymetrix and Incyte (and others) accuse each other of patent infringements

tions. DNA microarrays continue to gain popularity as a number of biotechnology companies aggressively pursue DNA chip technology enhancement and cost reduction.

In recent years a number of different methods for the microarrays have been developed. Electric fields have been used greatly to accelerate the hybridization of labelled target to immobilized sample oligonucleotides. The microarray was fabricated on a 1 cm^2 silicon chip. The silicon substrate was thermally oxidized and then platinized to form a 1 mm $\times$ 1 mm array of 25 microelectrodes. The electrodes were covered with a permeation layer of streptavidin-agarose, to which biotinylated DNA sample was coupled under a positive potential. Such a use of electric field to increase the transport rate of negatively charged probe leads to a ten-fold increase in the hybridization rate.

Another approach to perform and improve gene expression analysis is dynamic DNA hybridization (DDH) on a chip using paramagnetic beads (Fan *et al.* 1999). The advantages of this method are the dynamic supplies of both nucleic acid sample (target) and probe, and also use of the paramagnetic beads as a transportable solid support. It reduces hybridization time and makes the reaction more efficient. The magnetic coated beads are loaded with labelled capture sample or the capture samples are coated onto activated magnetic beads. In such a

microfabricated device, simultaneous analysis of many samples is possible. The microfluidic platform of the device is developed for automated analysis of nanolitre volumes. A pneumatic pumping apparatus transports probes and other reagents into the microfluidic device while hydrostatic pumping is used for the introduction of beads with samples. The sample/bead complex is introduced into the device in which hybridization takes place with a complementary probe. At the present time, the paramagnetic beads are extensively used for the preparation, separation and detection of biological molecules such as DNA, RNA and proteins. Their efficiency, simplicity and low cost are very favourable.

DNA chip is simple in concept, but generating probes on a solid array surface requires considerable expertise and technical expertise. Key matters in creating DNA arrays include: fidelity, reproducibility, ease of synthesis, flexibility, shelf-life, cost, hybridization conditions and steric considerations. As mentioned before, the major advantage of this technique is its high flexibility, which allows creation of a chip with any necessary sequences. But this approach has disadvantages that are difficult to resolve and which limit its usage. First is the time and resources needed to synthesize the required number of different oligonucleotides. Oligonucleotide arrays have longer shelf-lives, compared with cDNA chips, which may only be useable for a few weeks. Another difficulty is to extend the capability of the chip so that thousands of genes can be detected simultaneously. Further, fluorescence technology, which is the most commonly used detection method for array readouts, is reproducible, but is limited in sensitivity. That is why chemiluminescence, diode array detectors, direct electrical charge detection and piezoelectric readout are all being developed as alternative detection methods. Accelerated hybridization techniques are being developed by using electric field control.

Pointing out advantages and disadvantages of this technique reveals perspectives and directions of future research. The innovation of DNA chips opens up a window into the complex world of biology and provides a good tool for the forthcoming post-genomic project research.

Applications and potential use of the DNA chips

The GeneChip technology may be employed in diagnostics (mutation detection), gene discovery, gene expression and mapping (Wang *et al.* 1998). It is used to measure expression levels of genes in bacteria, plant, yeast, animal and human samples (Iyer *et al.* 1997; Oh and Liao 2000).

At the present time, the main large-scale application of microarrays is comparative expression analysis (see Chapters 8 and 9). The microarray technology enables the analysis of expression profiles for thousands of genes in parallel. Another application is the analysis of DNA variation on a genome-wide scale. Both of these applications have many common requirements.

By hybridization with labelled mRNA, cDNA, arrayed PCR products or oligonucleotides on a substrate have been successfully used for monitoring transcript levels, single nucleotide polymorphism (SNP) (Wang *et al.* 1998) or genomic variations between different strains (Behr *et al.* 1999). One of the most significant applications of this technique is, as mentioned above, gene expression profiling on the whole genomic scale. For example, the expression levels of the genes in the *Saccharomyces cerevisiae* genome have been successfully determined with both the DNA and oligonucleotide microarray technology. This technique has also been used to investigate physiological changes in human cells (Iyer *et al.* 1997). DNA microarray technology was applied to detect differential transcription profiles of a subset of the *Escherichia coli* genome.

The microarray technology is still a powerful economical tool for characterizing gene expression regulation and will prove to be useful for strain improvement and bioprocess development. It may prove to be useful for process diagnosis and process monitoring in bioreactors. Information obtained from DNA chip analysis may enable researchers to determine the impact of a drug on a cell or group of cells and consequently to determine the drug's efficacy or toxicity. Knowledge of gene expression profiles can also help researchers to identify new drug targets (Debouck and Goodfellow 1999).

The BioChip opens a new world of diagnostics based on genetics. This technology may be adequate to answer many medical questions. For example, gene expression profiles can be used for classification of tumours and for prognosis.

The technology finds increasing application in fundamental and applied research. The major feature of this technique is that it allows the simultaneous analysis of a great number of DNA sequences. As the Human Genome Project nears completion, a new era of genomic science is beginning. The GeneChip technology is a new technique that undoubtedly will substantially increase the speed of molecular biology research.

Furthermore, when sample preparation time and complexity decreases, it will be possible evolve current microarrays into more complete on-chip laboratories capable of performing all the necessary procedures to extract genetic materials from tissue or blood samples and analyse it as well. Perhaps more other applications of this technology will be achievable in the near future.

Concluding remarks

Over a decade of rapid advances in biology has swept an avalanche of genetic information into scientists' laps. But analysis of so vast an input, whether to deduce the inner workings of cells or to diagnose diseases, would be impractical without high-throughput technologies. Of these, DNA microarrays are in the lead. They allow scientists to look for the presence, productivity or sequence of thousands of genes at a time. Currently the power and width of DNA microarrays

technology has made it a predominant factor in genomics, transcriptomics, pharmacogenomics and system – biology, simultaneously becoming ever more important in pre-clinical research and even clinical study. Innovative efforts, coupling fundamental biological and chemical science with technological advances in the fields of micromachining and microfabrication should lead to even more powerful devices that will accelerate the realization of large-scale genetic testing. While offering remarkable tools for genetic analysis, proper applications of these new devices still require a solid intellectual input.

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