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DNA Chip Technologies

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Abstract The genome sequencing project has generated and will continue to generate enormous amounts of sequence data. Since the first complete genome sequence of bacterium *Haemophilus influenzae* was published in 1995, the complete genome sequences of 2 eukaryotic and about 22 prokaryotic organisms have been determined. Given this ever-increasing amounts of sequence information, new strategies are necessary to efficiently pursue the next phase of the genome project - the elucidation of gene expression patterns and gene product function on a whole genome scale. In order to assign functional information to the genome sequence, DNA chip technology was developed to efficiently identify the differential expression pattern of independent biological samples. DNA chip provides a new tool for genome expression analysis that may revolutionize many aspects of human life including new drug discovery and human disease diagnostics.

Keywords: DNA chip, genome, bioinformatics, functional genomics, lab-on-a-chip

INTRODUCTION

In 1995, *Haemophilus influenzae* became the first free-living organism to have its genome sequencing completed [1]. Since then, the genome sequences of 2 eukaryotic (*Saccharomyces cerevisiae* & *Caenorhabditis elegans*) and at least 22 prokaryotic organisms have been determined, representing a total of 140 megabases of DNA sequencing information [2]. Recently NHGRI (National Human Genome Research Institute) has announced that working draft (at least 90% of total genome) of the human genome sequence will be published by June of 2000. With sequencing data accumulating so rapidly, many researchers are entering the next phase of genome projects in which they functionally analyze sequence and other relevant data. However functional informations obtained by using classical techniques are too slow to keep up the speed of sequencing data. The list of classical techniques for gene expression assay are Northern blots [3], ribonuclease protection [4] and differential plaque hybridization [5]. For example, despite decades of yeast experiments using these classical methods, researchers estimate that less than half of the open reading frames (ORFs) identified in the yeast genome sequencing project have been experimentally characterized. Bioinformatists can infer functions for an additional third of those ORFs based on homology to other genes whose functions are known. Still, about 20% of the yeast ORFs have no known function. To reduce the gaps between sequence data and functional informations, more sophisticated methods of expression analysis have to be developed. Therefore, new methodology, so called DNA chip, has recently developed to allow rapid quantitation of expression levels of many genes in parallel [6]. Presented here is a discussion of the DNA chip technologies and how this technologies might facilitate in many aspects of biological research.

CONCEPT OF DNA CHIP TECHNOLOGY

Since the early 1990's there has been a growing hope that miniaturization of benchtop laboratory techniques will be achieved through the marriage of molecular biology and microelectronics and so revolutionize life science. The idea is to use microelectronics well established manufacturing technologies and guiding principles - parallelize, miniaturize, automate - to make molecular biology faster, smaller and cheaper (Fig. 1). The marriage was expected to produce such chimeras as the "DNA chips". The overview of DNA chip assay is shown in Fig. 1 and the brief description of the method is as follows. A biological sample is labelled with a fluorescent tag and reacted with a DNA chip. The location and extend of binding of the molecules on the chip provide a quantitative measure of the identity and amount of gene present in the sample. Biological information such as gene expression and polymorphism data at the genomic level can be used to accelerate many important areas of biological research.

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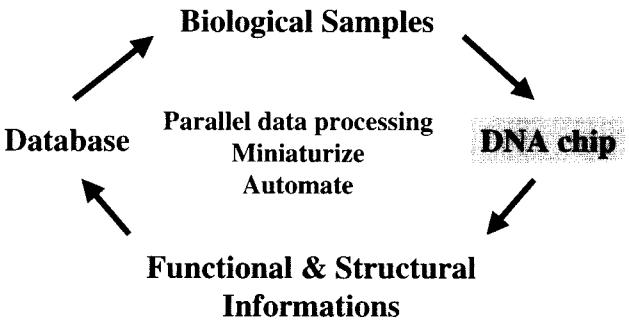

Fig. 1. Concept of DNA chip assays.

DNA CHIP FABRICATION TECHNOLOGIES

The DNA chip format is compatible with many advanced fabrication technologies. The four primary technologies used presently in DNA chip manufacture include microspotting, ink-jetting, photholithography and electronic addressing (Table 1) [7]. Each technology has specific advantages and disadvantages in DNA chip fabrication [8]. Detailed description of each of the technologies is provided in subsequent sections.

Pin Microarray

In 1995, Mark Schena and coworkers at Stanford University have demonstrated that about 1,000 gene expressions can be monitored by fluorescently labeling mRNA and then hybridizing the labeled mRNA to a cDNA microarray chip [6]. This new methodology exploits recent advances in high speed robotic printing of DNA samples, which allow the mass production of cDNA microarray chips for gene expression monitoring.

Table 1. DNA chip fabrication technologies

Fabrication technologies	Criterion	Type	Commercial vendors
Pin microarray	Microspotting	cDNA & oligonucleotide	Beecher instruments Biorobotics Cartesian technologies Genomic solutions Genetic microsystems Hyseq Incyte pharmaceuticals Molecular dynamics
Inkjet	Ink-jetting	cDNA & oligonucleotide	Cartesian technologies Incyte pharmaceuticals Packard instruments Rosetta
Photolitho-graphy	Photolitho-graphy	Oligonucleo-tide	Affymetrix
Electronic array	Electronic addressing	Oligonucleo-tide	Clinical micro sensors Nanogen

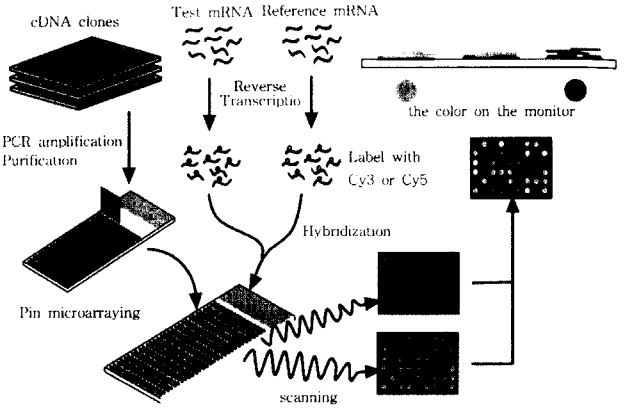

Fig. 2. cDNA microarray scheme.

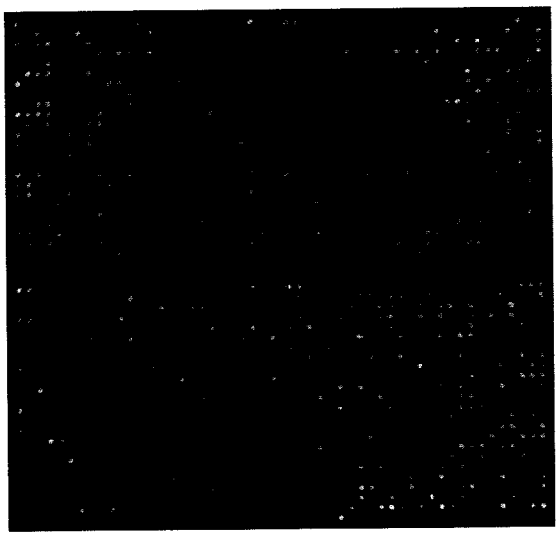

Fig. 3. cDNA microarray chip.

Fabrication, hybridization and scanning of a cDNA microarray chip is shown in Fig. 2 [9] and a brief description of the method is as follows. Templates for genes of interest are obtained from collection of cDNA clones and/or expressed sequence tags (ESTs), and amplified by polymerase chain reaction (PCR). Following purification and quality control, aliquots (~5 nL) are printed on coated glass microscope slides using a high speed robot called "microarrayer". This microarrayer has a printhead containing microspotting pins with uptake channels to hold defined volume of DNA samples. mRNA from both test and reference sample is fluorescently labelled with either Cy3- (green) and Cy5-dCTP (red) using a single round of reverse transcription. The fluorescent targets are pooled and allowed to hybridize under stringent conditions to the clones on the chip. After washing at high stringency, the DNA chip is scanned with a laser confocal scanner designed for DNA chips. Fluorescence intensity at each position on the chip provides an accurate measure of the expression of the cognate gene (Fig. 2) [9]. Fig. 3 shows a typical color image of gene expression microarray (unpublished data).

Fig. 4. Oligo chip.

Direct comparison of the scanned color image provides a rapid identification of genes whose expression is elevated or repressed in each sample. Unlike conventional methods, the use of a single DNA chip for the measurements of differential expression avoids complications inherent in comparing results from independent hybridization. Researchers in the National Institute of Health are now applying this technology to study gene expression in human cancers [11]. And also, this pin microarraying technology can be used for oligonucleotides instead of cDNA for detecting mutations (Fig. 4: unpublished data).

Ink-jetting

The ink-jetting technologies utilize similar versions of computer 'ink-jet printer' to dispense sub-nanoliter volumes of DNA samples to defined locations. A common jet method used in the DNA chip fabrication is piezoelectric capillary jets. Using this capillary jet, diameters of 25 μm are readily achieved [11]. The size of droplets from these devices depends upon the diameter of nozzle, the magnitude of the driving force and the physical properties of the DNA liquid in use.

Photolithography

For use in monitoring gene expression and polymorphism detection, scientists at Affymetrix in Santa Clara, USA, have synthesized high-density nucleic acid arrays on miniature solid-state supports [12]. To produce high-density nucleic acid arrays, Affymetrix used two techniques: photolithography and solid-phase DNA synthesis (Fig. 5) [13]. Synthetic linkers modified with photochemically removable protecting groups are attached to a glass substrate and direct light through a photolithographic mask to specific areas on the surface to produce localized photodeprotection. The first of series of chemical building blocks is incubated with the surface, and chemical coupling occurs at those sites that have been illuminated in the preceding step. This process is repeated, activating different sets of sites and coupling different bases up to about 25 mers. The amount of nucleic acid information encoded on the chip in the form of different probes is limited only by the physical

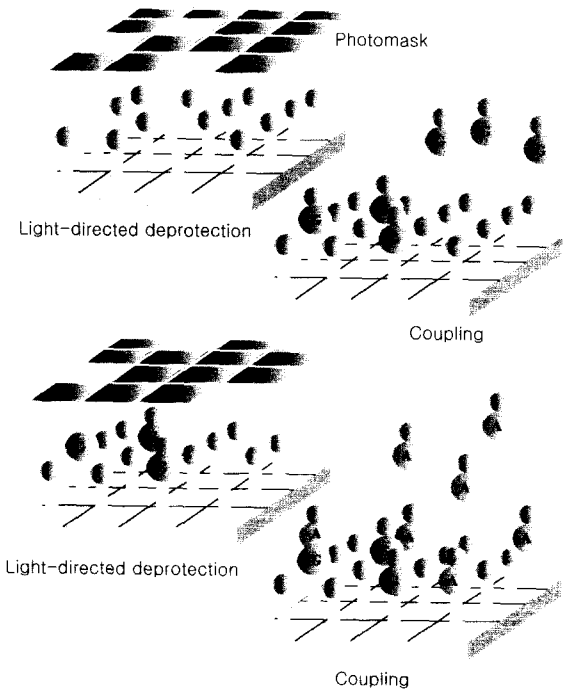

Fig. 5. Photolithography.

size of the array and the achievable lithographic resolution. Using this method, Affymetrix has created 400,000 different oligos on a small 1.28 × 1.28 cm chip. Compared with cDNA chip, oligonucleotide chip has an additional advantage of detecting single mutations. Therefore, Affymetrix has made more than 40 different expression chips for many different species and variant analysis chips for human CYP450, p53 and HIV [14]. Recently, Singh-Gasson and coworkers have successfully fabricated light-directed oligonucleotide chips using a digital micromirror array [15]. They have made a maskless array synthesizer (MAS) that replaces the chrome masks with virtual masks generated on a computer, which are relayed to a digital micromirror array. This instrument has been used to synthesize oligonucleotide chips containing more than 76,000 different oligos.

Electronic Addressing

Instead of using photolithography, researchers at San Diego-based Nanogen are manufacturing oligonucleotide chips by applying a controlled electric field [16]. Nanogen is developing microchip-based hybridization chips that utilize electric fields as an independent parameter to: (a) rapidly transport and selectively address DNA probes to any position on the chip surface, (b) accelerate the basic hybridization process by electronic concentration control and (c) rapidly discriminate single base mismatchs in the target DNA sequences by electronic stringency control [17]. Using this chip, target DNA fragments find their complementary oligo more quickly, and detection takes just minutes-rather

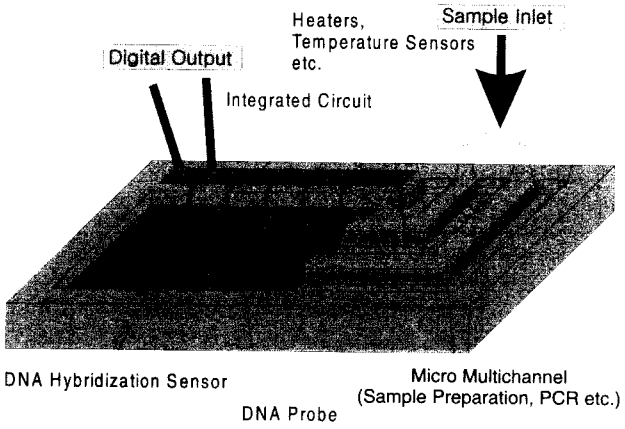

Fig. 6. Laboratory-on-a-chip.

than the hours needed with ordinary chips, which let the target DNA fragments diffuse randomly. And an altogether different approach is being taken by Clinical Micro Sensors (CMS) at Pasadena, USA. 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 chip. As you can see, over the last few years, several intriguing fabrication technologies have been developed to generate more efficient and powerful DNA chips.

PERSPECTS FOR LIFE SCIENCE

DNA chip could find broad applications in monitoring the differential expression and polymorphisms in human genes. This technology is able to allow changes in expression to be detected as a function of cell type, tissue source, physiological state or genetic background. DNA chip could also serve as a rapid detection method of identifying changes in specific gene sequences. Therefore, DNA chip can be possible to utilize a test for human disease diagnostics, whereby a sample of blood or a biopsy from a patient would provide a source of mRNA and genomic DNA for monitoring gene expression and mutation, respectively. To be successful in the application of DNA diagnostics, DNA chip must provide not only accurate results but also simple steps for easy use. A recent trend of DNA chip research is to develop integrated DNA analysis systems, DNA Laboratory-on-a-chip, which will provide the next generation of inexpensive DNA diagnostics. It will be low cost automated systems eventually providing methodologies for sample preparation, amplification, hybridization, and detection on a single chip. It can be possible through miniaturization and integration of all necessary components for sample analysis such as micropump, microvalve, microreactor, extractor, separator, and sensors using micro electro-mechanical system (MEMS) technologies. Fig. 6 shows the schematic diagram of conceptual Lab-on-a-chip utilizing MEMS technologies. The development of the next generation of DNA Lab-on-a-chip will open new era of personal DNA diagnostics because of low price, easy procedure, and most importantly more accurate results by removing possible human error factors. Another exciting application of DNA chip technology is in the area of pharmacogenomics, a new field in biomedicine focused at the interface between pharmacology and genomics [18]. Biological differences among individuals in a population are due to both genetic and environmental factors. Therefore, by providing massive and parallel DNA data of individuals, pharmaceutical companies will be able to find specific drugs for individuals, so called 'personal drugs'. To manage ever increasing chip data, bioinformatics is getting more critical to improve access to large data sets: reliable and effective tools for 'data mining' [19]. Therefore, the use of DNA chip technology will revolutionize many aspects of life science and human life in this new millenium.

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