

# DNA microarray technology and application

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## SUMMARY

*DNA microarrays (collections of DNA probes arranged on a shared base) have recently enlarged the spectrum of commercially available laboratory-ready kits in molecular biology. They are powerful new tools for the investigation of global changes in gene expression profiles in cells and tissues. Their assembly process is automatized and the DNA microarrays are further miniaturized. The DNA microarrays are used in search for various specific genes (e.g. connected with an infectious agent) or in gene polymorphism and expression analysis. They will be widely used to investigate expression of various genes connected with various diseases in order to find causes of these diseases and to enable their accurate treatment. Since the DNA microarray assembly technology has been based on methods widely used in the semiconductor industry, we can expect a rapid onset of the routine use of this revolutionary device.*

## INTRODUCTION

The proof of typical genetic information in the DNA taken from the patient can help to diagnose many clinical infectious or uninfected diseases. In addition, the activity of the gene can be assessed according to the mRNA content in the sample. However, until recently, hardly any other laboratory procedures than those for routine analysis of phenotypic expressions of these altered or causative genetic structures have been used. Later, probes and a polymerase chain reaction with thermostable DNA polymerase did make a breakthrough in a routine laboratory diagnostics of diseases caused by some difficult to cultivate pathogens (tuberculosis, chlamydia infections, AIDS, viral hepatitis). More advanced methods which are now employed mainly in the diagnostics of hereditary non-infectious diseases appear far too specialized. This overcautious approach should change in future when new mass manufactured technologies of DNA microarrays arrive.

## DNA MICROARRAY PRINCIPLE

Diagnostic molecular biology methods usually aim to find a specific segment of the nucleic acid which is typical for the searched for gene – i.e. to find a site with a particular order of bases specific for the examined gene (e.g. for the microbial one). Discovering such a structure in the examined material would prove presence of this gene in a patient's organism. It is detected using a short synthetically assembled single-chained complementary oligonucleotide – a chain of bases organised in a mirror order, to which the searched part of the nucleic acid would attach (hybridize) via A-T or G-C bonds. The diagnostic oligonucleotides may be called probes, primers or capture probes according to their purpose. DNA microarrays contain capture probes. In order to improve the method's sensitivity, the examined nucleic acid is first amplified (e.g. using the PCR).

Currently widely employed molecular biology laboratory-ready kits are usually aimed at a simple detection of the basic form of a single, or excep-

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tionally of a few nucleotide sequences or genes. From this point of view these kits are relatively expensive. Therefore, it is not feasible to introduce such procedures in places where other, cheaper and equally quick options of a comparable quality exist. The DNA microarray does not have the above described disadvantages of the individual diagnostic approach. Its construction (described below) enables parallel examinations in a specified broad area. Basically, the DNA microarray is an aid making a simple, ev. semiquantitative detection of many different genes possible using a collection of probes 'in a single box'. This might be one of the approaches to the DNA microarray. The other, completely new approach uses a large number of probes located in a DNA microarray to closely analyse a small area of a genom on the point mutations level, and, therefore, to study polymorphism of one or several genes. Both approaches have already been employed using commercially available DNA microarrays.

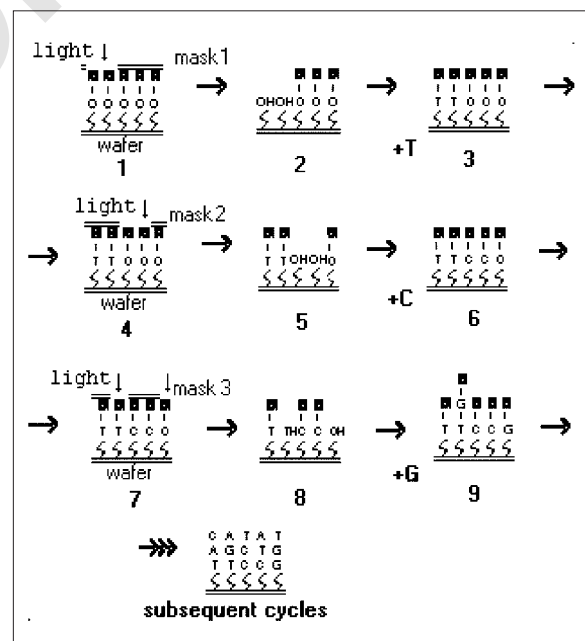
The DNA microarray is a collection of large numbers of various capture probes arranged on a shared base. In general, arrays are described as macroarrays or microarrays, the difference being the size of the sample spots. Macroarrays contain sample spot sizes of about 300 microns or larger and can be easily imaged by existing gel and blot scanners: For several years, preparations containing more, dozens or hundreds of probes on a nylon strip, have been available. Similarly, a microwell plate can be used, having a different capture probe in each hole. However, all this is still far too restrictive. On the other hand, a single Affymetrix GeneChip' array can carry up to 16000–100000 different probes. The coming generation of DNA microarrays developed by Affymetrix and Hewlett-Packard contains already 400000 different probes.

## DNA MICROARRAY ASSEMBLY

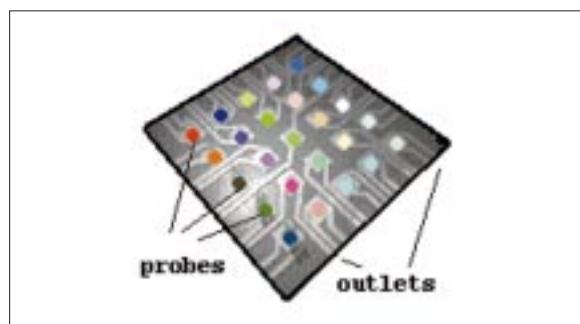
Some producers simply arrange appropriate probes on a wafer surface using a micromanipulator and anchor them chemically. The method's principle itself restricts the result. Therefore, manufacturers have aimed at further miniaturization and integration. In 1991–1993 a group of the Affymax company employees found the way [1]: The Affymetrix GeneChip DNA probe array synthesis is based on a photolithographic technology which is commonly used in semiconductor industry. Recently (1997) another, different way of the DNA microarray assembly has been patented. It uses electronic probe addressing [2].

## a) The photolithographic process

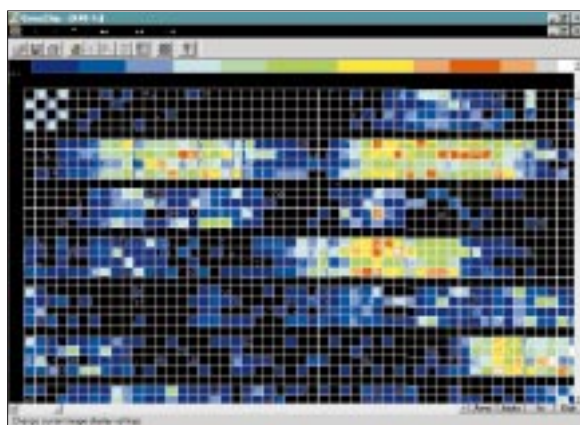
The assembly starts with a glass wafer of 12.8×12.8mm. Its surface is covered with a protective photosensitive layer which makes the wafer inert. Only those places where the protective layer has been lit subsequently are able to bind a deoxyribonucleotide containing a base (A, T, G, C): Following the light exposure the wafer is covered with a solution of a single particular deoxyribonucleotide. Other places were protected from the light by a lithographic mask. Therefore, the deoxyribonucleotide is bound exclusively to the previously lit places. Afterwards, the rest of the substance not bound is washed away. The deoxyribonucleotides had been previously modified, so that they, after binding to the wafer or to another deoxyribonucleotide, would bind with yet another deoxyribonucleotide only after the light exposure (light activated monomers [3]). Therefore, it is possible to repeat the cycle of masking – lighting – layering – incubation – washing as many times as necessary and by changing the masks to create a unique sequence of nucleotides for each DNA microarray spot (Figure 1). The mask shapes are designed according to the required surface array arrangement by a computer which also supervises the whole assembly process. Chains of a single-stranded DNA organized in square (90×90 μm) microscopic areas (spots) grow on the wafer sur-



**Figure 1.** Scheme of the DNA microarray photolithographic assembly process. A – adenine, T – thymine, C – cytosine, G – guanine



**Figure 2.** Scheme of the experimental electronically addressed DNA microarray containing 25 probes (the probes are marked in colours)



**Figure 3.** Intensity of the light emission of single spots in a part of the GENECHIP® (artificial colors – see scale above). GENECHIP® and the artificial colors depicted on the display are exclusive trademarks of Affymetrix, Inc. – USA. Trademarks and reproductions used with permission of Affymetrix, Inc. – USA.

face, a nucleotide by a nucleotide. Each spot, in the end, contains millions of identical probes. The length of each probe is currently 18–25 bases. Therefore, for instance, after 40 mask changes and related procedures on the same wafer, it is possible to synthesize such a number of different DNA microarrays which would otherwise require 10 million steps.

In practice, large numbers of identical DNA microarrays are assembled at once on large shared glass wafers. Individual DNA microarrays are then packaged in injection-molded plastic cartridges.

#### **b) Electronic addressing (Nanogen Inc. -Apex DNA analysis system with electronically addressable DNA microarrays)**

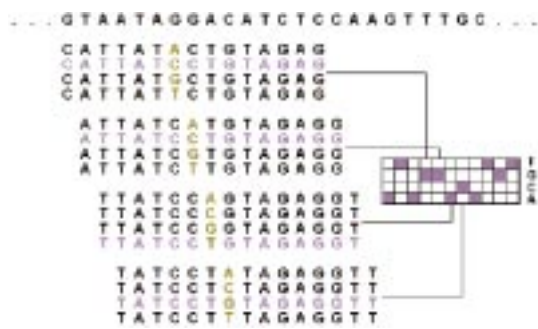
Construction of this DNA microarray requires advanced wafers. Such a wafer is an electronic

chip with many outlets, each of them controlling voltage of a particular spot on the wafer surface. (Figure 2). On the contrary to the photolithographic method where the probes are assembled one by one, a base after a base, in this method the whole oligonucleotide chains are arranged on the wafer, one after another. Entered positive voltage in a particular spot on the wafer specifies the probe's location during its assembly process. DNA has a negative charge, therefore, the probe travels to a positively charged spot where it gets attached. Density of these DNA microarrays is lower compared with the photolithography. On the other hand, these DNA microarrays can be recycled. Also they appear principally more suitable to construct special DNA microarrays directly in the laboratory.

#### **HOW THE DNA MICROARRAY WORKS**

Should there be too small amount of the examined DNA, at the beginning it is amplified. DNA is then transcribed into RNA. Commercial DNA microarrays usually work with RNA as a standard [4]. However, it is also possible to analyse single-stranded cDNA. The examined unknown RNA is expected to attach to the DNA microarray sites which contain probes with a complementary base order and to bind (hybridize) there. Prior to that, the examined RNA chain is labeled with a fluorescence marker to visualize the hybridized sites, and divided into shorter segments to ease binding. This RNA is applied into the DNA microarray cassette and then incubated. Subsequently, the unattached RNA is washed away. The special 'fluidics station' is used to control all the above procedures. Afterwards, the DNA microarray is scanned using a laser scan system with an argon laser light source which arises luminescence in the attached labeled RNA molecule. The sites where the labeled RNA bound to the DNA microarray surface emit light after being exposed to the laser beam. A microscope connected computer assesses fluorescence intensity for each individual DNA microarray site (Figure 3).

The particular DNA microarray spot fluorescence depends on quantity of attached RNA. DNA microarrays for routine microbiological laboratory diagnostics or for analysis of the activity of the important part of human genome can work in this way. However, this description cannot explain how point mutations in the examined genome section are examined; this is more complicated:



**Figure 4.** A quadruple of probes is located on the DNA microarray for each base. Spots with perfectly complemented probes cast the most intensive light signal.

Was the examined RNA a transcript of a mutated gene, its hybridization is incomplete: its chain base order would be complementary with the probe, except for the mutated part. Therefore, such RNA would attach to its probe only partly which would make the whole bond less mechanically resistant. However, it is possible to use also the probe copying the mutated gene resulting in a perfect bond (Figure 4.). The DNA microarray has so many spots that it can carry probes for all variants of the point mutations of the examined gene. At the beginning, all these probes hybridize with the examined gene. However, point mutations make the hybridization bonds unequally strong. The subsequent step provides stringency control: The DNA microarray is washed with a controlled stream of fluid to separate (more or less) RNA from the probes [5]. If the bonds were not sufficiently strong more of RNA molecules would break from the probes compared with spots where the probes perfectly completed the examined RNA. Such spots then cast a relatively weaker light signal. A computer compares the light signal intensity in the individual DNA microarray spots and assesses the most probable base order of the analysed DNA sample. This is how GeneChip HIV- PRT array, for instance, works. Various accessory information systems further process the data to clarify the picture, eventually they can compare them with previous experiments or with data from other world data banks.

The DNA microarrays assembled on electronic wafers (Apex) work differently: Their surface can be voltaged (outlets used in the DNA microarray assembly stay functional) which speeds hybridization process up. The analysed nucleic acid is attached to the probes in the same way as the probes were to the DNA microarray surface during the assembly process. Therefore, hybridization

takes only a few minutes. Furthermore, the sample can be directed to a particular probe group by putting a charge locally underneath it [6]. The stringency control is electronic: A nucleic acid which attached incompletely can be released by changing the charge under the probe from positive to negative. Perfectly hybridized probes would sustain the charge change.

## PERSPECTIVES IN THE DNA MICROARRAY TECHNOLOGY APPLICATION

The above mentioned procedures often are not new, in principle. They have just made the formerly employed methods extremely quicker and cheaper, which makes them predisposed for mass use in future. Those who have already worked with DNA microarrays expect them to make a breakthrough in medicine. New advances can be expected in diagnostics of genetically conditioned diseases. Completely new therapeutic procedures will be introduced when a DNA microarray analyses a relevant sequence of a human genome and then 'made-to-measure' drugs will be administered. The current commercially available GeneChip CYP 450 array is expected to open such possibilities in internal diseases therapy. It analyses 2D6 and 2C19 gene polymorphism for cytochrome c-450 which plays an important role in drug dynamics, e.g. in beta-blockers and antidepressives. It has been applied only experimentally, e.g. in cardiology, however, the first users congress was held in the United States in September 1998 [7]. A similar principle has been tested experimentally in AIDS treatment where GeneChip HIV-PRT array analyses viral protease genes and viral reverse transcriptase in a patient's captured actual HIV mutant. The analysis helps to optimize treatment procedures including administration of virus-coded enzyme inhibitors.

## NEGATIVE IMPLICATIONS

Negative implications of the DNA microarray technology have been considered: The DNA microarrays will make automatic analysis of individual human gene variants possible in the near future, which will lead to mass production and wide availability of cheap devices to control individual patients treatments. Such a device is also likely to be able to forecast future progress of a patient's state of health. These outlooks have already made individual pharmaceutical companies to invest enormous sums to develop new preparations which would not be aimed at treatment of current

diseases but at active prevention of eventual patient's diseases forecast by DNA microarrays [9]. However, who is going to ensure that the same data on patient's perspectives will not be misused or required as a part of job interviews? Will these have any influence on people's choice of a partner? Will not human embryos be selected? Arrival of the DNA microarrays cannot be stopped. Therefore, it will be necessary to take adequate measures to protect collected data and to outlaw inappropriate DNA microarray applications.

## INSTRUMENTATION AND THE CURRENT OFFER OF DNA MICROARRAYS

Currently, the DNA microarrays are thought a useful tool in research laboratories and reference centers. One Affymetrix GeneChip array cost between \$100–800. Other equipment include gene samples application, incubation and washing kit. Furthermore, reading DNA microarray instrumentation and software is needed. The current price of the whole functional device is \$230,000. There are plenty of other DNA microarrays manufacturers offering DNA microarrays of lower integration. The electronically addressed DNA microarrays are in a prototype stage.

## PERSPECTIVES

It is difficult to guess when the DNA microarrays will become a widely employed and substantial tool of modern medicine. Hopefully, it will be in 5–10 years. It also depends on the human genome analysis advance.

Another question is, which of the above basic modern technologies will prevail. According to the Nanogen Inc. management, however, they will not compete [10]. The photolithographic DNA microarrays have enormous capacity but are rather slow. Therefore, they are more suitable for gene polymorphism or gene expression analysis. On the other hand, the electronically addressed DNA microarrays have small capacity but are extremely fast. Therefore, they are predisposed for a simple routine molecular biology diagnosis, e.g. in microbiology. The following forecast illustrates the Nanogen Inc. Company management's idea of

perspectives in this diagnostic branch: whenever people feel sick, they will themselves take a smear or other sample and place the material on a Nanogen device at home. In a few minutes the sample will be analysed and the data sent via modem to a laboratory. After processing they will be sent back with an appropriate recommendation. This idea surely is influenced by commercial orientation of the forecaster. However, let us believe that the above described routine will be available to every general practitioner. The price of the DNA microarrays is expected to drop down in the future, the drop having no effect on enormous manufacturers' profits. The DNA microarray technology is based on the computer parts assembly process. If we compare the computer technology level and prices 10 years ago and today, we can easily imagine that the DNA microarrays could be soon commercialized in our region as well. The coming generation of medical doctors will take the DNA microarrays in their practice for granted.

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