Invited Editorial

DNA microarrays – a methodological breakthrough in genetics

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The end of the 20th century produced two breakthroughs in genetics. The first achievement involved sequencing complete genomes. To date, 74 entire prokaryotic and several eukaryotic genomes have been sequenced and over 200 prokaryotic and almost 160 eukaryotic genomes are the subject of sequencing projects. The other great achievement took place in methodology and concerned the first method of analysis of large fragments and even entire genomes. The method’s development was initiated in California in 1991 in Affymetrix – a small biotechnological company by a scientific team lead by Stefan Fodor (FODOR et al. 1991). The commercial name of this method offered by Affymetrix is GeneChip (http://www.affymetrix.com). The literature uses other names for this technology, such as DNA microarrays, DNA chip, biochip.

The success of the DNA microarray method was the result of its inventors being open to technical concepts offered by other sciences and the skill to apply them in genetic analysis. Fodor’s team successfully integrated achievements in DNA chemical synthesis, photolithography, minifabrication, automation and bioinformatics, and produced a new technology called DNA microarray (Figure 1) (FODOR 1997). The principle of the DNA microarray technique is that hybridization and its miniaturization replaced the currently common PCR amplification. DNA microarrays may be briefly defined as miniaturized parallel analytical devices containing libraries of oligonucleotides, robotically spotted (printed) or synthesized in situ on solid supports (glass, coated glass, silicon or plastic) in a such way that the identity of each oligonucleotide is determined by its

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location, designed to achieve a high throughput for specific assays. Commercially available DNA chips contain between 200 and 250000 oligonucleotides per square centimeter.

The DNA chip construction itself represents the method’s principles. DNA chips can be classified into two types: for DNA mutation detection and for gene expression analysis. The most frequently used strategies of DNA chip production are: the light-directed deprotection method applied to the commercial production of GeneChip series (Figure 2) and ink-jet printing (ROSE 2000).

Chips constructed by the light-directed deprotection method (Figure 3) have become the most popular. The nucleotide sequence (the so-called ‘pattern se-
and the possible sites of mutations producing specific phenotype effects are the basis of production of mutation-detecting chips. Each array consists of 5 probes of a sequence complementary to the pattern sequence, except the substitution site, where a significant mutation may be located. In this site, each probe contains alternative nucleotides A, C, T, G or a point deletion. In order to detect mutations in a specified locus, the examined DNA is denatured to obtain a single strand form, fluorescently labelled and placed on a chip for hy-

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**Figure 2. Step-by-step principle of DNA microarray production by light-directed deprotection method**

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bridization with the array. The hybridization signal is registered due to the fluorescence of a hybrid (analysed DNA – probe). Each probe is located in a specified chip position. The mutation type is identified by a read-out of the signal. A chip may contain either an array of probes to detect mutations in selected loci, known from phenotype effects, or it can contain a sufficient number of probes to analyse each nucleotide for mutations in the examined gene. The specificity of hybridization is based on thermodynamically perfect mismatch rule and therefore requires

Figure 3. Principle of DNA microarray action for mutation detection
Specificity of hybridization is controlled by the neighbouring probes (at least two) perfectly matching the target sequence. Grey squares indicate the hybridization signal. The sequences below chips 1, 2, 3, 4, 5 show the results of mutation detection.

Figure 4. Principle of DNA microarray used for gene expression analysis
careful probe designing with the use of advanced algorithms avoiding potential cross-hybridization of probes and undesired interaction of oligonucleotides to each other and to target sequence (SOUTHERN et al. 1999, RADDATZ, DEHIO 2001).

The principles of the gene expression chip for analysing mRNA transcripts in tissues are considerably simpler (Figure 4). In order to construct a gene expression analysis chip it is necessary to find mRNA in the examined genes, very frequently in the form of EST (expressed sequence tags) – short specific fragments of mRNA. The array consists of two probes of the sequences complementary to the examined mRNA with the exception of one nucleotide located in the centre of the array. The examined mRNA is isolated from a specific tissue and undergoes a reverse transcription, fluorescent labelling, denaturation and hybridization with the probes. Hybridization with a fully complementary probe proves the mRNA expression in the examined tissue. Lack of hybridization means no expression; the signal intensity shows the expression level.

The expression analysis chip may consist of a probe array to detect hundreds, thousands and even tens of thousands of different molecules of mRNA in an examined tissue.

The DNA microarray technology then involves several steps: genome analysis and probe design, chip synthesis, preparation of the examined DNA/RNA, DNA/RNA hybridization with the chip, chip scanning and results analysis. Both chip types have been quickly commercially applied. Currently, many other companies are developing the microarray technology and offer reagents, equipment and ready chips based on the human, mice or other organism genome sequences (http://www.gene-chips.com). The vast majority of research with the use of the DNA microarray technology allows biomedicine to analyse gene structure and expression (YANG 2000). This technology is relatively rarely used to detect mutation and is applied mainly to analyse genomes of some microorganisms (WESTIN et al. 2001) and to detect carcinogenic mutations (FAVIS et al. 2000, KALIONIEMI 2001).

The DNA microarray technology is being continuously improved. Clear progress has been made in making probe design programs (RADDATZ, DEHIO 2001), bioinformatic tools for analysis, interpretation and dissemination of results (LEMKIN et al. 2000, SHERLOCK et al. 2001) and standardization methods for the results obtained with the use of different types of DNA microarray technology (STAR, RASOOLY 2001, RUTH 2001, BRAZMA et al. 2002).

The DNA microarray technology can be successfully applied to examine the variability of structure and expression of various genes and genomes of farm animals. A wide range of factors encourage the use of this method for the analysis of polygenic traits in livestock, namely advanced genome mapping and sequencing programs, thorough phenotype databases, identification of major genes (http://locus.jouy.inra.fr; http://www.ri.bbsrc.ac.uk, http://tetra.gig.usda.gov) as
well as attempts to build expression libraries for some farm animal species (Rebeiz, Lewin 2000).

In order to design a chip for the analysis of the genetic background of variable protein content of milk, the trait that is nowadays preferred in breeding programs in dairy cattle, the probes representing at least a dozen of genes and the same number of anonymous sequences (micro-satellites) could be combined in the chip. These candidate sequences occur in the form of a series of multiple alleles directly or indirectly influencing this trait (Kamiński 2001). The list can be extended by the genes involved in mammary gland development and lactation (Malewski, Zwierchowski 2002).

Initially, the DNA microarray method seems to ‘x-ray’ a genome completely and leave nothing else to discover. However, after deeper examination, the technology provides information on genome structure and expression, and mainly its proteomics have yet to be explored (Lander 1999) (Figure 5). Combining these groups of information and determining their correlations may result in formulating the general theory of biological systems in the coming century.

This tempting future will, however, put science face to face with new ethical barriers. The dynamic development and wide dissemination of this technology may soon encounter strong resistance from the society, especially when this technology is associated with medical studies. The miniaturization of this method and the great speed of receipt of abundance of information on the genetic potential of a patient may also encourage the abuse of the DNA microarray technology.

Figure 5. Types of information necessary to formulate general theory of biological systems

- Gene interactions: allelic and non-allelic
- DNA sequences: structural and regulatory
- Biochemical reactions and pathways
- Physiological reactions and diseases
- Time and place of gene expression
- DNA polymorphism of species, breed or population
- Intermolecular interactions of proteins
- Localisation of proteins in the cell

General theory of biological system
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REFERENCES


Researchers have used DNA microarrays to drive genetic analyses for more than two decades. Essentially grids of thousands or even millions of tiny spots of DNA printed onto glass slides, DNA microarrays enabled scientists to massively parallelize their research. No longer did they need to probe one gene or transcript at a time; with microarrays, they could extend their studies to the whole-genome or transcriptome level with little extra effort. Naturally, the technology caught fire. And then along came next-generation DNA sequencing (NGS), a method that seemingly can do everything microarrays