DNA Microarrays**

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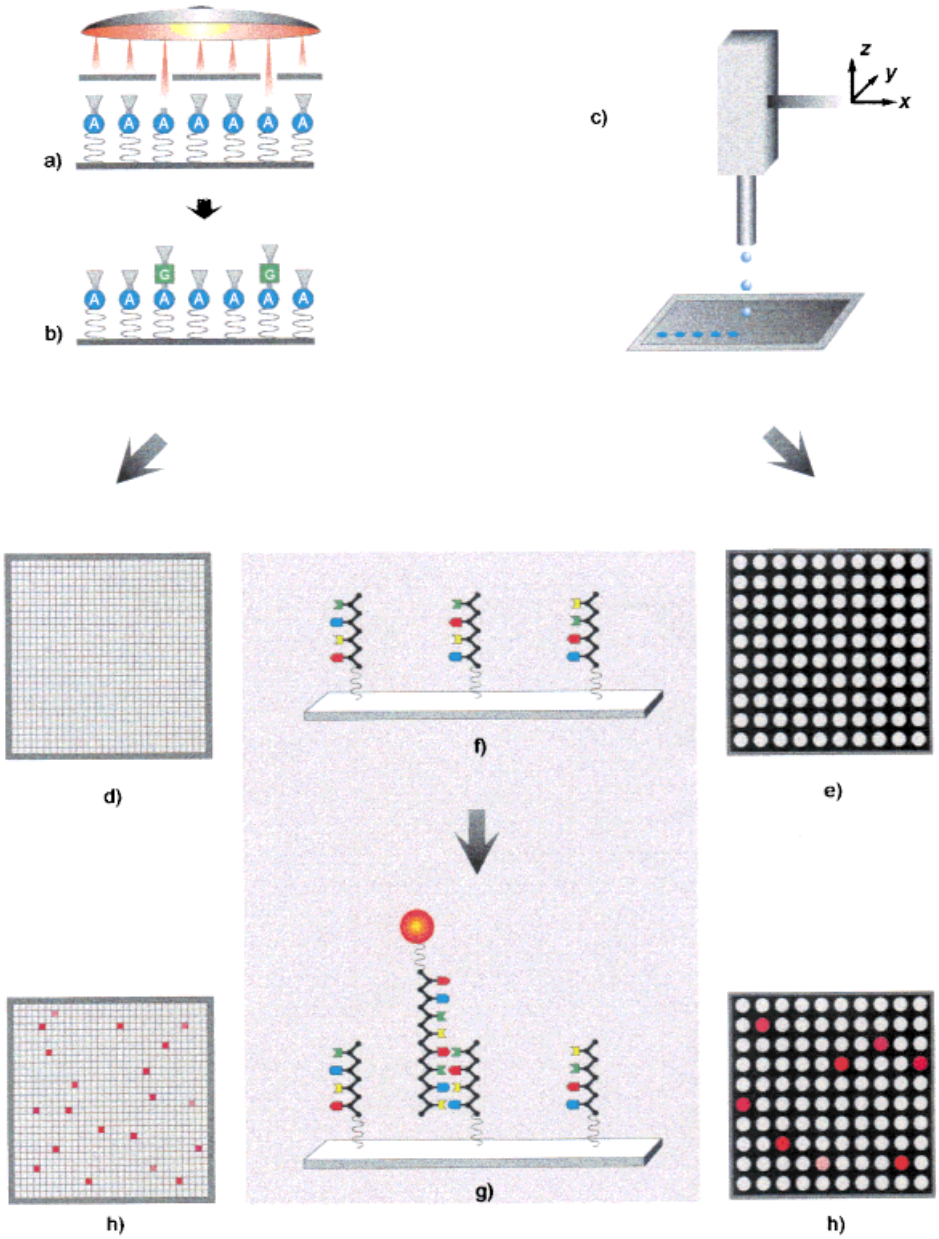
Two milestones characterize the way from the Southern Blot,[1] the first "array", to the nylon-filter-based screening of clone libraries, to today's DNA microarrays[2,3] that already host several million DNA fragments.[2e] The first was the use of nonporous solid supports, in particular glass surfaces, for the employment of fluorescence detection and miniaturization (Scheme 1). Up to 10000 DNA fragments, prepared by enzymatic or chemical syntheses, have been covalently immobilized on activated glass supports by means of automated dispensing or plotting devices.[2d, f, m, 4] The second landmark was the development of a combinatorial photolithographic process by Affymetrix,[2e] which was originally destined for peptide syntheses.[5] Highly structured lateral oligonucleotide libraries on glass supports are accessible by initally modifying the surface with photolabile protection groups.[6] Illumination through a microstructured photomask leads to the deprotection of selected areas, to which the first phosphoramidite building block is covalently attached. Since the coupled nucleotides also contain photolabile protection groups the iterative repetition of the process generates new patterns, which leads to two-dimensionally structured oligonucleotide arrays. As an example, an array comprised of 256 octanucleo-



Scheme 1. Comparison of microarray formats. High-densitiy oligonucleotide arrays (d) are attainable using Affymetrix photolithographic on-chip synthesis (a, b). The deposition of DNA fragments, previously synthesized by chemical or enymatic methods using automated spotting or dispensing devices, allows the production of microarrays with a lower density of hybridization spots (c, e). However, this technique allows for the immobilization of long pieces of DNA, for instance, obtained from clone libraries or by PCR. Following the hybridization of fluorophore-labeled cRNA or cDNA samples (f, g), the resulting hybridization patterns (h) are analyzed using a fluorescence scanner.

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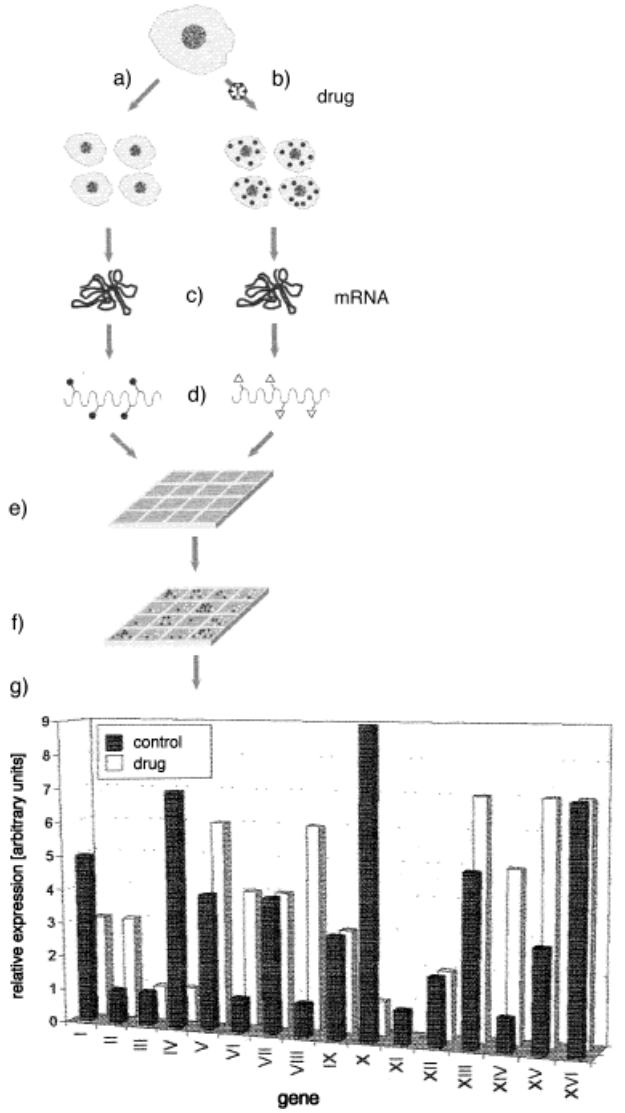
[**] We thank S. Hahner and M. Adler for help with the figures and critical discussions.

  1433-7851/99/3819-2865 $ 17.50+.50/0

tides on a surface area of 1.3×1.3 cm was synthezised by 16 reaction cycles in four hours.[7] Currently available fabrication routines allow the production of arrays containing more than 300 000 DNA oligomers to a length of up to 25 nucleotides. A microfluidics station, fluorescence GeneArray scanner, as well as processing and analysis software is required to run the GeneChip microarrays. The complete system is priced at about 175 000 US Dollars.[2e] As an alternative, the in-situ chemical synthesis of oligonucleotides on glass supports has been developed by Southern and co-workers.[8] Microarrays have been prepared that contain about 2000 different 17-mer oligonucleotides[8h] by using a stepwise movable device that allows the application of synthesis reagents to small areas of only a few square micrometers.

One initial motivation for the elaboration of DNA microarrays resulted from efforts during the early stages of the Human Genome Project aimed at developing powerful alternative methodologies for the sequencing of nucleic acids. "Sequencing by hybridization" (SBH) was considered as a promising approach.[9] This method allows the de-novo determination of an unknown nucleic acid sequence by means of hybridization analysis by using a comprehensive DNA array containing all possible 65 536 ($=4^8$) octanucleotides. During the experimental exploration it turned out that SBH is associated with severe unexpected practical problems. However, the DNA arrays were found to be suitable for immediate applications in the fields of gene expression analysis,[2c, e, f, m, 3] resequencing, and mutational analysis.[2h] The particular strength of microarray analysis results from the highly redundant measurement of many parallel hybridization events, which leads to an extraordinary level of assay validation. The probe redundancy does not mean that identical probe molecules are attached on several sites of the chip, but rather the presence of multiple probes of different sequence with specificity for the same nucleic acid target.

Total mRNA or, if there is insufficient sensitivity, polyadenylated RNA (poly(A)$^+$-RNA) is isolated, for instance, from different tissues for gene expression analysis. The material is enzymatically transcribed to cDNA and amplified either through a polymerase chain reaction (PCR) or isothermal amplification to incorporate labels, such as fluorophores. The sample material is then hybridized with a microarray containing DNA probes complementary to the genes of interest. Either short DNA oligonucleotides or long single-stranded cDNA fragments are used as array probes. A common experimental variation of enhanced performance is the differential expression analysis (Scheme 2). In this method the two mRNA samples to be compared are labeled with fluorophores that give rise to different colors. The competition of the two samples for the array-bound probe oligomers can be directly deduced from the colors obtained by hybridization. This process allows estimation of the relative abundance of particular transcripts. The analysis of the transcription activity of individual genes is not only useful to study whether and how external parameters influence cellular gene expression activity. Moreover, fundamental growth and differentiation processes, such as mitosis and meiosis, can be investigated.[2k] As a consequence of its relatively low genome



Scheme 2. Differential expression analysis, illustrated for the screening of drug effects. Cells are cultivated without (a) and (b) in the presence of a drug. The isolated mRNA (c) is amplified and labeled with fluorophores of different colors (d). A mixture of the two nucleic acid samples is hybridized to a microarray (e) containing probe oligomers complementary to the genes of interest. The competition for the array-bound probes leads to mixed colors (f), symbolized schematically by the varying occurrence of the two labels, which can be quantifed (g) by means of a fluorescence scanner.

complexity the yeast *Saccharomyces cerevisiae* is currently the eucaryotic model organism of choice. However, it becomes apparent that microarrays containing probes for all approximately 100 000 human genes will soon be available.[2a] Such arrays will further increase the importance of expression monitoring for the pharmaceutical industry as they will allow both detection of desease-causing genes as potential targets, and analysis of drug effects by means of "high-throughput-screening" (HTS).[2i]

In addition to the basic research-oriented expression analysis, the second main application of microarrays is the determination of nucleic acid sequences actually known with respect to mutational screenings.[2h] For this purpose, the microarrays accommodate subsets of probes containing the fully complementary oligonucleotides and also homologous

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probe molecules with single base exchanges in a central position. This format leads to the generation of highly characteristic hybridization patterns, thus enabling the determination of both mishybridization and the detection of base mutations.[2h] Genotyping microarrays currently being developed are capable of simultaneously reading several tens to hundreds of thousands of bases in order to allow for an efficient high-throughput. The analysis of point mutations (single nucleotide polymorphism, SNP) is not only important for population genetics,[2l] the identification of organisms, and in clinical diagnostics of cancer[2g] and viral diseases such as AIDS.[2e] Single nucleotide polymorphism analysis is also of great significance for the concept of pharmacogenomics.[2i] In addition, the reading of single nucleotides is used for the resequencing of highly variable genes. A well known example is the breast and ovarian cancer gene *brac1*, for which over 400 distinct mutations have been reported.[2h]

Despite the impressive technical advances in microarray development, there are still many basic questions within this new field of research, for instance, concerning the topology of DNA-functionalized surfaces and the details of the molecular interactions that occur at the interphase. DNA monolayers formed through self-assembly of thiol-modified oligonucleotides on gold substrates have recently been characterized.[10] Conventional hybridization assays using radiolabeled oligonucleotides as well as X-ray photoelectron spectroscopy (XPS),[10a] neutron reflectivity,[10b] electrochemical methods such as cyclovoltammetry,[10c–f] and scanning force microscopy[10e, 10g] have been used for this purpose. These studies reveal insights into the density of surface coverages and the orientation of the DNA fragments attached. For example, the single-stranded oligonucleotides within the monolayers are oriented horizontally to the surface, which suggests multiple contacts occur with the surface.[10a, 10b] In contrast, terminally attached double helical fragments are oriented almost perpendicular to the solid surface.[10b, 10f] Interestingly, single-stranded oligomers can also be "put upright" by treating the DNA-functionalized surface with short-chain thioalkanoles.[10a, b]

Southern and co-workers are also carrying out basic research with oligonucleotide arrays.[2b, 8] For instance, they have investigated how the shape, length, and base composition of the double helices during the formation process affects the efficiency of hybridization.[8c–f] Also studied were the dependency of surface densities and of the lengths of oligonucleotides and spacers on sterical effects.[8g] A severe problem of solid-phase hybridization is caused by the fact that the efficiency of duplex formation depends on both the melting temperature of the nucleic acids and the formation of intramolecular secondary structure. This is of general importance for microarray analytics, which requires uniform signal intensities that are independent of the nucleic acid sequence. To solve this problem the addition of quarternary ammonium additives to the hybridization buffer,[11] the incorporation of synthetic nucleotide analogues into the solid phase-bound oligomers,[12] and the semiempiric design of suitable sequence stretches[13] are being considered. Such studies are not only relevant for experimental microarray technology but also for other applications such as antisense techniques. For example, microarrays have been used to determine the sequence- and length-dependent hybridization efficiency of about 2000 surface-bound oligomers in order to select suitable antisense oligonucleotides capable of effectively binding a mRNA target,[8h] and to examine the intramolecular folding of an mRNA molecule.[8k]

Great efforts in microarray technology are devoted to technical improvements of reliable and highly sensitive detection methods, for instance, to enable the analysis of even trace amounts of mRNA, possibly without the necessity of a previous amplification step. In the field of DNA microarrays fluorophore labels have practically replaced radiolabels that were commonly used for highly sensitive detection and quantification purposes.[2d, 2e] The fluorescence scanners currently available allow the quantitation of subattomol amounts of fluorophores with a dynamic range of more than three decades.[2d] However, the use of labels in the detection of nucleic acid hybridization is generally associated with drawbacks. Homogeneously labeled sample materials are required, and stringent washing steps are necessary to remove unbound materials subsequent to hybridization. Instead of this end-point determination, a real-time hybridization analysis would be advantageous for obtaining additional data from the sequence- and temperature-dependent binding kinetics of individual oligomer probes attached to the surface. Optical[14] or mass-sensitive methods,[15] often based on layers of metallic transducers, are currently beeing investigated for their suitability as label-free detection methods. Although not yet sufficiently sensitive, the use of impedance spectroscopy[16] is very attractive since it would open a way to realize the visions of DNA chips with integrated electronics being used for signal analysis. In this context, studies concerning the hybridization of nucleic acids on microchips as directed by electric fields[17] shall be mentioned, which have recently been applied to SNP detection.[17d] Mass spectrometry[18] is a particularly powerful method for the "read out" of microarrays. Sequenome has developed an SNP analysis[18b] that is based on the enzymatic elongation of oligonucleotides bound to the solid phase subsequent to hybridization. The products are analyzed by matrix-assisted laser desorption ionization-time of flight mass spectrometry-(MALDI-TOF MS; Scheme 3). An entirely automated processing enables daily throughputs of about 10000 samples to be attained. Since MALDI detection allows the determination of the molecular weight of DNA oligomers with an accuracy of a few Daltons, the method is also convenient for Sanger sequencing at mass ranges up to 20 kDa, which is equal to about 60 nucleotides.[18c, 18d]

The current "gold rush" atmosphere in the field is probably a result of both the fascinating bioanalytical potential for basic research and also the excellent economic prospects of DNA microarray technology for the various industrial applications. To overcome the gap between desire and reality, however, serious technical obstacles remain to be solved, which range from the highly reproducable chip production to the necessity of performing the many thousands of hybridization reactions under identical reaction conditions. A current frontier in microarray analytics is the detection of length polymorphisms of micro- and minisatellite DNA, which is essential for forensics and paternity analyses. A solution to this problem

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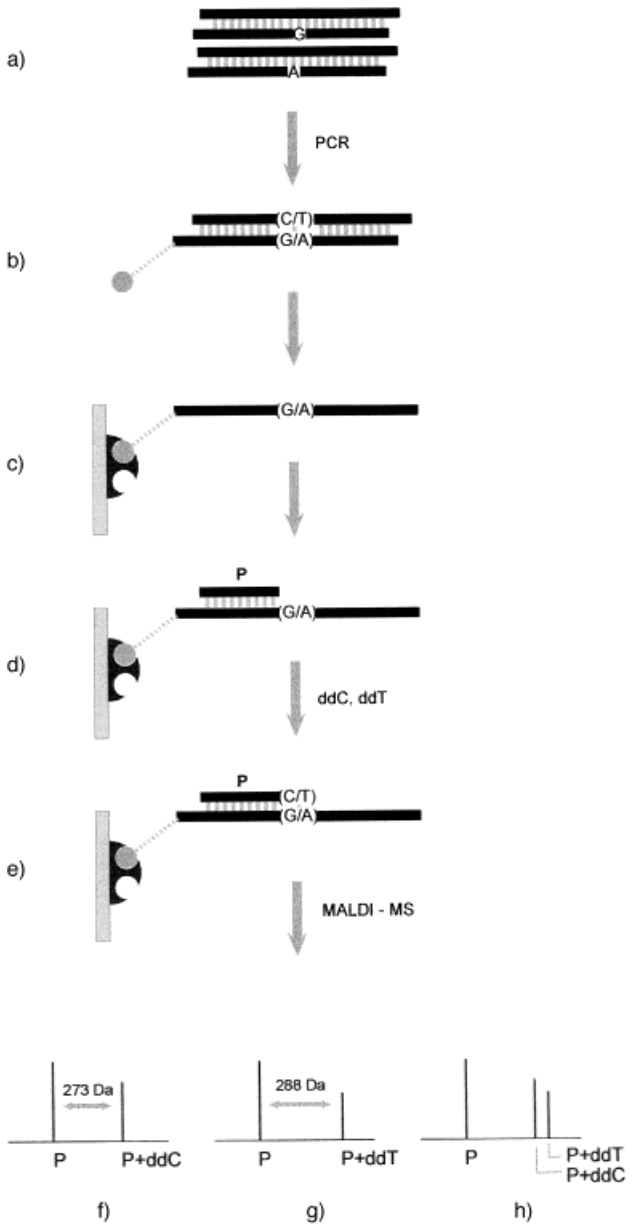

Scheme 3. Mass spectrometric analysis of SNPs. Genomic DNA (a) of a heterozygous proband containing a single point mutation (G→A) is amplified and functionalized with a biotin group by PCR (b). The DNA is denatured and immobilized at a streptavidin-coated surface (c), and an oligonucleotide probe P is added and hybridizes immediately upstream of the point mutation (d). Following, the enzymatic elongation of the probe with nucleotide terminators (ddC and ddT) is carried out, and the products are analyzed by MALDI-MS. The spectra shown schematically illustrate the cases of a healthy (f) and sick (g) homozygous as well as of a heterozygous proband (h).

may be provided by HTS based on mass spectrometry.[18e] Further improvements in detection sensitivity of microarray analytics might result from current advances in the area of single-molecule detection, and also chip-based amplification methods.[19] To take advantage of the full power of the novel microarray technology, however, it is necessary to emphasize the role of bioinformatics. As a result of the gigantic amounts of data emerging from array hybridizations, comprehensive hard- and software solutions are required for both the a-priory design of experiments and chips, and the analysis and standardization of the results. The latter is essential to allow for data mining by basic and commercial genome researchers,[2k] particularly if the next array generations already envisioned[2e] in fact accommodate several millions of oligomer probes. An increasingly important parameter that has not yet been substantially taken into account is the role of translation. This means that mRNA expression data needs to be correllated with the assortment of proteins actually present in the cell. Thus, it remains to be seen whether future developments of protein, antibody,[20] or even cell arrays[2a] will help to correllate genome and proteome[21] research. This may lead to a comprehension of the entire cellular processes, which will enable the exploitation and standardization of the multidimenisional relations between genotype and phenotype.

German version: *Angew. Chem.* **1999**, *111*, 3039 – 3043

Keywords: analytical methods · gene technology · microarrays · nucleic acids · oligonucleotides

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