

Protein arrays for gene expression and molecular interaction screening

Gerald Walter*, Konrad Büssow†, Dolores Cahill†, Angelika Lueking† and Hans Lehrach†

The array format has revolutionised biomedical experimentation and diagnostics, enabling ordered high-throughput analysis. During the past decade, classic solid phase substrates, such as microtitre plates, membrane filters and microscopic slides, were turned into high-density, chip-like structures. The concept of the arrayed library was central to this development which now extends from DNA to protein. The new and versatile protein array technology allows high-throughput screening for gene expression and molecular interactions. As a major platform for functional genomics, it is already on its way into medical diagnostics.

Addresses

*Biorchard AS, Nedre Skogvei 14, N-0281 Oslo, Norway;
e-mail: gerald@biorchard.com

†Max Planck Institute of Molecular Genetics, Ihnestrasse 73, D-14195 Berlin, Germany

Current Opinion in Microbiology 2000, 3:298–302

1369-5274/00/\$ – see front matter

© 2000 Elsevier Science Ltd. All rights reserved.

Abbreviations

CCD charge-coupled device

UPA universal protein array system

Introduction

With an ever increasing number of microbial genomes sequenced, our options for detailed analysis of infectious diseases and their causes multiply exponentially. Diagnosis and therapy of complex ailments, such as allergies, autoimmunity and cancer, benefit particularly from the fine-tuning potential of new array technology. The replacement of microbial growth as a diagnostic measure by quick and extremely sensitive multiplexed screening assays must be regarded as a major advance in microbiology.

Arrays are ordered arrangements of individual samples (e.g. proteins), enabling their parallel analysis. The organisational principle is partitioning, using either tubes, cavities (e.g. wells, as in microtitre plates) or discrete patches or spots on planar surfaces. Analytical efficiency is directly related to density, and miniaturisation has advanced considerably in recent years. Tubes can be arranged as capillary arrays and used in automated DNA sequencing and protein analysis. The 96-well microtitre plate provides the footprint for higher-density plates and is still the most widely used in immunoassays. Now, 384-well plates are taking over for many assays and are standard for storage and handling of clone libraries. 1536-well plates are available and etched glass and silicon wafers make substrates for ‘nanoplates’ (e.g. 9600-wells).

If large numbers of samples are to be analysed for their interaction with a single ligand, micro (spot) arrays on planar surfaces are the format of choice. The ideal surfaces are either filter membranes (e.g. nitrocellulose, nylon or polyvinylidene difluoride) or glass slides coated with various reagents (e.g. poly-L-lysine or polyacrylamide). Microarray (‘chip’) production is highly automated, using either pin-based or microdispensing liquid handling robots. Detection of ligand binding (or hybridisation) is achieved by radioactive or fluorescent labelling, using either a charge-coupled device (CCD) camera or a laser-scanner-based system. Software packages are available for image analysis and classification of results.

Here, we review the rise of the microarray technology to the proteins, the functional effectors of the genome. Originating from classic microtitre plate-based immunoassays on one hand and DNA microarrays on the other, a new field of genetic analysis has begun to unfold. Applications ranging from gene expression analysis to molecular activity and interaction studies are discussed. We speculate that future development will be mainly driven by increasing demands for high-throughput and miniaturisation, resulting in integrated, easy-to-use solutions for the diagnostics market.

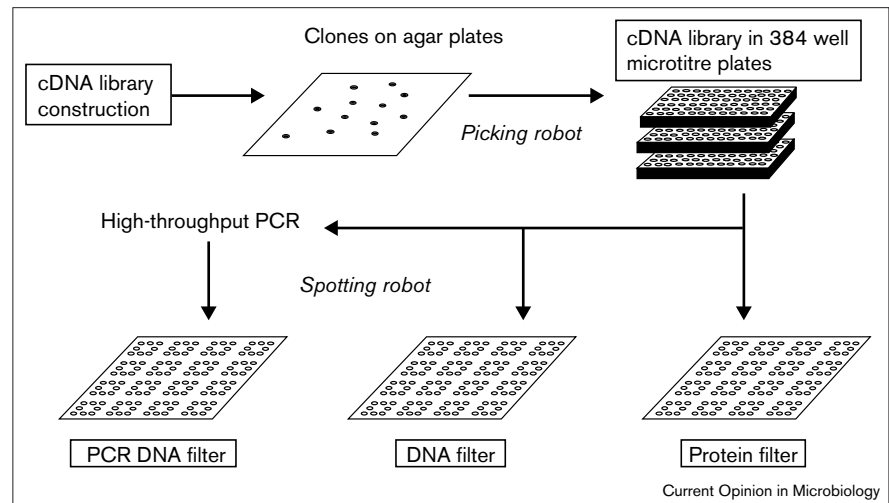
From microtitre plates to protein microarrays

The microtitre plate is a well-established protein array format that has been used in immunoassays for decades. Liquid-handling robots are employed to enable high-throughput screening. Array density is low, however, and assays take large amounts of reagents. Therefore, ‘nanoplates’ (100–1000 nl-well volumes, e.g. GeSiM mbH, Dresden, Germany, [<http://www.gesim.de>]; Orchid Biocomputer Inc, Princeton, NJ, USA, [<http://www.orchid.com>]) and other microarray formats were developed that exceed the density potential of the standard microtitre plate by far. Nevertheless, the 96-well microtitre plate has been the starting point for low-density protein arrays on filter membranes (e.g. the universal protein array system [UPA] developed by Ge [1**]).

Mendoza *et al.* [2*] have also maintained the footprint of the classic microtitre plate, while expanding greatly on density, by designing a microarray as an optically flat glass plate containing 96 wells formed by an enclosing hydrophobic Teflon mask. Inside the wells, the authors spotted arrays of 144 (4 × 36) elements each, using a 36-capillary-based print head attached to a precise, high-speed, X-Y-Z robot. Standard enzyme-linked immunosorbent assay (ELISA) techniques and a scanning CCD detector were employed for imaging of arrayed antigens. This array format appears quite promising as a

Figure 1

High-density arrays of cDNA libraries. Using a picking/spotting robot [21], cDNA clones are picked from agar plates into 384-well microtitre plates, and identical patterns are spotted at high density onto nylon or polyvinylidene difluoride filter membranes that are subsequently processed for DNA hybridisation (DNA filters) or protein detection (Protein filters), respectively [18*]. For microarrays, cDNA inserts are first amplified by high-throughput PCR [19], or proteins are expressed in microtitre plates before spotting [25**].



compromise between the high-density microarray and the microtitre plate for parallel incubation of many different analyte samples.

With similar intentions in mind, Rowe *et al.* [3] simulated the microtitre plate by applying capture antibodies and analytes onto microscopic slides, using flow chambers in a cross-wise fashion. Detection was again via fluorescent labels and CCD-based optical readout. Although still at a low-density stage (i.e. a 6 × 6 pattern), the technique has high-throughput potential as it involves automated image analysis and microfluidics, which is already becoming one of the future formats for enzyme activity and other assays [4] (Caliper Technologies, Mountain View, CA, USA, [http://www.calipertech.com]; Orchid Biocomputer Inc.). In order to manufacture three-dimensional arrays on a flat surface, Mirzabekov and co-workers [5] used a gel photo- or persulfate-induced co-polymerisation technique to produce oligonucleotide, DNA and protein microchips on polyacrylamide gel pads from 10 × 10 to 100 × 100 μm, separated by a hydrophobic glass surface. The three-dimensional polyacrylamide gel provides >100 times greater capacity for immobilisation compared with a two-dimensional glass support, thus increasing the sensitivity of measurements considerably [6].

An attractive method for fabricating antibody arrays using a micromolded hydrogel ‘stamper’ and an aminosilylated receiving surface was recently reported by Martin *et al.* [7*]. The stamper deposits protein (e.g. antibody) as a sub-monolayer, as shown by I¹²⁵ labelling and atomic force microscopy, whilst antibody activity was retained. Other approaches to protein microarrays have been reported using either photolithography of silane monolayers [8] or gold [9], combining microwells with microsphere sensors [10] or inkjetting onto polystyrene film [11]. These advances focus on the fabrication of miniaturised immunoassay formats by arraying of single proteins

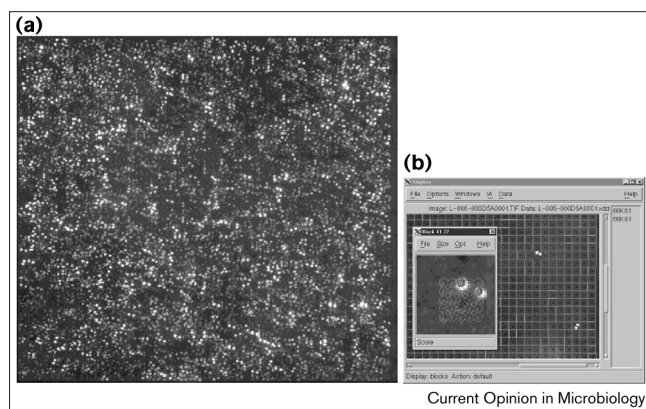
(e.g. bovine serum albumin, avidin or monoclonal antibodies). For capturing and analysis of specifically labelled proteins, sophisticated ligand-coated surfaces are available, such as the SELDI ProteinChip (CIPHERGEN Biosystems Inc., Palo Alto, CA, USA; [http://www.ciphergen.com]), XNA on Gold (INTERACTIVA Biotechnologie GmbH, Ulm, Germany, [http://www.interactiva.de]) and various BIACORE chips (Biacore AB, Uppsala, Sweden; [http://www.biacore.com]).

A particularly interesting application of the array principle is the recently developed tissue microarray for high-throughput molecular profiling of tumour specimens [12**]. The authors designed a robot (now commercially available from Beecher Instruments, Silver Spring, MD, USA; [http://www.beecherinstruments.com]) to punch cylinders (0.6 mm wide, 3–4 mm high) from 1000 individual tumour biopsies embedded in paraffin and to array them in a 45 × 20 mm paraffin block. On serial sections, tumours are then analysed in parallel by immunohistochemistry, fluorescence *in situ* hybridisation and RNA–RNA *in situ* hybridisation. This system allows the microscopic scanning of an immunohistochemistry array slide containing 645 specimens in less than two hours. It should be of great help for the simultaneous analysis of tumours from many different patients at different stages of disease, to establish the therapeutic importance of new candidate marker genes more rapidly [13].

From clone to gene to protein

Arrays are at the heart of genome research. Ninety-six-capillary electrophoresis arrays [14] are now available for high-throughput sequencing (PE Biosystems, Foster City, CA, USA, [http://www.pebio.com]; Molecular Dynamics Amersham Pharmacia Biotech Inc, Sunnyvale, CA, USA, [http://www.moleculardynamics.com]). High-density arrays of clone libraries were first introduced by the Genome Analysis Group at the Imperial Cancer Research

Figure 2



Protein expression screening on high-density filters. **(a)** Protein filters [18[•]] were screened for cDNA clones expressing recombinant fusion proteins recognised by the monoclonal RGS-His antibody (QIAGEN GmbH, Hilden, Germany). **(b)** Signals were scored and classified using the image analysis software Xdigitise (written by Huw Griffiths, Max Planck Institute of Molecular Genetics, Berlin, Germany, available on request).

Fund, London, about ten years ago [15]. They enable a new, organised approach to library handling and screening, encompassing multi-level data generation (reviewed in [16]). Each clone is individually addressable via its coordinates in a microtitre plate and respective spots on filter membranes or chips [17]. High-density arrays of cDNA libraries (Figure 1) can now be used to go from an individual clone to a specific gene and its protein product [18[•]]. Clone libraries become amenable to database integration including all steps from DNA sequencing to functional assays of gene products.

Databases can be built involving all kinds of relational data from the clinic to the pharmaceutical marketplace (see Resource Centre of the German Human Genome Project, RZPD, [http://www.rzpd.de]). The basic idea is the creation of resources as annotated systems that can be efficiently accessed and interrelated [19,20]. Ultimately, it is the synthetic description of life starting from individual genes and their actions. Experimentally, the exercise starts with the collection and organisation of large amounts of biological material as libraries of clones. This requires high-throughput work, using automated technology of constantly growing sophistication [21,22[•]]. Such technology allows gene activity monitoring by analysis of complex expression patterns, resulting in fingerprints of diseased versus normal or developmentally distinct tissues [23].

Starting from high-density filter membranes [15], DNA microarrays have been devised in chip format [24[•]]. The next step, profiling of protein products encoded by differentially expressed cDNA clones, requires a highly parallel approach to protein expression analysis, including the simultaneous expression of large numbers of

cDNA clones in an appropriate vector system and high-speed arraying of protein products. We [18[•]] have shown that the same arrayed cDNA expression libraries that are suitable for standard DNA hybridisation analysis can also be used as a source of recombinant proteins and can be screened for clonal protein expression at high throughput on automatically gridded high-density filters (Figure 2). Our human fetal brain cDNA expression library hEx1 (RZPD library no. 800, filters available from [http://www.rzpd.de]) connects recombinant proteins to clones identified by DNA hybridisation or sequencing, hence creating a direct link between the gene catalogue and a functional catalogue. We [25^{••}] have extended this approach to automated spotting of protein microarrays from liquid expression cultures, using either a new pin-based or a high-speed picolitre dispensing (inkjetting) head mounted onto a flat-bed gridding robot. Ninety-six proteins of the hEx1 library were expressed in liquid bacterial cultures, and solutions were spotted onto polyvinylidene difluoride filters, either as crude lysates or after purification by nickel-nitrilotriacetic acid (Ni-NTA)-immobilised metal affinity chromatography. Four thousand eight hundred samples were placed onto polyacrylamide-coated microscopic slides and simultaneously screened, using a hybridisation automat (Max Planck Institute of Molecular Genetics, Berlin, Germany), applying minimal amounts of reagents (less than 100 µl antibody solution — A Lueking, unpublished data). Sharp and well-localised signals allowed the detection of 250 attomol or 10 pg of a spotted test protein (e.g. GAPDH).

A high-throughput matrix-assisted laser desorption ionisation time-of-flight mass spectrometry technique (MALDI-TOF-MS) has been developed for robust, rapid and highly accurate quality control of proteins [26], and small deviations from expected masses, resulting from chemical modification or loss of single amino acids, now can be efficiently detected. An enzyme (GAPDH) activity assay was devised in a corresponding array format [26], providing a (biochemical) functional screening tool for human protein expression clone catalogues. A variety of further applications of protein arrays in proteomics have recently been proposed [27].

High-throughput molecular interaction screening

Protein arrays have been widely used for screening of molecular interactions. Ge [1^{••}] employed a low-density UPA system for studying interactions with protein, DNA, RNA and small chemical ligand probes. Testing interactions of the human protein p52 with 48 purified proteins spotted onto a nitrocellulose membrane, the author could distinguish high-affinity protein–protein interactions by washing the membrane with high salt concentrations (500–1000 mM potassium chloride). We screened high-density protein filters of the hEx1 library with antibodies against the human proteins GAPDH and heat shock protein 90- α (HSP90 α) and confirmed positives by cDNA probing and sequencing [18[•]].

hEx1 protein filters have now been used by a number of groups to detect expression clones using antibodies, single-chain Fv fragments or other protein–protein or nucleic acid–protein interaction screening. Ulrich Mahlkecht and colleagues (Department of Haematology and Oncology, University Clinic, Frankfurt am Main, Germany) used the filters to clone interaction partners of human HDAC-3 and the HIV Nef protein by Far-Western screening (U Mahlkecht, personal communication). For such experiments, proteins preferably should be in a ‘native’ state (e.g. be expressed in non-bacterial systems). In order to achieve this goal, a shuttle vector for dual protein expression in *Escherichia coli* and *Pichia pastoris* has recently been developed (A Lueking *et al.* unpublished data). Jörg Piontek (Department of Neurobiology, University of Heidelberg, Germany) screened the same filters with six different antibodies against components of the human neuronal membrane skeleton. All antibodies detected clones on the filters, but only positives detected with one of the antibodies matched the biochemical data of the antigen (J Piontek, personal communication). The hEx1 library is currently being used for profiling of sera from patients with Crohn’s Disease and ulcerative colitis, using sex-matched, age-matched normal sera as controls (D Cahill, unpublished data).

We [25**] have recently shown that protein microarrays enable very sensitive antibody specificity screening at high throughput. When protein microarrays displaying a test set of 92 hEx1 expression clones were screened with monoclonal antibodies (e.g. mouse anti-GAPDH, HSP90, tubulin- α), phage-displayed and soluble single-chain Fv fragments, different degrees of antibody cross-reactivity with nontarget proteins were observed. This suggests an interesting application of the technology for screening antibodies against arrays of potential antigens to detect common epitopes or to profile antibody specificity as a quality control measure. This seems particularly important for reagents that are to be used for immunohistochemistry or physiological studies on whole cells or tissues, where they face batteries of different structures. Using the same technology, antibodies with no known antigen specificity (e.g. lymphoma proteins) can be screened for binding to a highly diverse repertoire of protein molecules. As all of these proteins are expressed from isolated clones of arrayed cDNA libraries, the corresponding inserts can easily be sequenced to identify antigen-encoding genes. Such an approach can be applied to homology studies on protein families, defining binding domains, epitopes and interacting molecular motifs.

Conclusions

Protein arrays appear as new and versatile tools in functional genomics, enabling the translation of gene expression patterns of normal and diseased tissues into protein product catalogues. Protein function, such as

enzyme activity, antibody specificity or other ligand–receptor interactions and binding of nucleic acids or small molecules can be analysed on a whole-genome level. As the array technology develops, an ever-increasing variety of formats becomes available (e.g. nanoplates, patterned arrays, three-dimensional pads, flat-surface spot arrays or microfluidic chips), and proteins can be arrayed onto different surfaces (e.g. membrane filters, polystyrene film, glass, silane or gold). Various techniques are being developed for producing arrays, and robot-controlled, pin-based or inkjet printing heads are the preferred tools for manufacturing protein arrays. CCD cameras or laser scanners are used for signal detection; atomic force microscopy and mass spectrometry are upcoming options. The emerging future array systems will be used for high-throughput functional annotation of gene products, their involvements in molecular pathways, their response to medical treatment and become the doctor’s indispensable diagnostics tools.

Update

As arrays become so central to genomics research and their extension to protein analysis has only recently begun, a number of interesting new reports were published since this review was submitted. Mirzabekov and coworkers [28] have modified the polyacrylamide gel on their protein microchips to accommodate proteins of up to 400 kd in size. The chips could be used several times in different enzyme and immuno assays, and electrophoresis was used to speed up antigen–antibody interactions.

The group of Emmert at the National Cancer Institute, Bethesda, USA [29], extended on the use of tissue microarrays in cancer research. Their ‘layered expression scanning’ uses a series of capture membranes coated with antibodies or cDNA probes for multiplex protein or mRNA analysis. While cell or tissue samples were transferred through the layers, the two-dimensional relationship of cell populations was maintained, thereby producing a molecular profile of each cell type present.

A recent review from the Stanley Fields laboratory [30] reintroduces the arrayed library concept of 1991 [15], summarising functional studies on the approximately 6000 gene products of the completely sequenced yeast *Saccharomyces cerevisiae*. Martzen *et al.* [31] identified three previously unknown genes by pool array-based biochemical assays of their purified products. Uetz *et al.* [32] used two-hybrid arrays to identify protein–protein interactions.

Finally, protein arrays might become a preferred tool for recombinant antibody library screening. IM Tomlinson and co-workers at the Medical Research Council, Cambridge, UK (personal communication), have used the hEx1 protein filters to develop a system of ‘naive’ screening that could be applied to the high-throughput isolation of specific antibodies against many different targets in the human proteome.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Ge H: **UPA, a universal protein array system for quantitative detection of protein-protein, protein-DNA, protein-RNA and protein-ligand interactions.** *Nucleic Acids Res* 2000, **28**:e3, I-VII.
The paper describes an easy system for studying molecular interactions on membranes, indicating different affinities using low- or high-salt washes.
2. Mendoza LG, McQuary P, Mongan A, Gangadharan R, Brignac S, Eggers M: **High-throughput microarray-based enzyme-linked immunosorbent assay (ELISA).** *Biotechniques* 1999, **27**:778-788.
Protein microarrays were robotically printed within the microtitre well format, as useful for multi-analyte parallel incubations.
3. Rowe CA, Scruggs SB, Feldstein MJ, Golden JP, Ligler FS: **An array immunosensor for simultaneous detection of clinical analytes.** *Anal Chem* 1999, **71**:433-439.
4. Cohen CB, Chin DE, Jeong S, Nikiforov TT: **A microchip-based enzyme assay for protein kinase A.** *Anal Biochem* 1999, **273**:89-97.
5. Guschin D, Yershov G, Zaslavsky A, Gemmill A, Shick V, Proudnikov D, Arenkov P, Mirzabekov A: **Manual manufacturing of oligonucleotide, DNA, and protein microchips.** *Anal Biochem* 1997, **250**:203-211.
6. Parinov S, Barsky V, Yershov G, Kirillov E, Timofeev E, Belgovskiy A, Mirzabekov A: **DNA sequencing by hybridization to microchip octa- and decanucleotides extended by stacked pentanucleotides.** *Nucleic Acids Res* 1996, **24**:2998-3004.
7. Martin BD, Gaber BP, Patterson CH, Turner DC: **Direct protein microarray fabrication using a hydrogel 'stamper'.** *Langmuir* 1998, **14**:3971-3975.
A new, gel-based technology of array printing, offering great potential regarding economy and avoiding contamination problems.
8. Mooney JF, Hunt AJ, McIntosh JR, Liberko CA, Walba DM, Rogers CT: **Patterning of functional antibodies and other proteins by photolithography of silane monolayers.** *Proc Natl Acad Sci USA* 1996, **93**:12287-12291.
9. Jones VW, Kenseth JR, Porter MD, Mosher CL, Henderson E: **Microminiaturized immunoassays using atomic force microscopy and compositionally patterned antigen arrays.** *Anal Chem* 1998, **70**:1233-1241.
10. Michael KL, Taylor LC, Schultz SL, Walt DR: **Randomly ordered addressable high-density optical sensor arrays.** *Anal Chem* 1998, **70**:1242-1248.
11. Ekins RP: **Ligand assays: from electrophoresis to miniaturized microarrays.** *Clin Chem* 1998, **44**:2015-2030.
12. Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, Kallioniemi OP: **Tissue microarrays for high-throughput molecular profiling of tumour specimens.** *Nat Med* 1998, **4**:844-847.
This technology of tumour typing on serial sections, automatically cut and arrayed from tissue core biopsies is already widely used in cancer research.
13. Theillet C: **Full speed ahead for tumour screening.** *Nat Med* 1998, **4**:767-768.
14. Behr S, Matzig M, Levin A, Eickhoff H, Heller C: **A fully automated multicapillary electrophoresis device for DNA analysis.** *Electrophoresis* 1999, **20**:1492-1507.
15. Lennon GG, Lehrach H: **Hybridization analyses of arrayed cDNA libraries.** *Trends Genet* 1991, **7**:314-317.
16. Clark MD, Panopoulou GD, Cahill DJ, Büssov K, Lehrach H: **Construction and analysis of arrayed cDNA libraries.** *Methods Enzymol* 1999, **303**:205-233.

17. Eickhoff H, Ivanov I, Lehrach H: **Miniaturized equipment and potential of microsystem technology.** In *Technical System Management in Microsystem Technology: A Powerful Tool for Biomolecular Studies*. Edited by Saluz HP. Basel: Birkhäuser Verlag; in press.
18. Büssov K, Cahill D, Nietfeld W, Bancroft D, Scherzinger E, Lehrach H, Walter G: **A method for global protein expression and antibody screening on high-density filters of an arrayed cDNA library.** *Nucleic Acids Res* 1998, **26**:5007-5008.
The paper describes the first use of high-density filters for parallel DNA hybridisation, protein expression and antibody screening.
19. Meier-Ewert S, Maier E, Ahmadi A, Curtis J, Lehrach H: **An automated approach to generating expressed sequence catalogues.** *Nature* 1993, **361**:375-376.
20. Zehetner G, Lehrach H: **The reference library system – sharing biological material and experimental data.** *Nature* 1994, **367**:489-491.
21. Maier E, Bancroft DR, Lehrach H: In *Automation Technologies for Genome Characterization*. Edited by Beugelsdijk TJ. New York: John Wiley & Sons, Inc.; 1997:65-88.
22. Bowtell DD: **Options available – from start to finish – for obtaining expression data by microarray.** *Nat Genet* 1999, **21**(suppl 1):25-32.
This paper is part of a dedicated supplement on the matter and gives a broad overview over the latest microarray technology.
23. Meier ES, Lange J, Gerst H, Herwig R, Schmitt A, Freund J, Elge T, Mott R, Herrmann B, Lehrach H: **Comparative gene expression profiling by oligonucleotide fingerprinting.** *Nucleic Acids Res* 1998, **26**:2216-2223.
24. Schena M, Heller RA, Theriault TP, Konrad K, Lachenmeier E, Davis RW: **Microarrays: biotechnology's discovery platform for functional genomics.** *Trends Biotechnol* 1998, **16**:301-306.
The paper lists a wide range of applications of the new microarray technology for gene expression analysis.
25. Lueking A, Horn M, Eickhoff H, Büssov K, Lehrach H, Walter G: **Protein microarrays for gene expression and antibody screening.** *Anal Biochem* 1999, **270**:103-111.
Here is the first paper on robotically printing microarrays from lysates of a cDNA expression library and their use for high-throughput molecular interaction screening.
26. Büssov K, Nordhoff E, Lübbert C, Lehrach H, Walter G: **A human cDNA library for high-throughput protein expression screening.** *Genomics* 2000, **65**:1-8.
27. Cahill D, Nordhoff E, Klose J, O'Brien J, Eickhoff H, Lehrach H: **Bridging genomics and proteomics.** In *Proteomics*. Edited by Pennington S, Dunn M. Oxford: BIOS Scientific Publishers Ltd; 2000.
28. Arenkov P, Kukhtin A, Gemmill A, Voloshchuk S, Chupeeva V, Mirzabekov A: **Protein microchips: use for immunoassay and enzymatic reactions.** *Anal Biochem* 2000, **278**:123-131.
29. Englert CR, Baibakov GV, Emmert BM: **Layered expression scanning: rapid molecular profiling of tumor samples.** *Cancer Res* 2000, **60**:1526-1530.
30. Emili AQ, Cagney G: **Large-scale functional analysis using peptide or protein arrays.** *Nat Biotechnol* 2000, **18**:393-397.
31. Martzen MR, McCraith SM, Spinelli SL, Torres FM, Fields S, Grayhack EJ, Phizicky EM: **A biochemical genomics approach for identifying genes by the activity of their products.** *Science* 1999, **286**:1153-1155.
32. Uetz P, Giot L, Cagney G, Mansfield TA, Judson RS, Knight JR, Lockshon D, Narayan V, Srinivasan M, Pochart P *et al.*: **A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*.** *Nature* 2000, **403**:623-627.