Review Article

An gene expression pattern

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Abstract

DNA microarray technology has become a powerful tool in the arsenal of the molecular biologist. Capitalizing on high-precision robotics and the wealth of DNA sequences annotated from the genomes of a large number of organisms, the manufacture of microarrays is now possible for the average academic laboratory with the funds and motivation. Microarray production requires attention to both biological and physical resources, including DNA libraries, robotics, and qualified personnel. Although the fabrication of microarrays is a very labor-intensive process, production of quality microarrays individually tailored on a project-by-project basis will help researchers shed light on future scientific questions.

Key words: DNA Library, gene microarray, microarray, robotic printer.

INTRODUCTION

In the past ten years, the use of microarrays has gone from a cutting edge novelty to a well-defined technique in most molecular biology laboratories. With the availability of affordable, high-precision robotics, the production of high-density microarrays is accessible to anyone with the determination, will, and funding. Ever since Patrick Brown laboratory at Stanford University popularized the method, the allure to print one's own microarrays has been enticing.[1,2]

The principle is simple and is derived from what we already know about RNA and DNA hybridization. mRNA is isolated from a given sample.[3] Then, when cDNA synthesis is initiated, the first strand of the cDNA is labeled with the tag. This forms the pool of target sequences. The next step is to hybridize the labeled cDNA to a microarray.

DNA MICROARRAY LIBRARY

The most important aspect of building a microarray, which often becomes overshadowed by the technological hardware issues, is the DNA library. In the beginning, microarrays were manufactured with cDNA assembled from available clone libraries. Generally, these libraries were gathered as part of larger genomic sequencing efforts and then made available to groups printing microarrays. Typically, the DNA was cloned in bacterial vectors with universal primers that allowed PCR amplification of the libraries in order to generate high concentration of pure DNA that corresponded to an expressed gene.[1,3,4]

Today, research groups are increasingly switching to presynthesized, long oligonucleotide libraries as the printing libraries of choice. The companies supplying large oligonucleotide expression libraries have been winnowed down to Operon and Illumina. As the genomic sequence information becomes more complete, oligos can be designed for any known gene for sequenced organisms. Oligonucleotides of 60 to 70 bases in length show the best sensitivity and specificity.[1,3,5]

Moreover, oligonucleotide libraries are easier to maintain. Because they can always be resynthesized, they can be digitally archived in a computer database, so there is no need to keep a permanent physical copy in a -80°C freezer. Use of oligonucleotide libraries also eliminates the possibility of cross-contamination during PCR or bacterial propagation. As human error cannot be eliminated, cross-contamination of oligo libraries

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might occur by well-to-well splashing caused by careless handling.[1,3]

One detail of microarray DNA libraries of any type that is often overlooked is the care and maintenance of the plate sets. For any library of significant size (>10 000 features), it is highly advisable to have access to a liquid handling system for microwell plates. Although printing from 96-well plates is possible, 384-well microtiter plates are required for an array to be printed with reasonable speed. Access to many thermal cyclers (>eight 96-well cyclers) is needed for a moderate throughput of samples, so that the library can be completed in a timely manner.[1,4] At some point in the process, one will have to transfer four 96-well plates into one 384-well plate. In order to accomplish this without error, a liquid handling robot with a 96 pipette-tip head is recommended. Speed and flexibility of the liquid handler are the primary concerns, followed closely by reliability and quality service.[1]

Oligo libraries come from the manufacturer in aliquots that should already be normalized by mass. One can follow the recommended printing concentrations from the manufacturers. Oligo libraries are easier to print than the cDNA libraries in general, because oligos are more even in concentration and viscosity.[1,5]

ROBOTIC PRINTERS

A printing robot needs to have motion control in three axes with an accuracy of +/- 5 JLlm. There are two approaches to obtaining a printing robot: Self-assembly or commercial purchase.[1]

Self-assembly of a printing robot requires access to talented individuals with abilities in both electronics and engineering. For those who are less inclined toward engineering, several printing robots are available commercially, though they all have their strengths and weaknesses. From a purely statistical viewpoint, one would expect that the more moving parts a machine has, the more likely that one of those parts will fail. But experience shows that the quality of the components and the care in construction and engineering are often a better predictor of reliability.[1,6,7]

For either approach, the first consideration should be the desired throughput of the arrayer. Arrayers are available that will print 25, 50, 100, or >200 slides in a print run. If fewer than 50 arrays at a time are needed, a smaller instrument may be sufficient. Keep in mind that a high throughput machine can always be used to print a smaller number of arrays than the full capacity.[1]

A second critical criterion of any arrayer is the pin-washing station. In order to limit or eliminate potential carryover, the washing station must be able to thoroughly remove all the DNA from one sample before picking up the next one. In addition, failure to completely clean and dry the pins before the next sampling could lead to carryover and/or pin failure. Finally, regardless of the printing arrayer used, the calibration of the robot is absolutely critical.[8] The tolerances needed are very tight, and every micron out of "true" can cause problems. Quite often what may seem to be a printing failure is in reality caused by poor calibration.[1]

Additionally, the optimal location for the printing robot is a dust-free environment; preferably the entire room should be supplied with high efficiency particulate air (HEPA)-filtered air. Even if the robot has a dust-free enclosure, the slides have to be loaded by hand, with access doors allowing dust infiltration. A single piece of dirt or dust can clog a printing pin and ruin the whole print.[1,2,6,7]

PRINTING PINS

The printing pin is the heart of the entire microarray manufacturing process. Once the robot is calibrated and the DNA library is at the proper concentration in the appropriate plates, the pins must reliably print every spot on every array.[1]

Several types of printing pins employing different technologies are available. These include the ring-and-pin system; piezoelectric spotters; ink-jet printers; and quill-type split pins. The ring-and-pin system employs a ring that picks up a droplet of solution and a pin that passes through the drop to deposit the solution on the substrate.[9] The main drawbacks to this system is that it withdraws 1/l ml of solution, and the spots are relatively large >200 pm, making this suitable only for low-density arrays.[1]

Both the piezoelectric spotters and ink-jet printers are noncontact printing systems. Although these systems have many potential advantages, they are typically complicated with the concomitant problems of maintenance and reliability. The ink-jet system in particular has been successfully used by Agilent Technologies to manufacture their commercially available microarray, and there are even designs available for in-house custom fabrication.[1,4,8,9]

Although one may want to investigate different printing technologies, the simplest, most robust method utilizes contact printing with a quill-type printing pin. The quill-type printing pin operates on the same technological

principle i.e drawing up DNA solution instead of ink in the slot through capillary action and depositing a spot by contacting the surface of the substrate,. The liquid in the pin must make contact with the substrate so that the spot will be drawn out and left behind through surface tension.[1,4,9,10]

The principle of capillary action that makes the quill type pin so robust and simple is also its Achilles heel. If any dirt or dust accumulates in the slot, the pin will not draw up the DNA solution. If the two tines of the pin are not perfectly even and do not touch the substrate at the same moment, the liquid may not touch the substrate and no spot will be deposited.[1]

Even using as much care as possible when setting up a print run, there will always be the possibility of a piece of dust getting in and clogging the pin. To try to determine the cause of a pin failure requires the removal of the pin and visual inspection, employing a high-quality stereo dissecting scope. Although there will be times when there is no apparent cause of failure, often the problem might be noticeable, such as an offending dust particle trapped in the slot or a bent tip. If the problem is a piece of dust, it can be carefully removed.[1,4,11]

If the printing robot operates within proper tolerances, the amount of "wobble" the pins will exhibit during the print run will be determined by the alignment and tolerances of the pin in the print head. Although some robots have a test-printing mode as a separate function, other instruments will require making a dummy run. The type of DNA used is not critical, as long as it is sheared to a small size simulating the DNA library. The concentration should be similar to the library, as spot morphology is affected by the concentration of DNA.[1,9,10]

Each type of robot has unique wash/dry stations, and so the optimal number of wash cycles and timing parameters must be determined through trial and error. During a test print, the minimum spot-to-spot spacing achievable with the selected pin/buffer/substrate combination should be determined.[1]

MICROARRAY SLIDE SUBSTRATES

The essential choice in choosing a suitable substrate is whether to coat slides in-house or buy commercially prepared slides. The slide must be clean and dust free, enhance active binding of DNA to the surface, and be sufficiently hydrophobic. The more hydrophobic the slide surface is, the smaller the spots will be. Smaller spots are required in order to achieve high-density arrays. Although the DNA binding capacity of the substrate is clearly important, it is difficult to measure experimentally. The best indication of substrate performance is to empirically determine the signal-to-noise ratio.[12,13]

Several slide coatings are in use, with the most common types being poly-L-lysine, aminosilane, and epoxy. Poly-L-lysine and aminosilane give a positive charge to the slide surface, allowing the negatively charged DNA to bind to the slide electrostatically. With epoxy coatings, the epoxy group binds covalently to DNA, especially to amino-modified oligonucleotides.[1,3,4]

Two commonly used printing buffers are 50% dimethyl sulfoxide (DMSO) and 3X SSC. The advocates of 50% DMSO like to print with it because it leaves spots with consistency round and even spot morphology. DMSO buffers are also used to reduce the evaporation rate of the solution in the printing plates. The major objection to printing with DMSO buffers is that the spots tend to be much larger than with aqueous salt buffers, and the final spot size is tremendously affected by the ambient humidity.[14] To print a high-density array with DMSO buffer, the humidity may need to be kept below 30%, which is exceedingly difficult unless one is printing in an arid environment. And, if the humidity increases in the middle of a print run, the spots can start to run together.[1]

Depending on the parameters to be affected, many additives may be added to printing buffers, such as betaine, ethylene glycol, or detergents. 1.5M betaine can be added to the print buffer, which will reduce the evaporation rate of the spot on the slide. This will presumably increase the time for the DNA to bind to the substrate in the aqueous environment, and thereby increase amount of DNA bound to the slide. Ethylene glycol reduces evaporation rates. Detergents (Sodium dodecyl sulfate (SDS), sarcosyl, Tween, Triton, etc.) are added to increase the spot size and improve wetting (and wicking) of the print pin. Very small amounts of detergents can make large increases in spot size, so the optimal concentration will be between 0.001 and 0.05%.[1,5,12]

The final concentration of detergent that will make the desired spot size must be empirically determined for the chosen substrate.[3-6,11]

Whichever method is used, die blocking step is critical to ensure low background in the final hybridization. If there is incomplete or improper blocking, all of the previous steps in the array manufacturing process will prove to have been fruitless.[1]

PRINCIPLES OF GENE MICROARRAY TECHNOLOGY

Determining the level at which genes are expressed is called microarray expression analysis, and the arrays used in this kind of analysis are called "expression chips." The basic concept of this microarray analysis is the following: RNA is harvested from a sample of interest (e.g., cell lines, tissue biopsy) and labeled to generate the target, i.e., the free nucleic acid sample whose identity or abundance is to be detected. The target is then hybridized to the probe DNA sequences corresponding to specific genes that have been affixed, in a known configuration, onto a solid matrix.[3,6,15]

Hybridization between probe and target provides a quantitative measure of the abundance of a particular sequence in the target population. This information is captured digitally and subjected to various analyses to extract biological information. Comparison of hybridization patterns enables the identification of mRNAs that differ in abundance in two or more target samples.[6,7,12,13]

Microarray technology was introduced in the 1990s, although the certain techniques similar to microarray technology were first conceived and developed in the 1980s.[1,14]

Since then, both commercial and academic groups have developed a number of different microarray platforms and there are now numerous high-density platforms available which differ in terms of probe content, design, deposition technology, labeling, and hybridization protocols. Regarding probe types, possible choices include spotted cDNA sequences or polymerase chain reaction (PCR) products, and short or long oligonucleotides ranging from 25 to 70 base pairs.[16-18] However, two major platforms for high-density microarray manufacture are in common use. Both methods share the feature of a solid support "chip" to which hundreds of thousands of gene fragments are attached. The first utilizes robotic deposition or "spotting" of DNA molecules that can be in the form of PCR-amplified complementary DNA (cDNA), presynthesized oligonucleotides, or genomic DNA like plasmids or bacterial artificial chromosomes. These spotted arrays are referred to as "cDNA microarrays."[12,15,19,20]

The second technology was developed by Afrymetrix™, using 25-mer oligonucleotides synthesized in situ by a photolithographic process similar to manufacture of computer chips in which up to 1.3 million different oligonucleotide probes are synthesized on each array. Each oligonucleotide is located in a specific area on the array called a probe cell and each probe cell contains hundreds of thousands to millions of copies of a given oligonucleotide.[1,5,11,14,16,17]

Beside the different immobilized probe used to detect specific mRNA transcripts, the main difference between the two types of arrays is the number of biological samples used within a single chip experiment.[21,22] cDNA microarrays are two-channel arrays, with both a reference and experimental sample analyzed in the same chip. Samples are labeled with two fluorescent dyes, generally Cy3 (green) and Cy5 (red), and the chip scanner measures the amount of the two signals and eventually gives the ratio of the two intensities.[5,7,23] Typical cDNA microarray experiments compare a normal cell or tissue samples with a treated or pathological sample.[6,12,18,19]

Oligonucleotide-based arrays are one-channel arrays that give an absolute measurement of mRNA binding, and this result can be directly compared with the results of other oligonucleotide microarray experiments. The key point for this DNA array platform is the targeted design of probe sets.[24,25] Using as little as 200 to 300 bases of gene, cDNA or expressed sequence tag (EST) sequence, independent 25-mer oligonucleotides are selected to serve as unique, sequence-specific detectors.[2,7,9,11,20,21] The arrays are designed in silico, and as a result, it is not necessary to prepare, verify, quantitate, and catalogue a large number of cDNAs, PCR products, and clones, and there is no risk of a misidentified tube, clone, cDNA, or spot. Although the binding of the probe to the target is constituted by an oligonucleotide only 25 base pair long, Affymetrix™ technology achieves a high grade of specificity by using set multiple probe pairs for each probe, consisting of perfect match (PM) oligonucleotides and corresponding mismatch (MM) oligonucleotides, used as control for nonspecific binding. For each probe designed to be perfectly complementary to a target sequence (PM), a partner probe is generated that is identical except for a single base MM in its center, the MM oligonucleotide.[15,19,21,26,27] This probe MM strategy, along with the use of multiple probes for each transcript, helps to identify and minimize the effects of nonspecific hybridization and background signal. Moreover, the use of multiple independent detectors for the same molecule greatly improves signal-to-noise ratios, improves the accuracy of RNA quantitation, reduces the effects of cross-hybridization, and drastically decreases the rate of false positives.[28] In addition, short-chain oligonucleotides with single points of constraint are probably more accessible for hybridization to target than cDNA probes.[4,5,29,30]

The latest generation of Gene Chip expression arrays is represented by arrays with smaller feature size (11 microns), allowing the expression of all known transcripts of an organism to be analyzed on a single array.[2,7,31,32]

The application of microarray technology to immunology is apparent. One could easily ask what is the difference between T cells and B cells. Or what is the difference between an activated T cell and a resting T cell? The list of possible comparisons is immense. To begin to answer some of the interesting immunology questions, Louis Staudt and co-workers at the National Institutes of Health (NIH) have developed an array they term "Lymphochip." The Lymphochip is an array that consists of more than 10 000 human genes and is enriched in genes expressed in lymphoid cells.[5,13,17,33,34] It also includes genes from normal as well as transformed lymphocytes. This particular microarray has provided a great deal of useful information, including a profile of T cells compared with B cells, plasma cells compared with germinal center B cells, and gene expression patterns induced by various signaling pathways.[18-21,23,35]

MICROARRAY ANALYSIS AS A DIAGNOSTIC TOOL FOR HUMAN DISEASES

It is almost impossible to distinguish visually between B and T cells without molecular analysis. Similarly, it can be quite difficult to distinguish one tumor from another. Two of the best-known acute leukemias are AML, which arises from a myeloid precursor (hence the name, acute myeloid leukemia), and ALL (acute lymphoid leukemia), which arises from lymphoid precursors.[1,8,36,37] Until recently, these two diseases could be diagnosed with some degree of confidence using a combination of surface phenotyping, karyotypic analysis, and histochemical analysis, but no single test was conclusive; reliable diagnosis depended upon the expertise of the clinician.[18,20,24]

The difference between an ALL diagnosis and an AML diagnosis can mean the difference between life and death. ALL responds best to corticosteroids and chemotherapeutics such as vincristine and methotrexate. AML is usually treated with daunorubicin and cytarabine.[38,39] The cure rates are dramatically diminished if the less appropriate treatment is delivered due to misdiagnosis. In 1999, a breakthrough in diagnosis of these two leukemias was achieved using microarray technology. Todd Golub, Eric Lander, and their colleagues isolated RNA from 38 samples of acute leukemia, labeled the RNA with biotin, and hybridized the biotinylated RNA to commercial high-density microarrays that contained oligonucleotides corresponding to some 6 817 human genes.[3,6,9,13,25] Whenever the biotin-labeled RNA recognized a homologous oligonucleotide, hybridization occurred. Analysis revealed a group of 50 genes that were highly associated with either AML or ALL when compared with control samples. These 50 genes were then used to sample nucleic acid from 34 independent leukemias as well as samples from 24 presumed-normal human bone marrow or blood samples. The result of microarray analysis is clearly classified a tumor as ALL or AML and suggested that the treatments for AML and ALL can be targeted more precisely.[40,41] For example, an AML expressing genes x, y, and z might respond to one treatment modality better than an AML that expresses a, b, and c. Several pharmaceutical companies have established research groups to evaluate different treatments for tumors based on the tumor's microarray profile. This designer-approach to oncology is expected to produce much more effective treatments of individual tumors, and ultimately, enhanced survival rates.[33,38,40] Microarray analysis is likely to be very useful in the diagnosis of tumors of the immune system. Most notably, a laboratory at the NIH has developed a specialized DNA microarray containing more than 10 000 human cDNAs that are enriched for genes expressed in lymphocytes.[26,27] Some of these cDNAs are from genes of known function, others are unknown cDNAs derived from normal or malignantly transformed lymphocyte cDNA libraries. This specialized array is called the "Lymphochip" because the lymphocyte cDNAs are arrayed on a silicon wafer. The group at NIH asked whether they could use the Lymphochip to divide the B-cell leukemia known as diffuse large B-cell lymphoma (DLBCL) into subgroups, an important question because this type of lymphoma has a highly variable clinical course, with some patients responding well to treatment, while others respond poorly. Earlier attempts to define subgroups within this group had been unsuccessful.[5,14,27,38] A definition of subgroups within DLBCL could be useful in designing more effective treatments. Using the Lymphochip, the group at NCI identified two genotypically distinct subgroups of DLBCL. One group was comprised of tumors expressing genes characteristic of germinal-center B cells and was called "germinal-center–B-like DLBCL."[2,5,28,29] The other group more resembled activated B cells and was termed "activated B-like DLBCL." Significantly, patients with germinal-center–B-like DLBCL had a higher survival rate than those with activated B-like DLBCL. Normally, all patients with DLBCL receive multi-agent chemotherapy. Patients who do not respond well to chemotherapy are then considered for bone-marrow transplantation. The data obtained from this study suggest that patients with activated B-like DLBCL will not respond as well to chemotherapy and may be better served by bone-marrow transplantation shortly after diagnosis. As a direct result of this work, ongoing clinical trials are evaluating how best to treat patients with activated B-like DLBCL.[18,19,21-24,41,42]

Gene profiling is not restricted to diagnosis of cancer. This technology provides us with a unique opportunity to examine differences between any distinct populations

of cells. One can compare which genes are expressed in common or differentially in a native T cell and a memory T cell. What is the difference between a normal T cell and a T cell dying by apoptosis? Comparisons like these will be a rich source of insight into differences in cell populations. The key to using this valuable information will be the development of tools to analyze the vast quantities of data that can be obtained from this new approach.[5,8,14,29,30]

CONCLUSION

By using gene microarray technology, scientists can determine in a single experiment the expression levels of thousands of genes within a given sample. DNA microarray technology is evolving rapidly and there are now numerous high-density platforms available which differ in terms of probe content, design, deposition technology, labeling, and hybridization protocols. However, two major platforms for high-density microarray manufacture are in common use. The first utilizes robotic deposition or "spotting" of DNA molecules, while the second uses short oligonucleotides synthesized *in situ*. It is true that manufacturing microarrays is a very labor-intensive process, even with all of the robotic equipment to be had. However, manufacturing microarrays in a research laboratory is easier now than ever before, thanks to the wealth of information and available resources. As more genomic information becomes annotated, there will be more opportunities to mine this wealth of data, and microarrays will continue to be an invaluable tool.

By using gene microarray technology, scientists can determine in a single experiment the expression levels of thousands of genes within a given sample. DNA microarray technology is evolving rapidly and there are now numerous high-density platforms available which differ in terms of probe content, design, deposition technology, labeling, and hybridization protocols.

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REFERENCES

1. Mocellin S, Mandruzzato S. Microarray Technology and Cancer Gene Profiling. Vol. 593. Landes Bioscience / Springer Science+Business Media. Germany: LLC dual imprint / Springer Science+Business Media, LLC series. Advances in Experimental Medicine and Biology; 2007. p. 1-24.
2. Hughes TR. Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer. Nat Biotechnol 2001;19:342-7.
3. Lausted C. POSaM: A fast, flexible, open-source, inkjet oligonucleotide synthesizer and microarrayer. Genome Biol 2004;5:R58.
4. Brown PO, Botstein D. Exploring the new world of the genome with DNA microarrays. Nat Genet 1999;21(1 Suppl):33-7.
5. Southern E, Mir K, Shchepinov M. Molecular interactions on microarrays. Nat Genet 1999;21(1Suppl):5-9.
6. Alizadeh AA, Eisen MB, Davis RE. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000;403:503-11.
7. Fodor SP. Light-directed, spatially addressable parallel chemical synthesis. Science 1991;251:767-73.
8. Churchill GA. Fundamentals of experimental design for cDNA microarrays. Nat Genet 2002;32(Suppl):490-5.
9. Fodor SP, Rava RP, Huang XC, Pease AC, Holmes CP, Adams CL. Multiplexed biochemical assays with biological chips. Nature 1993;364:555-6.
10. Lee ML, Kuo FC, Whitmore GA. Importance of replication in microarray gene expression studies: Statistical methods and evidence from repetitive cDNA hybridizations. Proc Natl Acad Sci USA 2000;97:9834-9.
11. Mocellin S, Provenzano M, Rossi CR. DNA array-based gene profiling: From surgical specimen to the molecular portrait of Cancer. Ann Surg 2005;241:l6-26 .
12. Khan J, Wei JS, Ringner M. Classification and diagnostic prediction of cancers using gene expression profiling and artificial neural networks. Nat Med 2001;7:673-9.
13. Golub TR, Slonim DK, Tamayo P. Molecular classification of cancer: Class discovery and class prediction by gene expression monitoring. Science 1999;286:531-7.
14. Alizadeh AA, Eisen MB, Davis RE. Distinct types of diffuse large B -cell lymphoma identified by gene expression profiling. Nature 2000;403:503-11.
15. Heller RA, Schena M, Chai A. Discovery and analysis of inflammatory disease-related genes using cDNA microarrays. Proc Nat Acad Sci USA 1997;94:2150-5.
16. Rogge L, Bianchi E, Biffi M. Transcript imaging of the development of human T helper cells using oligonucleotide arrays. Nat Genet 2000;25:96-101.
17. Crescenzi M, Giuliani A. The main biological determinants of tumor line taxonomy elucidated by a principal component analysis of microarray data. FEBS Lett 2001;507:114-8.
18. Rabson AB, Weissmann D. From microarray to bedside: Targeting NF-kappaB for therapy of lymphomas. Clin Cancer Res 2006;11:2-6.
19. Suarez-Farinas M, Noggle S, Heke M, Hemmati-Brivanlou A, Magnasco MO. Comparing independent microarray studies: The case of human embryonic stem cells. BMC Genomics 2005;6:99.
20. Kane MD, Jatkoe TA, Stumpf CR, Lu J, Thomas JD, Madore SJ. Assessment of the sensitivity and specificity of oligonucleotide (50 mer) microarrays. Nucleic Acids Res 2000;28:4552-7.
21. Diehl F. Manufacturing DNA microarrays of high spot homogeneity and reduced background signal. Nucleic Acids Res 2001;29:E38.
22. Liu ET, Karuturi KR. Microarrays and clinical investigations. N Engl J Med 2004;350:1595-7.
23. Wang K, Gan L, Jeffery E, Gayle M, Gown AM, Skelly M, *et al*. Monitoring gene expression profile changes in ovarian carcinomas using cDNA microarrays. Gene (Amst.) 2008;229:101-8.
24. Kallioniemi OP, Wagner U, Kononen J. Tissue microarray technology for high-throughput molecular profiling of cancer. Hum Mol Genet 2005;10:657-62.
25. Yoshitake Y, Nakatsura T, Monji M. Proliferation potential-related protein, an ideal esophageal cancer antigen for immunotherapy, identified using complementary DNA microarray analysis. Clin Cancer Res 2004;10:6437-48.
26. Chen Z, Gordon JR, Zhang X. Analysis of the gene expression profiles of immature versus mature bone marrow-derived dendritic cells using DNA arrays. Biochem Biophys Res Commun 2002;290: 66-72.
27. Suciu-Foca Cortesini N, Piazza F, Ho E. Distinct mRNA microarray profiles of tolerogenic dendritic cells. Hum Immunol 2007;62:

1065-72.
28. Chen Z, Dehm S, Bonham K. DNA array and biological characterization of the impact of the maturation status of mouse dendritic cells on their phenotype and antitumor vaccination efficacy. Cell Immunol 2001;214:60-71.
29. Zhang X, Chen Z, Huang H. DNA microarray analysis of the gene expression profiles of naive versus activated tumor-specific T cells. Life Sci 2005;71:3005-17.
30. Houseman BT, Mrksich M. Carbohydrate arrays for the evaluation of protein binding and enzymatic modification. Chem Biol 2002;9:443-54.
31. Kononen J, Bubendorf L, Kallionimeni A. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Hum Immunol 2008;4:844-7.
32. Baghdoyan S, Roupioz Y, Pitaval A. Quantitative analysis of highly parallel transfection in cell microarrays. Nucleic Acids Res 2004;32:e77.
33. Schena M. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 1995;270:467-70.
34. Schena M. Parallel human genome analysis: Microarray-based expression monitoring of 1000 genes. Proc Nat Acad Sci USA 1996;93:10614-9.
35. Kuruvilla FG, Shamji AF, Sternson SM. Dissecting glucose signalling with diversity-oriented synthesis and small-molecule microarrays. Nature 2002;416:653-7.
36. Lockhart DJ, Dong H, Byrne MC. Expression monitoring by hybridization to high-density oligonucleotide arrays. Nat Biotechnol 2006;14:1675-80.
37. Pietu G, Alibert O, Guichard V. Novel gene transcripts preferentially expressed in human muscles revealed by quantitative hybridization of a high density cDNA array. Genome Res 1996;6:492-503.
38. Hess KR, Zhang W, Baggerly KA. Microarrays: Handling the deluge of data and extracting reliable information. Trends Biotechnol 2001;19:463-8.
39. Duggan DJ . Expression profiling using cDNA microarrays. Nat Genet 1999;21(1 Suppl):10-4.
40. Velculescu VE, Zhang L, Vogelstein B. Serial analysis of gene expression. Science 1995;270:484-7.
41. DeRisi J, Penland L, Brown PO, Bittner ML, Meltzer PS, Ray M, *et al*. Use of a cDNA microarray to analyse gene expression patterns in human cancer. Nat Genet 2006;l4:457-60.
42. Pease AC. Light-generated oligonucleotide arrays for rapid DNA sequence analysis. Proc Natl Acad Sci USA 1994;91:5022-6.

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