DNA microarrays and Gene expression analysis

Written by Administrator
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Microarrays have provided a means by which microspots of DNA, protein or small organic compounds can be probed with possible binding ligands. Advances in techniques—particularly fluorescence based approaches for detecting the incidence of interactions, this has enabled the simultaneous analysis of thousands of variables in a single experiment.

Array based gene expression analysis (immobilized DNA probes hybridized to RNA or cDNA targets) has become an important primary tool in many research projects. Gene expression microarrays have been used in numerous applications, including identifying novel genes associated with certain cancers, classifying tumors, and predicting patient outcome.

The research is based on the fact that the abundance of a particular species of mRNA is indirectly proportional to the amount of protein in the cell.

Expression analysis for the quantitative gene expression of many genes can be performed using either one- or two-colour fluorescent schemes. One-colour analysis is primarily used for arrays prepared by photolithography. Affymetrix (http://www.affymetrix.com) patented this process under the trade name GeneChip™ (Fodor, S.P.A. et al., 1995). In this method, expression profiles for each sample are generated on a different chip using a single fluorescent label, such as phycoerythrin, and the different images are then compared. A two-colour analysis protocol has subsequently been developed, whereby two RNA samples are labelled separately with different fluorescent dyes, for example cyanine 3 and cyanine 5 (M. Schena et al., 1995).

These labelled probes hybridize to a printed array of cDNA; when the microarray is scanned, the fluorescent signals can be overlaid to visualize genes that have been activated or repressed.
DNA microarrays can be fabricated using short oligonucleotides (15–25 nucleotides), long oligonucleotides (50–120 nucleotides) or PCR-amplified cDNA (100–3,000 base-pairs). In situ oligonucleotide synthesis on a solid support involves the use of photolithography to build up each element of the array, nucleotide by nucleotide up to 20 bases (R.J. Lipshutz et al., 1999). Alternatively, longer nucleotides and cDNA can be spotted directly onto glass slides. Between 10,000 and 30,000 spots can be mechanically deposited onto a single glass slide by robotic instrumentation designed to print from metal pins or ink-jets.

In the drug discovery and therapeutics arena, microarrays have been used for expression analysis of cells or tissues in different disease states, single nucleotide polymorphism (SNP) analysis, pharmacogenomics and toxicogenomics. The information obtained from these studies can be used to design arrays that assist in the selection of custom and rational drug design.

Current uses of DNA microarrays

Although the DNA microarray is being supplemented by other technologies, it is currently used, and will continue to be developed, in many areas of drug discovery. In the study of cancer, the ultimate goal will be to link the data obtained from DNA microarrays to the proteomic and metabolomic findings because the function of the cell is affected more by the proteins and metabolites within it, rather than the mRNA levels per se. A complete characterization of some of the more complex cancers will result in new drug targets, improved diagnosis and more successful treatment.

DNA microarrays have had a significant impact on our understanding of normal and abnormal cell biochemistry and, thus, on the choice of targets for drug design. However, their use has not been restricted to human cell biology and is also being developed in many other drug-related fields. Community profiling of bacteria could lead to new avenues of research in preventative medicine. In this area, the DNA microarray is an ideal tool for the identification of bacterial species in a mixed population: the DNA or cDNA from an entire bacterial population can be isolated and hybridized to an array of 16S ribosomal DNA fragments giving information on both the abundance and identity of the bacteria in a particular environment. The standard application of such microarray technology to the medical microbiology lab would certainly aid epidemiology and diagnosis, if costs can be reduced significantly.
Currently, Affymetrix produces oligonucleotide microarrays encompassing a wide range of organisms and providing a variety of functions. These arrays include expression analysis for the following organisms: human, murine, rat, E. coli, Arabidopsis, yeast, Drosophila. Furthermore, genotyping can be performed using SNP analysis. This oligonucleotide platform has a variety of potential functions, and has more recently become commonly used.

A. Advantages of Spotted Arrays

1. Flexibility of the microarray and the ability to customize or "direct" the microarray composition.
2. The cost of production of the microarrays can be less if produced locally.
3. The use of clone sets for follow-up studies.

B. Disadvantages of Spotted Arrays

1. Tremendous cost for equipment, including spotters, robots, and scanners.
2. Technical difficulties with spotted arrays and quality control for generating spotted arrays.

A. Advantages of Affymetrix Arrays

1. There are multiple arrays available, as previously described.
2. Data between experiments can be normalized and used for comparison. Large expression databases can be generated. Some of these databases are commercially available, such as with GeneLogic: http://www.genelogic.com/
3. Equipment, scanners, and data analysis tools are readily available.

B. Disadvantages of Affymetrix Arrays
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1. The arrays are somewhat expensive, and pricing is based on volume of use.
2. Changing of the microarray platform can sometimes be problematic and provide difficulty in databases that are built on previous platforms of Affymetrix arrays. Sources of assistance for platform comparison are becoming available from private sources as well as from Affymetrix (NetAffx)
   http://www.affymetrix.com/analysis/index.affx

There are several scientific problems related to the expression analysis of microarrays. The basic categories are on the hardware/biological, software and conceptual parts of the analysis.

Hardware/biological problems the following are considered:

- RNA degradation of the specimen, the quality of construction of the microarray, the quality of reading with the current technology, repeatability of the experiment

While the software problems are,

- annotation problems while using different or updated databases, variety of analysis software with different degrees of detail and difficulty to ascertain the better technique for analysis.

Furthermore the conceptual problems that might arise are:

- The experimental design, the across-platform comparison, the multiple testing problem, the clustering method, choosing of the appropriate normalization technique.
Several sites are available to provide updates on the technology of microarrays:
http://www.bioarraynews.com/index.htm
http://www.genomeweb.com/
http://www.bio-itworld.com/subscribe/
http://www.genpromag.com/scripts/default.asp
http://www.microarrays.org

Reference:


2. Designing a Microarray Core Facility Mark W. Geraci, M.D. University of Colorado Health Sciences Center Denver, CO