UNIVERSIDAD DE MÁLAGA

Microarrays as a functional approach to the transcriptome

Barrionuevo Moreno, Ignacio; Del Espino Pérez, Ángel

Abstract

Knowing a cell's transcriptome is a fundamental requisite in order to analyze its response to the environment. Microarrays have supposed a revolution on this field as they are able to yield an overview of gene expression at any environmental condition on a genome-wide scale.
This technique consists in the hybridisation of a nucleic acid sample, previously marked, with a probe (which might be made up of cDNA, oligonucleotides or PCR products) anchored to a solid surface (made of glass, plastic, silicon...) giving as a result a dot grid which reveals, after image analysis, which genes are being expressed. Nevertheless, this only can be achieved if information on the species genome has been generated.
Different kinds of expression microarrays exist attending to the probe's nature and the method used in its synthesis. In this poster two of these will be treated:
Spotted Microarrays, for which the probe is synthesised prior to its fixation to the array and allow the analysis of two targets simultaneously. They can be easily customized, but lack high reproducibility and sensitivity.
Oligonucleotide Microarrays, which are characterized by the direct printing of the probe on the array. In this case the probes consist on, invariably, oligonucleotides that are complementary to a small fraction of the gene it is representing at the microarray. Their application is somewhat restricted. This fact, however, makes them more reproducible.
Currently, the approach towards the transcriptome studies from the Next Generation Sequencing technologies offers a large volume of information in a short amount of time needing less previous information on the target organism than that needed by microarrays, but their expensive price limits their use. The versatility of the latter, together with their reduced costs in comparison to other techniques, makes them an interesting resource in applications that may need less complexity.

Types

Oligonucleotide

The paradigm of this kind of microarray is Affymetrix's GeneChip®, in which the probe is synthesised directly over the solid surface, which often is a silicon chip, using a photolithographic-like method in order to elongate only the desired chains in each nucleotide applying cycle.

Each gene or, more precisely, each sequence of interest, is represented in the microarray by a probe set, consisting on a series of probe cells covered each with perfectly matching 25 nt oligonucleotides against different sections of the sequence of interest. Each one of this "perfect match" (PM) probe cells is accompanied by a "mismatch" (MM) probe cell, differing from the PM oligonucleotide in the 13th nucleotide. Together, PM and MM probe cells make a probe pair.

These microarrays can give absolute expression level values thanks to the accuracy and precision in their fabrication and the comparative method of the probe set, which also make them much more reproducibility. On the other hand, the hi-tech manufacturing processes imply a high cost, and the chips are not customizable.

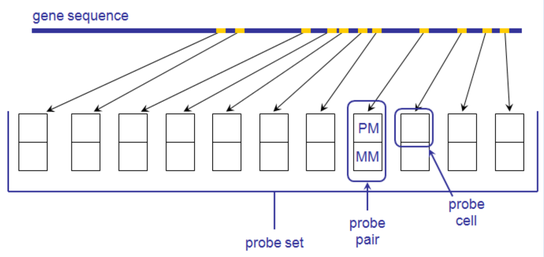


Figure 1. Conceptual representation of the design of an oligonucleotide microarray probe set.

Spotted

This type of microarray, as was mentioned, is based on the anchoring of a pre-synthesised probe on the solid surface, which might typically be a glass slide. Said probe may consist of the whole fragmented genome (genome-wide analysis) or whichever partial library, especially a cDNA one, usually PCR-amplified. Its deposition is achieved by using robotically controlled fine-tipped pins or an ink-jet printing-like method.

The target is normally derived from a mRNA extraction of a certain tissue/cell type under a specific environmental condition, and is hybridised with the sample. Target samples of two different origins can be marked with two distinct fluorophores, thus enabling to compare the expression and its relative levels between the conditions and/or the tissue/cell type.

Due to their manufacturing process this type of microarrays are highly customizable and can be adjusted to a varied set of experimental designs. Nevertheless, this same production, because of the great relevance of human manipulation, induces a low level of reproducibility among other inconvenients.

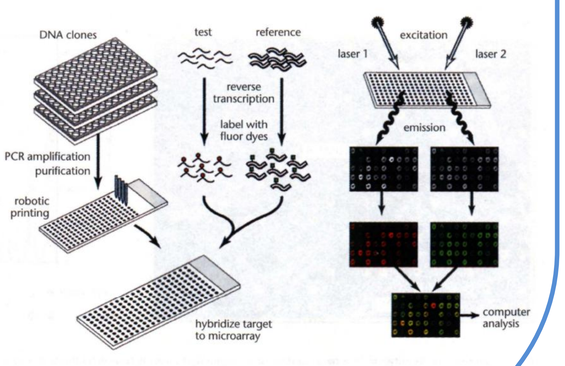
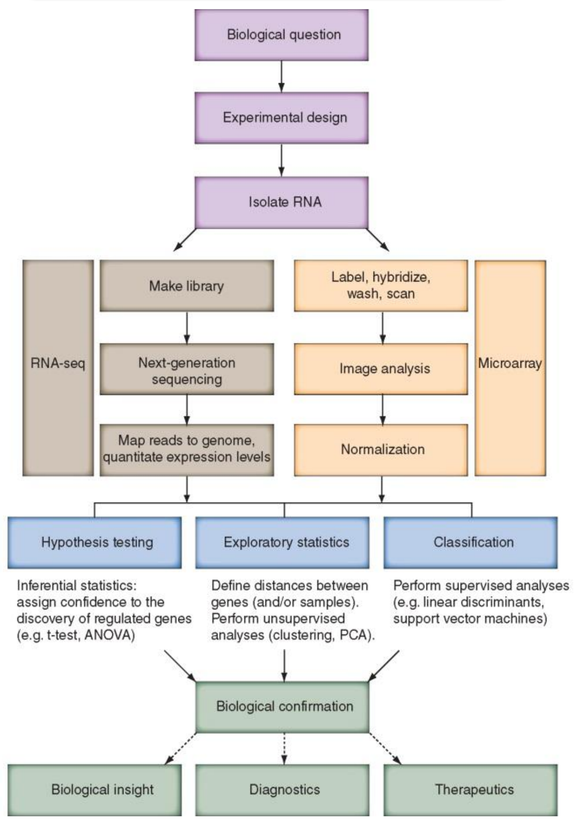


Figure 2. Diagram showing the development of a spotted microarray experiment.

Transcriptome analysis overview



Applications

• Transcriptome studies: The expression levels of even the whole genome can be known, thus knowing the expression profile of a cell under certain conditions.

• Comparing between types, environments, developmental cues, mutations: This might enable the diagnosis of specific mutations or, even, gene expression-modifying illnesses according to the transcriptome of the affected tissue.

• Gene discovery: The function of a gene may be inferred from the time or the place where it is overexpressed or underexpressed or the known genes with which it forms an expression profile cluster, among other examples.

• Classification/Identification: By knowing the expression profile a certain mutation or a certain species gives, the analysis of any evidence left by it can yield the identification of the origin of said evidence.

Conclusions

In summary, the microarrays, despite being a very versatile tool on the study of the transcriptome from multiple approximations , have been relegated to a second place by the Next Generation Sequencing technologies due to the big quantity of data that this techniques generate in a shorter amount of time compared to what microarrays yield. Nevertheless, microarrays are not totally obsolete, their current strength comes from the fact that their price is low and they have been refined for years, allowing their use in routine applications, such as clinical practice or other.

Bibliography

-Richard et al.: **Comparison of gene expression microarray data with count-based RNA measurements informs microarray interpretation**. BMC Genomics 2014 15:649.
-Kogenaru et al.: **RNA-seq and microarray complement each other in transcriptome profiling**. BMC Genomics 2012 13:629.
-Leimena et al. **Comparative Analysis of *Lactobacillus plantarum WCFS1* Transcriptomes by Using DNA Microarray and Next-Generation Sequencing Technologies.** Applied and Environmental Microbiology 2012 10.1128
-Grubaugh ND, McMenamy SS, Turell MJ, Lee JS **Multi-Gene Detection and Identification of Mosquito-Borne RNA Viruses Using an Oligonucleotide Microarray.** PLoS Negl Trop Dis 2013 10.1371.