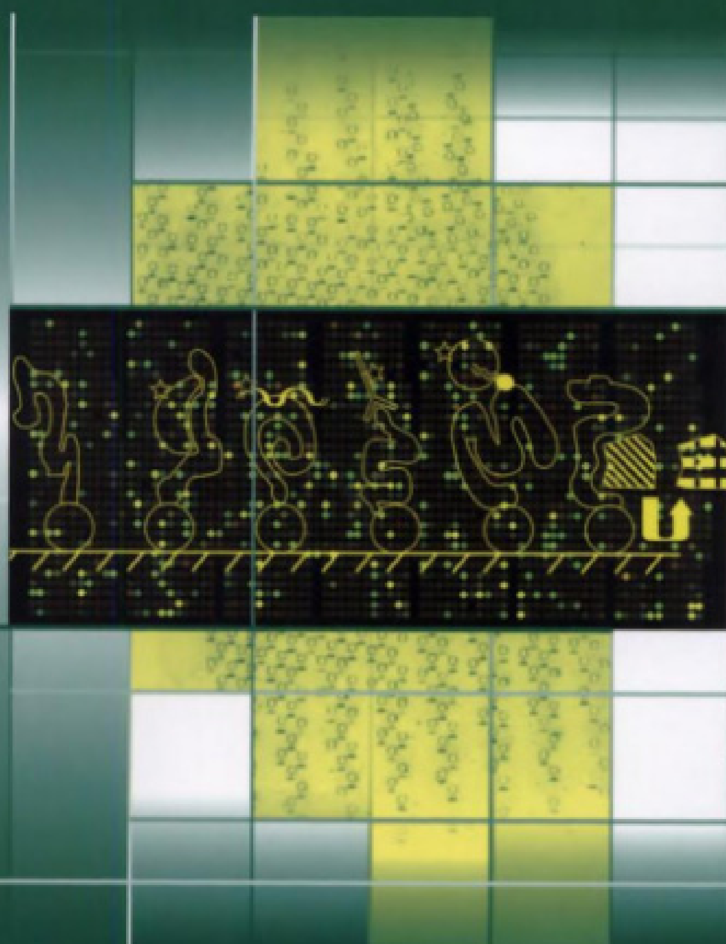
Microarrays & Microplates

Applications in Biomedical Sciences

S. Ye and I.N.M. Day (Eds)



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Expression Microarrays

2

Carl Whatling, Shu Ye and Per Eriksson

2.1 Introduction

Rapid progress in the field of functional genomics has made it possible to monitor the expression of thousands of genes simultaneously. Not only does this have the advantage of high-throughput analysis, but also the increase in the number of data points has a direct effect on the accuracy and relevance of the information generated. A variety of technological developments have made this possible, including expressed sequence tag (EST) analysis (Adams *et al.*, 1991; Okubo *et al.*, 1992), differential display (Liang & Pardee, 1992), serial analysis of gene expression (SAGE; Velculescu *et al.*, 1995) and a variety of array-based technologies (Schena *et al.*, 1995, 1996; Lockhart, 1996). Of the various options, much progress has been made in the development and refinement of array technologies, reflected by the abundance of diverse publications in which this technology has been employed (e.g. Chabas *et al.*, 2001; Hedge *et al.*, 2001; Hoffmann *et al.*, 2001, Kato-Maeda *et al.*, 2001; Thimm *et al.*, 2001). The purpose of this chapter is to provide an overview of the principles and techniques of expression arrays, with particular emphasis on the use of cDNA expression microarrays.

2.2 Microarrays in overview

The term array refers to the regular arrangement of oligonucleotide or cDNA representations of genes on a solid support such as a nylon membrane, plastic or glass microscope slide. In the latter case, it is possible to array samples very close together such that the term 'microarray' can be applied. Gene expression is monitored following hybridization with a probe generated from RNA in which each expressed gene should be represented in relative proportion to its transcript level. In principle, it should be possible to measure absolute levels of expression for each represented gene by quantifying the amount of a single probe that hybridizes to the arrayed DNAs. However, a variety of factors entail that hybridization of probe to target is not equivalent for every expressed transcript. This includes consideration of the target sequences, the labelling method and the hybridization conditions (Eisen & Brown, 1999). For such reasons, it has become standard to quantify expression levels by measuring the relative level of hybridization between two different probes. By convention, probe is prepared from two related RNA pools corresponding to a reference and test condition, and the measurement made is the amount of test probe relative to reference probe that binds to each arrayed DNA.

Probes are prepared from RNA pools by reverse transcription to generate first-strand cDNAs. Two methods have been used in particular to label

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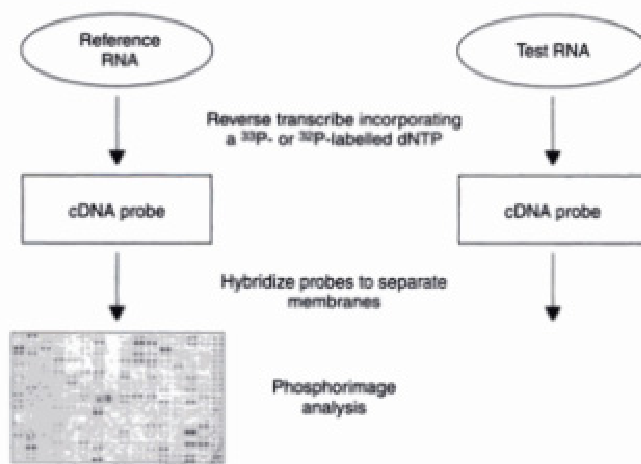

Figure 2.1

Preparation of radioactively labelled probes and hybridization to membrane arrays.

probe, dependent upon the type of array (*Figure 2.1*). For arrays printed on nylon membranes, it is conventional to incorporate a ^{32}P- or ^{33}P-labelled dNTP into the cDNA. ^{33}P is generally preferred because it is a less energetic emitter, reducing the risk of strong hybridization signals interfering with the detection of adjacent signals on the array. The use of the same label to prepare both test and reference probe entails that two identical arrays are hybridized in parallel, followed by detection using phosphorimage analysis. When glass microarrays are used, reference and test probe are synthesized using a different fluor-labelled UTP (*Plate 1*). This has the advantage that both probes can be combined and hybridized simultaneously to the microarray. Following detection of fluorescence using a laser confocal microscope or CCD camera, it is therefore possible to measure the relative level of hybridization between test and reference probe on the same arrayed DNA. Both types of array system have been evaluated and used successfully. However, the microarray format offers three particular advantages over membrane arrays (Schena & Davis, 1999):

1. *Miniaturization*. The solid glass surface used for microarrays is non-porous allowing the deposition of low amounts of material in a defined area. It is thus possible to print arrays at a high density, facilitating a high level of representation within a small area. As a consequence, small reaction volumes are possible, allowing a high concentration of probe and increased hybridization kinetics. This is also aided by the non-porous nature of the support as probe does not have to diffuse into pores to reach arrayed target.
2. *Uniformity*. The solid support provides a uniform attachment surface facilitating a highly defined geometric arrangement of the arrayed targets. This renders the arrays amenable to analysis by digital image

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