Robust and efficient synthetic method for forming DNA microarrays

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ABSTRACT

The field of DNA microarray technology has necessitated the cooperative efforts of interdisciplinary scientific teams to achieve its primary goal of rapidly measuring global gene expression patterns. A collaborative effort was established to produce a chemically reactive surface on glass slide substrates to which unmodified DNA will covalently bind for improvement of cDNA microarray technology. Using the *p*-aminophenyl trimethoxysilane (ATMS)/diazotization chemistry that was developed, microarrays were fabricated and analyzed. This immobilization method produced uniform spots containing equivalent or greater amounts of DNA than commercially available immobilization techniques. In addition, hybridization analyses of microarrays made with ATMS/diazotization chemistry showed very sensitive detection of the target sequence, two to three orders of magnitude more sensitive than the commercial chemistries. Repeated stripping and re-hybridization of these slides showed that DNA loss was minimal, allowing multiple rounds of hybridization. Thus, the ATMS/diazotization chemistry facilitated covalent binding of unmodified DNA, and the reusable microarrays that were produced showed enhanced levels of hybridization and very low background fluorescence.

INTRODUCTION

Accompanying the development of several high-throughput technologies that make it possible to sequence complex genomes is the challenge to not only identify genes, but to understand the function and expression of those genes. cDNA microarray technology, first developed by Patrick Brown and his colleagues at Stanford University (1), allows for the high-throughput measurement of expression patterns of thousands of genes simultaneously. Microarrays utilize a high-speed precision robot to affix thousands of DNA samples onto a solid support (a glass slide, chip or nylon membrane). This allows high spot densities to be obtained, thus increasing the number of samples that can be analyzed at once. The slides are simultaneously probed with fluorescently labeled cDNAs generated from mRNA isolated from cells or tissues in two different physiological, developmental or disease states (2–6). Each population of cDNAs is labeled with a different fluorescent dye, allowing direct comparisons on a single array. The relative intensities of the two fluorescent dyes within a spot correspond to the relative expression levels of the genes, reflected by the two RNA populations used to make the labeled cDNA. The use of microarrays, therefore, allows the expression of thousands of genes to be assayed in a single experiment.

While DNA microarray technology was initially developed to detect global patterns of gene expression, it has many other potential applications including identification of complex genetic diseases (7), mutation/polymorphism detection (8), and drug discovery and toxicology studies (9). Recently, tissue microarrays were used for the molecular profiling of tumor specimens (10).

The major steps of DNA microarray technology include the manufacture of microarrays, the fluorescent labeling of cDNA probes, hybridization of the probes to the immobilized target DNA and the subsequent analysis of the hybridization results.

There are two predominant methods for producing DNA microarrays. The first was developed by Stephen Fodor and colleagues in the early 1990s (11), and it is often referred to as the Affymetrix method (Affymetrix Inc., Santa Clara, CA). In this process, DNA oligonucleotides are synthesized directly onto the microarray or DNA chip using photolabile protecting groups and masks to direct the selective addition of nucleotides.

In the second common method for producing microarrays, pre-existing DNA fragments are spotted onto a glass or membrane support using a precision robot (1). Both of these systems commonly permit only single usage of the DNA arrays. Reusability of microarrays would eliminate the variance between arrays, which are often presumed identical; the variance ultimately affects the experimental reliability of microarray-based analyses. The surface chemistry of the glass substrate is a major determinant of the stability of DNA attachment throughout the hybridization and washing steps.

The most popular substrates for spotting DNA are polylysine- and aminosilane-coated glass slides (12). These slides

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