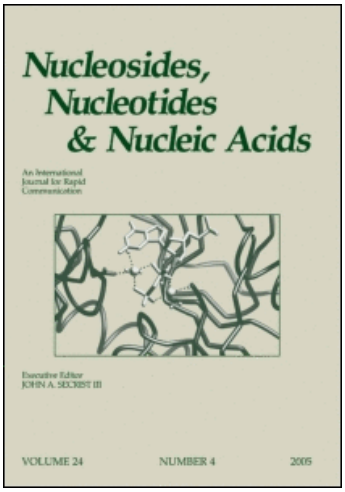

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New Developments in Oligomer Array Technologies

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NEW DEVELOPMENTS IN OLIGOMER ARRAY TECHNOLOGIES

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ABSTRACT

Modified phosphoramidite chemistry allows the generation of oligonucleotide arrays containing molecules of high purity. Such arrays are being used for experiments on the determination of expression levels in yeast and for investigations of the hybridization behaviour of modified oligonucleotides such as phosphorothioate derivatives.

In recent years the conception of utilizing oligonucleotide libraries as diagnostic tools in molecular biology and medicine for hybridization studies such as positional, mutational or transcriptional analyses has developed from a mere idea to some initial practical applications [e.g., 1, 2]. Different strategies have emerged to create large arrays of immobilized oligonucleotides. Either pre-synthesized oligonucleotides are spotted onto a surface [3] or the oligomers are directly synthesized on glass [4] or polypropylene membranes [5]. The most ambitious application of the oligomer chip technology is the *Sequencing by Hybridization* (SBH) technique [6-9]. By this method, the DNA sequence of an individual could be compared to a given master sequence that previously has been determined for the respective organism. However, several problems concerning the practical application of this technology remain to be solved. Pirrung and Bradley [10] have shown that the reliability of hybridizations to arrays of short oligonucleotides depends strongly on the quality of the surface-bound oligomers, which still seems to be problematic with regard to the use of photo-cleavable protecting groups during *in situ* oligomer synthesis.

Recently, we reported the establishment of a modified phosphoramidite chemistry that in combination with specially derivatized polypropylene membranes allows oligonucleotide arrays not only to be used as hybridization substrates but in addition permits the separation of the biopolymers from the support medium to be used in (subsequent) analyses, especially as primer molecules in biological reactions like the polymerase chain reaction and DNA sequencing [11]. This phosphoramidite chemistry provides for a direct quality

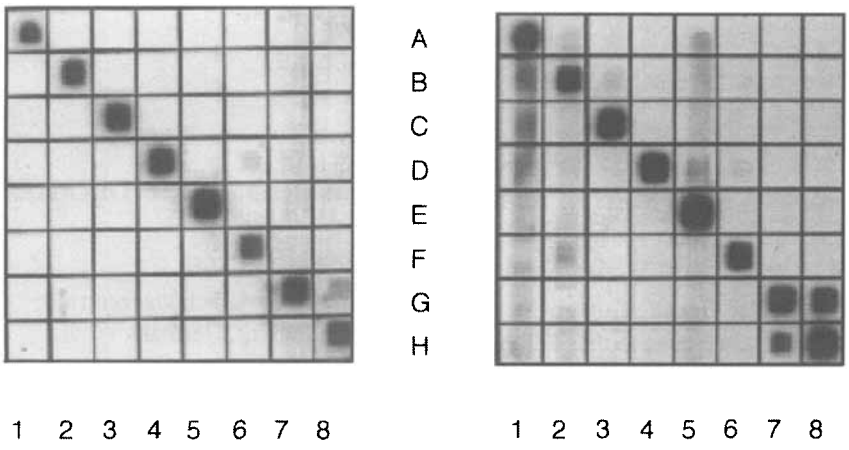

FIG.1: Two grids each containing the same set of 64 different 21-mer oligonucleotides representing cell cycle regulated genes of the yeast *Saccharomyces cerevisiae* were hybridized with radioactively labeled probe. Oligomers positioned in the diagonal positions of the arrays were entirely complementary to the probe, while the other squares contained mismatched oligonucleotides. Hybridization was carried out overnight in 600 mM NaCl, 60 mM sodium citrate, pH 7.5, and 7.2% sodium N-lauroylsarcosine at 4°C. On the grid made of phosphorothioate modified oligonucleotides, sufficient discrimination was achieved by washing at 50°C in the same buffer (left). In the case of the phosphodiester grid (right), the temperature had to be raised to 58°C to obtain an equivalent degree of discrimination.

assessment of the oligomers synthesized on the array and might also lead to an improved light-induced synthesis of oligonucleotide arrays [12].

Besides work concerned with developments toward SBH [13] and analyses of complex expression patterns of yeast [14], oligomer-arrays were used to study in parallel the hybridization behaviour of oligonucleotides made from different nucleoside derivatives. Such an approach might be a promising tool for studies on the design of appropriately modified oligonucleotides that could act as potent candidates in applications such as the antisense strategy. We investigated the hybridization of arrays containing phosphorothioates, for example, which are the first oligonucleotide-drug to reach clinical trials [15], and compared them to array-bound phosphodiester compounds. The phosphodiester array was prepared using the synthesis protocol described recently in detail [11]. The phosphorothioate array was synthesized in the same manner, with the exception of the oxidation step in which a 0.5 M solution of Beaucage reagent [16] in acetonitrile was used for sulfurization instead of tert.-BuOOH. After deprotection, both types of arrays were used for parallel hybridization experiments (Fig.1). As known from experiments in

solution [17], the duplex-stabilities of the phosphorothioate oligonucleotides was found to be lower than the equivalent phosphodiester sequences, while the specificity of the duplex formation was not significantly different in the cases analyzed. Effects such as the influence of neighboring nucleotides on mismatch hybridization could be detected and are being studied further for a thorough and quantitative understanding of oligomer hybridization. At position F1 of the hybridization shown in figure 1, for example, the background signal caused by mismatch hybridization under the non-stringent washing conditions is of lower intensity compared to the signal obtained on the rest of the F lane, although the matching part of the sequence should be identical. In addition to such analyses, investigations are in progress to hybridize very complex probes like total yeast RNA preparations to arrays containing oligonucleotide analogues which form more stable hybrids than standard DNA-DNA duplexes [18].

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