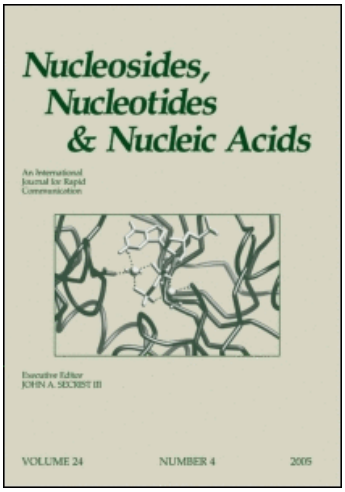

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Picomole Syntheses of High Quality Oligonucleotide Primers in Combination with the Preparation of Oligonucleotide Arrays

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PICOMOLE SYNTHESES OF HIGH QUALITY OLIGONUCLEOTIDE PRIMERS IN COMBINATION WITH THE PREPARATION OF OLIGONUCLEOTIDE ARRAYS

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ABSTRACT

The establishment of a new synthesis procedure for the preparation of oligonucleotide arrays is described. A modified phosphoramidite chemistry allowed the *in situ* synthesis of oligomer arrays on specially derivatized polypropylene membranes which can be used both for hybridisation experiments and for the isolation of the individual oligonucleotides.

Over the last years there has been much progress in the automation of DNA-synthesis. Today, the preparation of synthetic oligonucleotides needed in physical, biological and biomedical applications is usually executed with automated DNA-synthesisers. These machines produce oligonucleotides in the nanomole up to micromole range. However, for many applications in molecular biology, notably the polymerase chain reaction (PCR) and DNA sequencing, oligomer quantities in the picomole range are usually an adequate amount, but a great many different oligonucleotides are needed. This has led to procedures aiming at the simultaneous production of different oligonucleotides in little quantities [1,2].

Recently, another consequential technology in molecular biology was developed using ordered arrays of oligonucleotides to serve as analytic and diagnostic tools for DNA analyses [3-5]. Technically most challenging within this "oligomer-chip" technology" is the sequencing by hybridisation (SBH) strategy, whereby the sequence of an unknown nucleic acid is reconstructed from its hybridisation binding pattern on a matrix which contains a comprehensive set of short oligonucleotide sequences (e.g., all 65.536 octamers).

During our work concerned with developments towards a practical application of the SBH technique [6, 7], it was apparent to us that such oligomer-chips by design would be an ideal source for large numbers of different oligonucleotides, if quantity and purity of each individual oligomer would be sufficient for biological reactions. For practical reasons, a modification of the synthesis chemistry was aimed at that would allow the use of the oligomer-grids both for hybridisation experiments and for the isolation of the individual

compounds, sufficient in amount and purity for PCR-amplification and DNA-sequencing [8].

Usually, for oligonucleotide synthesis the traditional ß-cyanoethyl phosphoramidite chemistry is used; after completion of the oligonucleotide chain elongation process, the protecting groups are removed by an incubation in concentrated aqueous ammonia at 60°C. Different between the construction of oligomer arrays and the synthesis of free oligonucleotides in commercial machines is the anchoring to the solid support at the 3'-terminal nucleoside. While for the latter a succinate linker is intentionally cleaved simultaneous to the biopolymer deprotection, the arrayed oligonucleotides must remain attached to the solid support after the deprotection process for hybridisation experiments. In earlier experiments, this was achieved by linkage *via* a stable phosphoester [9] or phosphoramidate [10] bond.

For the preparation of oligonucleotide arrays that combine usage in hybridisation screening experiments with the capability of cleaving the oligonucleotides off the solid phase, nucleobase-deprotection had to be decoupled from the release reaction [11], with nevertheless stable binding during oligomer synthesis and any subsequent hybridisation experiment. This goal was reached by using the common succinate linker between the 3'-terminal nucleoside and a methylamino-derivatized polypropylene membrane in combination with an alternative phosphoramidite approach, the so-called "NPE/NPEOC-strategy", which on CPG and polystyrene supports had led to oligonucleotide products of high purity [12]. This synthesis strategy is using the ß-eliminating base-protecting groups 2-(nitrophenylethyl) (NPE) and 2-(4-nitrophenyl)ethoxycarbonyl (NPEOC). These functions allow the deprotection of the biopolymer while the oligonucleotide remains attached to the polypropylene (FIG. 1).

Oligonucleotide arrays were synthesised directly on methylamino-modified polypropylene sheets (8 x 8 cm^2) using an 8-channel reaction chamber connected to a commercial DNA-synthesizer. By removing the polypropylene sheet after several cycles, rotation by 90° and continued synthesis, this simple instrument allowed the synthesis of up to 64 oligomers. Besides being reliable substrates for hybridisation experiments, the oligomer-arrays were used as a source for the isolation of the various, individual oligonucleotides [8].

Step-wise improvements of the synthesis conditions e.g. replacing THF as capping solvent for acetonitrile led to oligomer primers in picomole range (about 10-25 picomole/cm^2) sufficient in amount and purity (data not shown) to work without further purification in PCR and DNA-sequencing reactions, with the results being identical to controls with commercially obtained primer molecules.

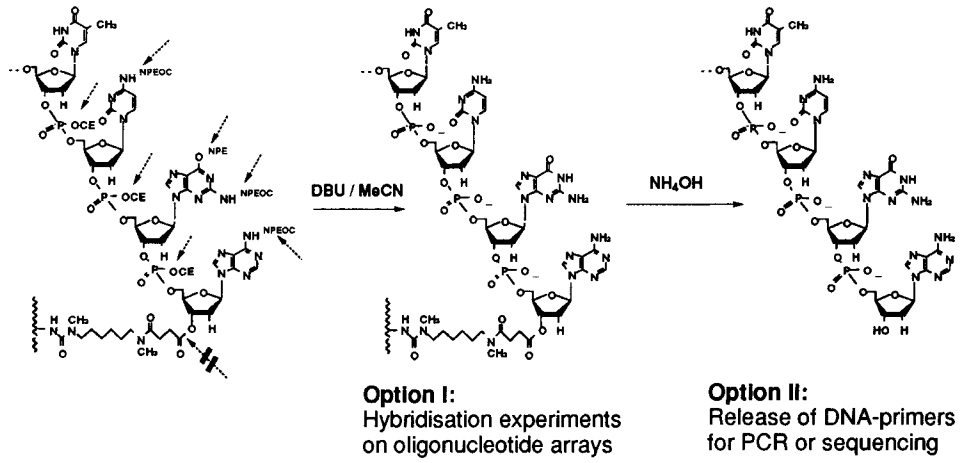

FIG.1: The "NPE/NPEOC"-strategy allows alternative use of oligonucleotide arrays for either hybridisation experiments or as a source for the isolation of individual primer molecules.

In conclusion we have demonstrated, that the modified phosphoramidite chemistry in combination with polypropylene membranes as solid support allows oligonucleotide arrays to be used as substrates in hybridisation experiments or for the isolation of individual, biologically active oligonucleotides in the picomole range. Recent investigations [13] indicated that the reliability of hybridisations to arrays of short oligonucleotides depends strongly on the quality of the array-bound oligomers. Hence, the high-quality oligonucleotides on the chip and as confirmed by the independent control mechanism made possible by the presented technique are paramount for accurate SBH analysis.

The efficient separation of nucleobase deprotection and release from the solid support, the apparent stability of the succinate linker during hybridisation, and the high quality of the oligomer molecules also suggest an alternative application of the arrays, cleaving off the oligonucleotides only after the performance of hybridisation experiments. Thereby, an oligomer could first serve as a detector molecule in complex screening experiments, immediately followed by its use for an isolation of the appropriate DNA-fragment by PCR or a direct sequence analysis of the region. Also, oligonucleotides identified by hybridisation of a given DNA could in turn be used as probes for the identification of homologous fragments, for example, an approach that would simplify and speed up comparative genome analyses.

Recent investigations have shown that this new approach is also applicable to create arrays containing modified oligonucleotides, e.g. phosphorthioates which might be a promising tool with regard to the design of antisense oligonucleotides.

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