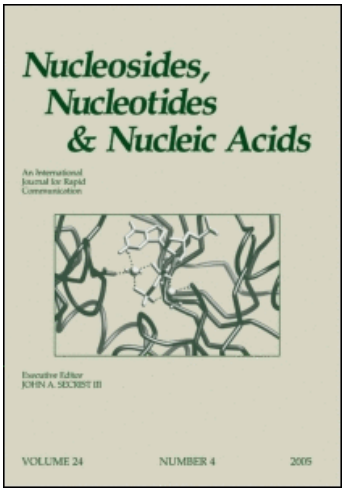

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A Simple Solid-Phase Based Purification Procedure for Oligodeoxynucleotides

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A SIMPLE SOLID-PHASE BASED PURIFICATION PROCEDURE FOR OLIGODEOXYNUCLEOTIDES

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ABSTRACT : A two-cartridge method for routine purification of DNA oligomers has been investigated. The full-length target oligonucleotides are purified using a method that select for intact 3'- and 5'-termini. The procedure results in purified DNA without the use of PAGE gels or HPLC.

During chemical synthesis depurinated sites are introduced into the oligomer due to the prolonged exposure to acid. The standard ammonium hydroxide deprotection step (18 hours at 60 °C) cleaves these sites generating truncated oligomers that contain the 5'-dimethoxytrityl (DMT) group. Methods have been devised to simplify DNA purification [1], but they are not fully satisfactory since they do not fully take into account the complicating nature of the cleavage products. An improved purification scheme involving cleavage of abasic sites with lysine in the solid-supported oligomer prior to complete deprotection has been reported [2].

A new purification method is outlined that further simplify the routine purification of DNA oligomers. The full-length target oligonucleotide is purified using methods that select for intact 3'- and 5'-termini. The thiol group was chosen as capture moiety, since it reacts rapidly with activated thiol supports (CPG-S-S-Pyridyl) [3]. The oligomers were synthesized on a special support, DMT-CM1-C3-S-S-C3-O-succ-CPG (Glen Research, VA), and CM1 is a special linker that can be cleaved regenerating a free 3'-phosphate terminus [4]. The oligomer is deprotected with ammonium hydroxide, and the

-C3-S-S-C3- linkage cleaved by treatment with dithiothreitol (DTT). The key step in the new strategy is capture of oligomers with intact 3' ends. Only oligomers that contain the 3'-SH capture moiety will react with a special CPG-based capture support, CPG-S-S-Pyridyl, and be retained. 5'-DMT truncated oligomers, lacking the 3' thiol capture moiety, are not captured. Captured oligomers are specifically released after periodate oxidation and cleavage of CM1 linker [4], and the full-length oligomer with a 5'-DMT is purified using a Reverse Phase cartridge (Baker Phenyl SPE).

To test the scheme three oligomers, 5'-DMT-T_{35}-3'-CM1-C3-S-S-C3-OH, 5'-DMT-T_{20}, and 5'-pT_{15}-3'-CM1-C3-S-S-C3-OH, were synthesized and deprotected separately. The test mixture contained equimolar amounts of the three oligomers, and they simulate the cleavage products resulting from the degradation of a partially depurinated oligomer, T_{20}-A-T_{15} [2]. The purification steps were analyzed by Polyacrylamide Gel Electrophoresis (PAGE).

The first Baker Phenyl cartridge effectively removed excess DTT and some non-DMT oligomers, whereas 5'-pT_{15}-CM1-C_3-SH was only partially removed, since the 3'-CM1-C3-SH moiety is hydrophobic and interferes in the RP cartridge purification step. The capture step retained the 3'-SH oligomers in high yield on CPG-S-S-Pyridyl, and 5'-DMT-T_{20} was then completely removed by washing with high salt buffer. After release of oligomers from the capture support the second Baker Phenyl RP cartridge purification step effectively resolved the two released oligomers. The product, 5'-DMT-T_{35}-3'-p, was obtained substantially free of the contaminating oligomers.

In the purification of a mixed-sequence oligomer the combination of 3'-SH capture and 5'-DMT ON Reverse Phase cartridge purification effectively isolated the full-length product substantially free of contaminating oligomers.

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