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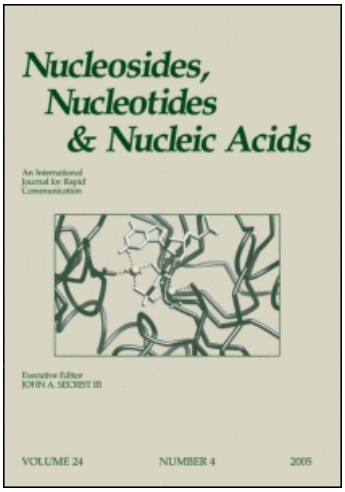

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Biotinylation of Oligonucleotides and Their Use as Polymerase Chain Reaction Primers

M. Bengtström[a]; L. Harju[a]; A-C. Syvhen[a]
[a] Orion Corp., Orion Pharmaceutica, Biotechnology, Helsinki, Finland

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NUCLEOSIDES & NUCLEOTIDES, 10(1-3), 507-509 (1991)

BIOTINYLATION OF OLIGONUCLEOTIDES AND THEIR USE AS POLYMERASE CHAIN REACTION PRIMERS

Bengtström,M.*, Harju,L. and Syvänen,A-C.
Orion Corp., Orion Pharmaceutica, Biotechnology, Valimotie 7, 00380 Helsinki, Finland

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ABSTRACT: Oligonucleotides aminated at the 5′-end were biotinylated with a water soluble N-hydroxysuccinimide ester of biotin in large scale. The biotinylated oligonucleotides were used as polymerase chain reaction (PCR) primers.

Biotinylated oligonucleotides can be used to capture specific DNA sequences from complex mixtures taking advantage of the strong affinity of the streptavidin-biotin interaction. We have set up an efficient biotinylation method for oligonucleotides in large scale using a water soluble sulphonated N-hydroxysuccinimide ester of biotin. The oligonucleotides were synthesized by the ß-cyanoethyl phosphoramidite method on an Applied Biosystems (ABI) oligonucleotide synthesizer, 381A, using an optimized cycle[1]. The free 5′-hydroxy group was automatically aminated using a linker molecule, Aminolink 2 (ABI). 0.1-7 µmoles of the amino-oligonucleotides were biotinylated in 2 hours at 37 °C using a 40 molar excess of 50 mM sulfo-NHS-biotin (Pierce) in 100 mM phosphate buffer, pH 8.5, FIG. 1.

The biotinylated oligonucleotides were purified on a RP18 HPLC-column. The purity was confirmed by incubating the biotinylated oligonucleotide with streptavidin-agarose particles and analyzing the filtrate by HPLC.[2]

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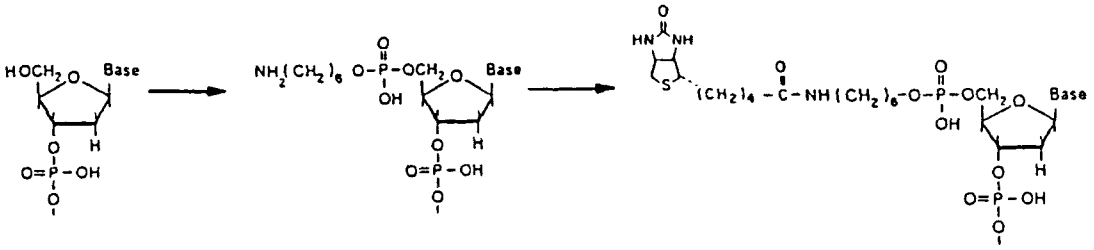

Fig. 1

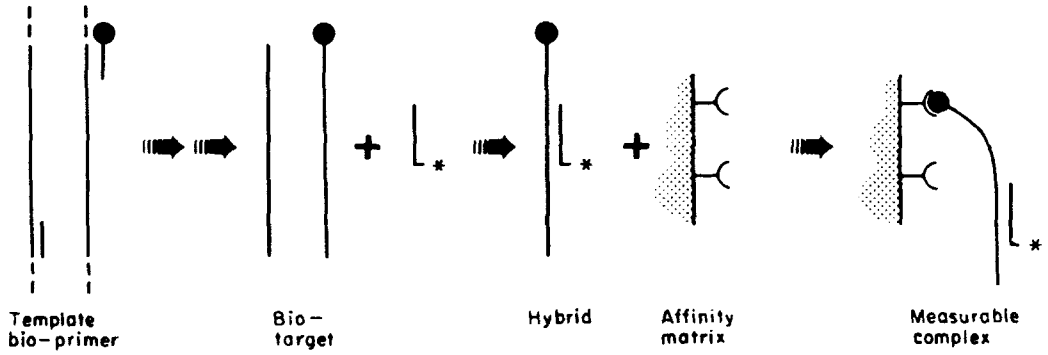

Fig. 2

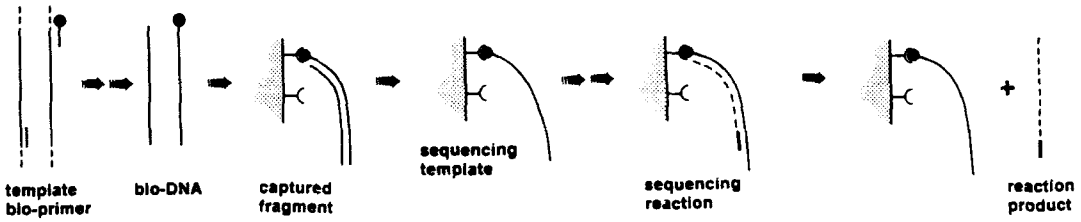

Fig. 3

An assay for detection of viral DNA was designed. In the first step biotin is introduced into copies of the viral DNA using the 5'-biotinylated oligonucleotides as PCR primers. A labelelled probe is then hybridized to the biotinylated DNA. The formed hybrids are captured on an avidin-coated affinity matrix. The matrix is washed and the collected hybrids are measured, FIG 2.[3]

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A solid phase sequencing method was devised for analysis of nucleotide variations in human genes. The biotinylated DNA fragments synthesized by PCR are captured on an avidin-matrix and the non-biotinylated strand is removed. The remaining DNA strand is sequenced directly on this solid support[4], FIG.3.

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