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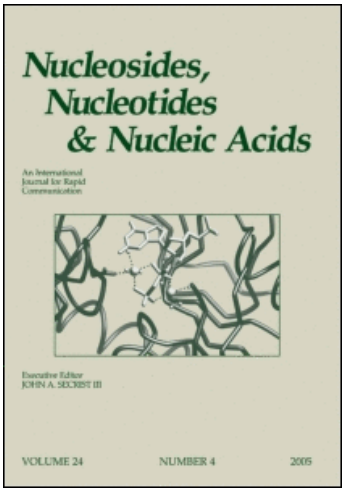

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A Simple Preparation of 5'-Biotinylated Oligonucleotides and Their use as Primers in Dideoxy-Sequencing of DNA

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A SIMPLE PREPARATION OF 5'-BIOTINYLATED OLIGONUCLEOTIDES AND THEIR USE AS PRIMERS IN DIDEOXY-SEQUENCING OF DNA

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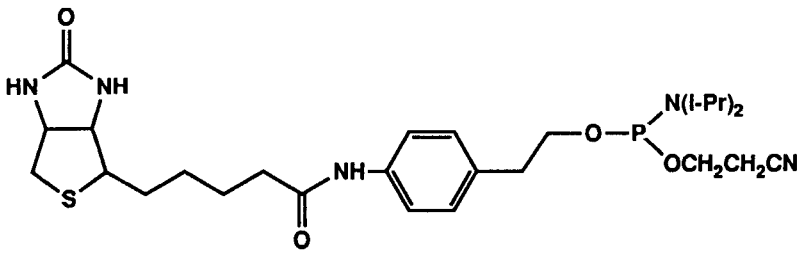
5'-Biotinylated oligonucleotides have proved valuable in a number of applications in molecular biology, particularly as hybridization probes and as primers for PCR[1]. This report describes a simple, efficient method for the preparation of 5'-biotinylated oligonucleotides and their use in a new application, as primers in an improved method for dideoxy sequencing of DNA.

Typically, 5'-biotinylated oligonucleotides are prepared by the solid phase synthesis of oligonucleotides carrying a reactive aliphatic amine which is then derivatized by an active ester of biotin[2]. This method requires specialized reagents as well as reaction and purification steps beyond those used in automated solid phase synthesis. We have developed a biotin phosphoramidite reagent **1** which can be directly used with automated synthesizers to prepare 5'-biotinylated oligonucleotides. *This reagent makes biotinylated oligonucleotides as accessible as ordinary oligonucleotides.*



1

Since our original report[3,4] describing the synthesis of **1**, we have found that biotinylated oligonucleotides are most conveniently prepared by attaching a bottle containing a 0.2 M solution of **1** in anhydrous DMF to the open port of a DNA synthesizer and programing the instrument to introduce it as the terminal 5'-residue. The instrument's standard synthesis cycle followed by standard ammonia treatment affords biotinylated oligonucleotides in high yield.

In the Sanger dideoxy method of DNA sequencing, a template-directed extension of an oligonucleotide primer by a DNA polymerase generates a set of primer extension products which are then analyzed by gel electrophoresis. The electrophoretic analysis of these extension products is often complicated by the presence of other components which comprise the vast bulk of the reaction mixture and which are difficult to remove. These components include: template DNA, which comprises greater than 99% of the DNA that is loaded onto the sequencing gel; spurious extension products arising from contaminating primers; and unincorporated radioisotopicly or fluorescently labeled nucleosides.

We have developed a new sequencing protocol in which these and other interfering components of the Sanger reaction are easily removed prior to electrophoresis. The extension reaction is run using a 5'-biotinylated oligonucleotide primer producing essentially the same mixture as a conventional Sanger reaction. The DNA is then denatured with heat, and streptavidin-coated magnetic beads are added. The biotinylated DNA fragments are captured by the beads and the removal of the template DNA, enzyme, substrates, buffer etc. is accomplished simply by using a magnet to pull the beads to the side of the tube and pipetting off the liquid phase. The beads remaining in the tube are then warmed in formamide which cleanly dissociates the complex, and the formamide solution containing *only the labeled extension products* is loaded on the sequencing gel.

A robotic work station which will have the ability of performing all the manipulations involved in our biotinylated primer-based sequencing procedure is being developed. The combination of this work station with our previously described automated DNA sequencing system[5] will allow complete automation of Sanger sequencing from running the primer extension reactions to electrophoretic analysis and sequence determination.

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REFERENCES

1. L. G. Mitchell and C. R.Merril, *Anal. Biochem.* **1989**, *178*, 239.
2. For example: S. Agrawal, C. Christodoulou, and M. J. Gait, *Nucleic Acids Res.* **1986**, *14*, 6227.
3. A. J. Cocuzza, *Tetrahedron Lett.* **1989**, *30*, 6287.
4. An alternative biotin phosphoramidite reagent similar to **1** has been reported: A. M. Alves, D. Holland, and M. D. Edge, *Tetrahedron Lett.* **1989**, *30*, 3089.
5. J. M. Prober, G. L. Trainor, R. L. Dam, F. W. Hobbs, C. W. Robertson, R. J. Zagursky, A. J. Cocuzza, M. A. Jensen, and K. Baumeister, *Science* **1987**, *238*, 336.