Glen Research REPORT

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DNA Purification

While advances in oligonucleotide synthesis have allowed rapid, facile and efficient production of DNA fragments, purification remains time-consuming and occasionally ineffective. In some applications, there is no need for purified product. However, if purification is necessary, what is the optimum method available for a specific set of circumstances? In this article, we review the options currently available for DNA purification.

The two main techniques used for the purification of synthetic oligonucleotides are polyacrylamide gel electrophoresis (PAGE) and high performance liquid chromatography (HPLC). While the choice of technique may depend simply on previous experience or access to equipment, there are some basic advantages to both techniques.

PAGE

PAGE is probably most often used for the analysis of synthetic oligonucleotides. However, with UV shadowing, it allows the rapid purification of small quantities of synthetic DNA and is especially useful for parallel purification of several sequences. PAGE is

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Modification of Oligonucleotides - An Update

In the most recent issues of the *Glen Research Report*, several techniques for the chemical modification of synthetic oligonucleotides have been discussed. Over the last few months, we have received feedback from researchers and we feel that some of their comments may be of general interest.

5'-Thiol-Modifier

In response to several requests, we are happy to offer the 5'-Thiol-Modifier C6 designed to allow the production of thiol specific probes[1] and attachment of proteins to oligonucleotides. We chose the 6 carbon linker arm which has proved to be most popular in the 5'-amino-modifier series. A trityl group has been used to protect the thiol group. However, this group is stable to acid deprotection, requiring oxidative cleavage with, for example, silver nitrate.

Biotinylation Kit

Labelling with biotin has become a standard technique in DNA chemistry. We are happy to introduce a kit shown to generate the most sensitive assay results. The biotin includes a 14 carbon (XX) spacer terminated in NHS ester for attachment to a primary amine at the 5'-terminus. The kit includes a protocol and all reagents necessary for biotinylation at the primary amine.

5'-Amino-Modifiers

Glen Research 5'-Amino-Modifiers have proved to be quite popular and have performed admirably in a variety of situations. The six carbon linker has proved to be the most useful in a variety of applications. Consequently, we have added 0.5g packs of this product to our catalog. Several requests were obtained for a two carbon linker due to its applications in DNA sequencing. Accordingly, we have also added this item to our catalog; details are shown below.

Phosphate Modification

Phosphorothioates

Of the phosphate modifications accessible using H-phosphonate chemistry, far and away the most popular seems to be phosphorothioates with applications in anti-viral research. Oligophosphorothioates can be prepared by either phosphoramidite or H-phosphonate chemistry. Phosphoramidite chemistry is the most popular method, although the sulfurization step for converting phosphite to phosphorothioate is slow and, by necessity, must be performed once per cycle. In this regard, H-phosphonate chemistry seems more practical because the sulfurization process occurs faster and needs to be performed only once per synthesis.

The greatest advantage of the H-phosphonate method, however, may be in the preparation of oligonucleotides containing a mixture of phosphorothioate and phosphodiester linkages. Typically, two phosphorothioate linkages are prepared adjacent to the 3'-terminus to protect against exonuclease hydrolysis. These linkages are unlikely to survive a large number of iodine oxidations. Thus, with phosphoramidite chemistry, the length of sequence that can be prepared is limited. Using H-phosphonate chemistry, however, a single oxidation step converts all internucleotide H-phosphonate linkages to phosphodiester linkages. The phosphorothioate linkages, therefore, are more likely to survive when H-phosphonate chemistry is used.

Phosphoramidates

Feedback from several researchers has indicated that phosphoramidate linkages can be conveniently and successfully prepared from internucleotide H-phosphonate linkages. Oligonucleotides containing these modifications may be an interesting alternative to those containing methyl phosphonate linkages. By varying the choice of the amine used to prepare these linkages, it is possible to modify the physical properties of the resulting oligonucleotides.

Phosphotriesters

While these linkages are prepared in a manner analogous to phosphoramidates, it is now clear that the synthesis is difficult to perform because of the necessity of maintaining absolutely anhydrous conditions. Even traces of moisture lead to the production of a mixture of phosphotriester and phosphodiester linkages. It is, therefore, doubtful that this procedure will lead to reproducible and sensible results.

RNA Supports

The main difficulty encountered by researchers using RNA supports has been the packing of synthesis columns. We now offer these supports packed in disposable columns suitable for use in ABI and Du Pont/Vega instruments as well as Biosearch instruments. Ordering information is shown below.

In our review of the use of RNA supports for the production of oligodeoxynucleotides modified at the 3'-terminus, we omitted to reference a paper[2] by Zudhir Agrawal and Mike Gait. In this issue we correct our omission and thank Dr. Agrawal for bringing it to our attention.

References: 1. B.A. Connolly and P. Rider, *Nucleic Acids Res.*, 1985, **13**, 4485.
2. S. Agrawal, C. Christodoulou, and M.J. Gait, *Nucleic Acids Res.*, 1986, **14**, 6227.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price ($)
5'-Chemical Phosphorylation Reagent	10-1900-90	100µM	60.00
5'-Amino-Modifier C2	10-1902-90	100µM	50.00
5'-Amino-Modifier C3	10-1903-90	100µM	50.00
5'-Amino-Modifier C6	10-1906-90 10-1906-05	100µM 0.5g	60.00 360.00
5'-Amino-Modifier C12	10-1912-90	100µM	90.00
5'-Thiol-Modifier C6	10-1926-90	100µM	60.00
5'-Branched Modifier C3	10-1950-90	100µM	90.00
Biotin-XX-NHS Ester Labelling Kit	50-1950-01	ea.	115.00
A-CPG 500 column, 1µmole C-CPG 500 column, 1µmole G-CPG 500 column, 1µmole U-CPG 500 column, 1µmole A,C,G,U-CPG 500 column, 1µmole	20-3100-11 20-3110-11 20-3120-11 20-3130-11 20-3140-41	Pk/1 Pk/1 Pk/1 Pk/1 Pk/4	25.00 25.00 25.00 25.00 100.00

DNA Purification

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appropriate for the purification of sequences of any length. However, there are some drawbacks to using PAGE for purification. For example, because of the handling required, the product yield may be quite low and, when purifying longer fragments, it may be necessary to enrich and desalt the crude product prior to purification.

HPLC

One of the most attractive techniques for purification is RP HPLC with the dimethoxytrityl (DMT) group still attached at the 5'-terminus of the synthetic oligonucleotide. With this technique, failure sequences (containing no DMT groups) are virtually unretained on the column while the product is retained and eluted later. In general, a set of components with the DMT group still attached is revealed. Normally, the product accounts for greater .han 95% of these components. The buffers used in this RP HPLC technique are volatile so lyophilization affords the product rapidly and in good yield after removal of the DMT group with acetic acid.

RP HPLC can also be used for the purification of fully deprotected oligonucleotides. The technique retains the advantage of rapid recovery of the purified product simply by lyophilization of the volatile solute.

Recently, several HPLC column manufacturers have introduced ion exchange HPLC columns specifically for the analysis and purification of synthetic oligonucleotides. These columns are capable of superb resolution of components in the product mixture and, in time, are likely ɔ provide new insights into the synthesis process. The columns have a high capacity and even analytical columns may be used in a preparative mode. Semi-preparative columns are available for the purification of larger quantities of product.

Cartridges and Columns

Reverse phase cartridges have been popular[1] for some time for rapid and inexpensive purification of synthetic oligonucleotides. The technique involves evaporating the ammonia used for deprotection (since the silica gel based support is not stable to high pH), redissolving the crude mixture (with DMT group still attached), and introducing the mixture onto the cartridge. The purification procedure is similar to that described above for RP HPLC columns. The DMT groups are later removed by treatment with acetic acid prior to lyophilization to give the purified product.

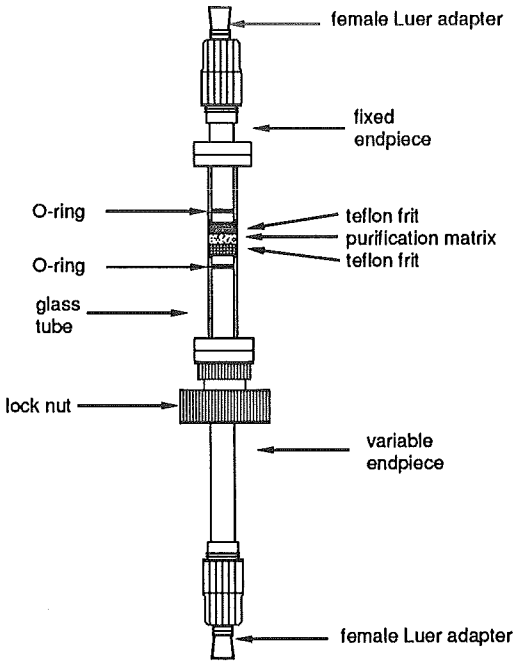

Figure 1: Reusable Purification Column

Several disadvantages, however, exist with this technique. During the step to concentrate the ammonia, a second base, for example, triethylamine, must be added to maintain a basic solution and avoid the loss of the DMT group. In addition, the acetic acid deprotection necessitates two lyophilization steps after purification and an optional extraction to remove dimethoxytritanol from the product.

These disadvantages can be overcome by using a polymer reverse phase (RP) packing in a cartridge or column. Because these resins are stable in the pH range 1-13, the ammonia solution, diluted with water, can be added directly to the purification matrix. After elution of failure sequences, the DMT group can be removed and washed from the matrix. The fully deprotected product can then be eluted and isolated by lyophilization.

Glen Research is delighted to offer Polymer RP1 in bulk for use in the preparation of cartridges and columns for purification of synthetic oligonucleotides. We also offer columns and fittings for DNA purification on a variety of synthesis scales. A typical configuration is shown in Figure 1. Ordering information is shown below.

Reference: 1. K.M. Lo, S.S. Jones, N.R.Hackett,and H.G. Khorana, *Proc. Natl. Acad. Sci. USA*, 1984, **81**, 2285.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price ($)
Polymer RP1	60-1000-05	5mL	70.00
(75Å pore, 12-20µM)	60-1000-20	20mL	175.00
Reusable Purification Column	60-6600-10	ea.	80.00
Fritted Discs (2µM)	60-6653-20	20/pk	9.30
Cartridges	(Inquire for availability)		

LITERATURE REVIEWS

Elimination of Base Modification - Improved Capping Reagent

Throughout the history of oligonucleotide synthesis, there are reports of modification of guanine at the 6-position. This modification has been apparent in both solution- and solid-phase phosphotriester chemistry as well as in solid-phase phosphoramidite chemistry. The most effective way to eliminate this modification may be to protect the 6-position of guanine. However, the additional cost of fully-protected 2'-deoxyGuanosine monomers may limit their acceptance for routine DNA synthesis.

Despite much speculation on the cause and nature of the modification at the 6-position of guanine, no formal study had been carried out. However, recently two researchers at Applied Biosystems, Inc. reported[1] that the initial event in the process of modification of guanine is the phosphitylation of the oxygen at the 6-position. This adduct is partially cleaved by acetate ion in the capping mix but, after iodine oxidation, a more stable phosphotriester species is formed. In the capping step of a subsequent cycle, the phosphotriester adduct is displaced by 4,4-dimethylaminopyridine (DMAP) (present in the capping mix) to form a fluorescent species. Ammonia in the deprotection step leads to the substitution of a portion of the DMAP containing adducts to form 2,6-diaminopurine, albeit in relatively low levels.

Following an exhaustive series of experiments, the authors are able to offer a solution to the problem of modification of the 6-position of guanine. By substituting N-methylimidazole (MeIm) for DMAP as the capping catalyst, the presence of fluorescent impurities and 2,6-diaminopurine in the product sequences is virtually eliminated.

Improved Oxidant for Synthesis

A recent report[2] describes an oxidant with a significantly improved shelf life for automated oligonucleotide synthesis. The most popular oxidant used in phosphoramidite chemistry is 0.1M I_2 in THF/2,6-lutidine/H_2O (40:10:1). For the most part this solution has proved to be effective and stable. However, solutions which are exposed to the atmosphere may develop a black oily residue. This problem may be alleviated by the use of 0.05M I_2 in THF/Water/Pyridine (7:2:1). This solution has proved to be effective in DNA synthesis.

References: 1. J.S. Eadie and D.S. Davidson, *Nucleic Acids Res.*, 1987, **15**, 8333.
2. R.T. Pon, *Nucleic Acids Res.*, 1987, **15**, 7203.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Cap Mix B			
10% MeIm in THF	40-4120-45	45mL	16.00
	40-4120-50	100mL	24.00
	40-4120-51	150mL	30.00
Oxidizing Solution			
0.05M I_2 in THF/H_2O/Pyridine (7:2:1)	40-4130-51	150mL	16.00
	40-4130-52	200mL	20.00
	40-4130-55	250mL	24.00
	40-4130-60	960mL	48.00

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