One-step labelling of oligonucleotides with fluoresceine during automated synthesis

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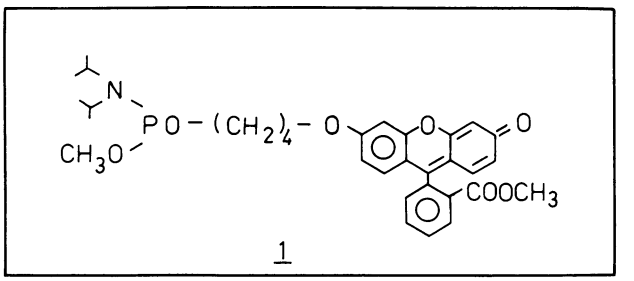
Fluorescent or biotin labelling of an oligonucleotide at it's 5'-end usually involves two steps. First, a N-protected aminoalkyl phosphoramidite derivative (as for instance Aminolink 2 from Applied Biosystems) is added to the 5'-end of an oligonucleotide during automated DNA synthesis. After removal of all protecting groups the NHS ester of an appropriate fluorescent dye or biotin is coupled to the 5'-amino group overnight followed by careful purification of the labelled oligonucleotide from the excess of dye or biotin using reverese phase HPLC or PAGE (1). Recently, the synthesis of phosphoramidite derivatives of different fluorophores including bathophenanthroline-Ru (II) complexes or psoralene acting as coupling components in the automated oligonucleotide synthesis has also been described (2–4).

To reduce the necessary steps in fluorescent labelling for automated DNA sequencing, we have synthesized a phosphoramidite **1** which enables us to label oligonucleotides with fluoresceine during automated DNA synthesis. Thus, fluoresceine methylester was alkylated with 4-chloro(4,4'-dimethoxytrityl)butanol-1 in the presence of K_2CO_3 and KI in DMF for 17 hrs (4). After removal of the trityl group with 1% TFA in chloroform the product was phosphitylated by standard procedures with bis(diisopropylamino)methoxyphosphine yielding 50% of **1**. Phosphonylation of the above obtained fluoresceine derivative led to an H-phosphonate in reasonable yields. The resulting amidite **1** (0.1 M solution in dry acetonitrile) has been used for the automated synthesis of different M13mp18 sequencing primers using β-cyanoethyl phosphoramidite chemistry and a 380 B DNA sysnthesizer from Applied Biosystems. Cleavage from the support and deprotection was performed with 25% aqueous ammonia for 36 hrs at room temperature. The crude product was purified by PAGE and the labelled primer is visible as a pale green fluorescent band at 310 nm. Elution and desalting using RP 18 cartridges yielded the desired product. Figure 1 shows the elution profile of the unlabelled (A) and labelled (B) 14mer oligonucleotide d(CCCAGTCACGAC). UV-spectra show two maxima at 260 nm and 454 nm, respectively, in a ratio of 2:1.

In contrast to the above described two-step procedure, fluorescent labelling of the 5'-end of the primer in our scheme was directly achieved with **1** during DNA synthesis in the last coupling cycle. Coupling yields were as high as with the normal phosphoramidites. After deprotection and removal of ammonia by lyophilization using a speed vac or by ethanol precipitation fluorescent labelled oligonucleotides can be directly used for automated dideoxy DNA sequencing using fluorescent detection. Our labelling technique provides significant advantages when applied to primer walking sequencing strategies.

REFERENCES

1. Applied Biosystems (1989) *User Bulletin for DNA Sequencer Model 370* **No. 11**.
2. DeNapoli,L., Mayol,L., Piccialli,G. and Santacroce,L. (1988) *Tetrahedron* **44**, 215–220.
3. Bannwarth,W. and Schmith,D. (1989) *Tetrahedron Lett.* **30**, 1513–1516.
4. Pieles,U. and Englisch,U. (1989) *Nucl. Acids Res.* **17**, 285–299.



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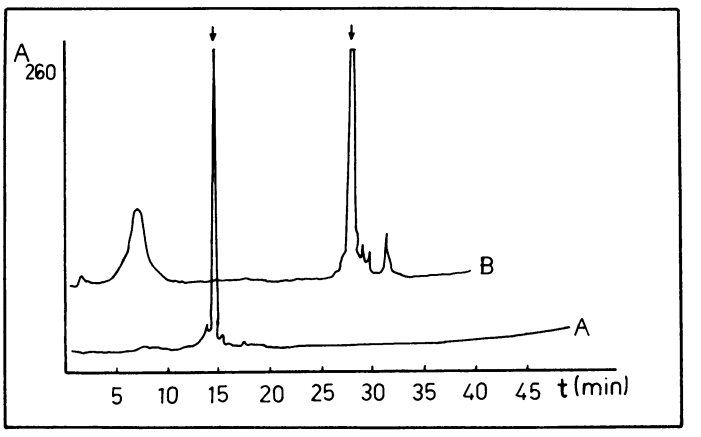

HPLC on RP 5 C18 (analytical column, 125 mm) linear gradient of 10% buffer B to 50% in 50 min, flow rate 1 ml/min. Buffer A: 98% 0.1 M triethyl ammonium acetate (pH 7.0)/50% acetonitrile (v/v); buffer B: 50% 0.1 M triethyl ammonium acetate (pH 7.0)/50% acetonitrile (v/v).