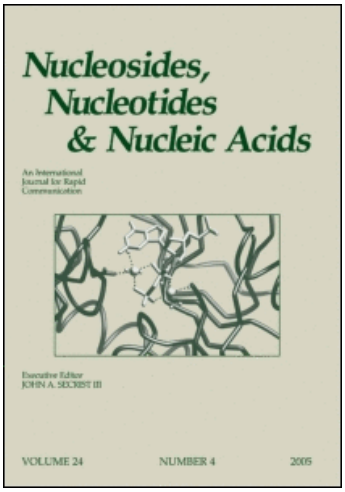


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# Oligonucleotide Microsynthesis of A 200-mer and of One-Dimensional Arrays on a Surface Hydroxylated Polypropylene Tape

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# OLIGONUCLEOTIDE MICROSYNTHESIS OF A 200-MER AND OF ONE-DIMENSIONAL ARRAYS ON A SURFACE HYDROXYLATED POLYPROPYLENE TAPE

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**ABSTRACT:** For the synthesis of multiple oligonucleotides on a single solid support we used a surface hydroxylated non-porous polypropylene tape. This tape was first oxidized and then reduced to introduce hydroxyl groups on the surface of polypropylene. The polypropylene tapes had a capacity sufficient to detect the loading of individual panels by hybridization with fluorescently labeled targets and a very good signal/noise ratio. The nucleotide loading capacity of the tapes was determined to be up to 7.1 nmol/cm$^2$. The excellent accessibility of the tape was demonstrated by the microsynthesis and gel analysis of an oligonucleotide up to 200 bases.

## INTRODUCTION

Different carriers and various methods of functionalization (anchoring) have been described for the synthesis of oligonucleotides irreversibly bound to a solid support surface. Thus, Maskos and Southern[1] used glass plates with a linker of hexaethylene glycol tethered to the surface. Pease and coworkers[2] used a silicium wafer, to which oligonucleotides were linked, the chain elongation being preceded by partial photochemical removal of protecting groups. Recently, Chu et al. described the plasma functionalization of polypropylene tapes with direct introduction of amino groups[3] and Matson, Rampal and Coassin used this method for the preparation of a two-dimensional library of oligonucleotides[4] and the preparation of tri- and dinucleotide short tandem repeat scanning strips with very low disturbing background signals[5].

Here, we describe a procedure for the synthesis of oligonucleotides on polypropylene surfaces yielding relatively highly loaded polypropylene tapes with hydroxyl groups on

the surface and the application of such tapes to site-specific oligonucleotide microsynthesis, as well as to the preparation of a one-dimensional oligonucleotide library.

## MATERIALS AND METHODS

Polypropylene tapes of thickness 75 μm containing no additives were a gift from Lenzing AG, A-4860 Lenzing, Austria. The chemicals used in the reactions to introduce hydroxyl groups on the polypropylene surface were purchased from Aldrich, Deisenhofen, FRG, the phosphoramidites ( e.g. FluorePrime Fluorescein Amidite for fluorescent labeling) were purchased from Millipore, Eschborn, FRG, all other DNA synthesis materials from Roth, Karlsruhe, FRG.

### Preparation of surface functionalized polypropylene tapes

The preparation of the support was done by slightly modifying the method of Lee et. al.[6], who previously described a reaction for introducing OH-groups onto the surface of polypropylene tapes.



Tapes of polypropylene were oxidized by treatment with 5 g of chromium(VI)oxide, dissolved in 50 ml of acetic acid and 50 ml of acetic anhydride at room temperature for 15h. After removing the oxidation solution, the samples were washed with 100 ml of 1M NaOH (2x), 1M HCL (2x), water (2x), methanol (3x), and dichloromethane (3x), in this order, and then dried at 20°C. The samples change their color from clear transparent to a yellowish transparent tint during the oxidation procedure. The dried oxidized tape samples were then placed into a septum tube and flushed with argon for at least 15 min. Via syringe we added 100 ml THF and 15 ml of THF borane complex ( 1.0 M in THF). The reaction was run for 6h. The yellow tint of the tapes disappeared immediately, when the borane solution was added, as described in the publication of Lee et. al.[6] After 6 h the borane solution was removed, and without intermediate washing a mixture of 60 ml $H_2O_2$ and 60 ml 3M NaOH was added. The color of the reaction solution turned to yellow/brown, and the temperature inside the tube increased. The reaction was allowed to proceed for 15 h; during the first hour gas emission could be observed. The peroxide solution was then removed, and the samples were washed with 100 ml each of water (2x), 1 M HCL (3x), water (3x), methanol (3x), and dichloromethane (3x), in this order. The samples were dried and stored at room temperature without further precautions.

Nucleosides were loaded to the functionalized tapes either by direct coupling of the 3'-terminal nucleoside phosphoramidite or by attachement via a phosphorylating amidite[7], using the previously described synthesis unit[8]. The loading capacity, calculated by meas-

urement of the vis-absorption of trityl release during the first synthesis steps, was up to 7.1 nmol/cm$^2$. The conditions for hybridization are given in 'RESULTS'.

# RESULTS

## Oligonucleotide Microsynthesis

To demonstrate the very good accessibility and the high coupling efficiencies we synthesized $dT_{200}$ on the surface of the tape. With the use of the phosphorylating amidite[7] at the first coupling step the long oligonucleotide could be recovered from the tape after the ammonia treatment. The crude product was analyzed after a desalting procedure with a NAP column in a polyacrylamide slab gel. The synthesis product of the $dT_{200}$ is shown in the first lane of FIG. 1. The second lane contains length standards for comparison with the synthesized length of 200 bases. The chemically prepared, radiolabeled oligonucleotide shows mainly one spot with a minimum of by-products of shorter chain length. Starting from an initial loading of 15 nmol nucleoside, the total yield of purified $dT_{200}$ was calculated to be 2nmol. This corresponds to an average yield of 99.0% per chain elongation. Since deprotection and desalting lead to a loss of product, the overall coupling efficiency should be beyond 99%.

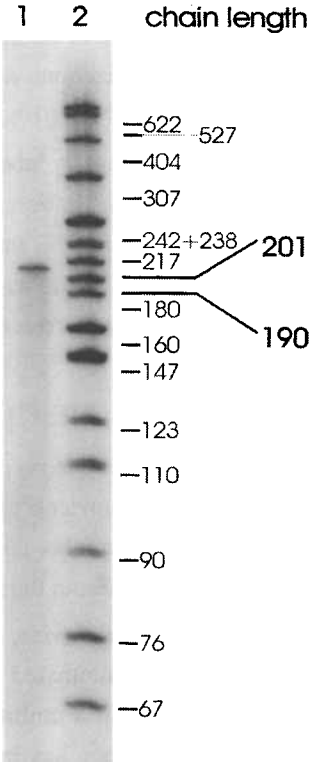


**FIG. 1:** Autoradiography of the analysis of crude $dT_{200}$ synthesized on a functionalized polypropylene tape by PAGE (lane 1). Lane 2: length standards (pBR 322 MspI digest)

## Hybridization experiments

The use of the oligonucleotide-loaded polypropylene tapes for hybridization experiments was done to determine the stringency and specificity of the immobilized oligonucleotides in hybridization reactions. Our tape contained, in addition to the correct antisense (panel 2) and sense (panel 1) oligonucleotides, sequences containing mismatches, as shown in Table 1. The target had the same sequence as in Panel 1 of Table 1, labeled with fluorescein. The hybridizations were done in a solution containing 6 x SSC + 0.01 % SDS and 50 µl (100 pmol/µl) of fluorescently labeled target. In the first experiment the hybridization was done at 48°C for 2 h (FIG. 2A) and at 5°C for 15 h (FIG. 2B). At this temperature the target was shown to bind to sequences containing a single mismatch and, with decreasing fluorescence intensities, to the other mismatched oligonucleotides.

**TABLE 1** Sequences on the tape in FIG. 2

| *Panel* | *5' Sequence 3'** |
|---|---|
| 1 | tga act gaa ctg act |
| 2 | agt cag ttc agt tca |
| 3 | agt cac aag tgt tca |
| 4 | gtt ca |
| 5 | agt cag aag agt tca |
| 6 | agt cag tac agt tca |
| 7 | tca gtg ttc agt tca |

* The underlined monomers are mismatched to the target.



## DISCUSSION AND CONCLUSIONS

In this publication we report on a rapid and efficient method for the surface functionalization of polypropylene tapes for use in oligonucleotide synthesis. As compared to work described from other laboratories[1,3,4,5], our approach is characterized by the use of simple chemistry without the requirement of sophisticated apparatus.

A simple modular device, as we have published it previously[8], can be combined with every commercial automated DNA synthesizer and, thus, serve to produce a polypropylene tape loaded with a multitude of different oligonucleotide sequences. The relatively high degree of surface loading of up to 7.1 nmol/cm$^2$, as compared to the loading reported for glass slides (0.108 nmol/cm$^2$)[1], together with a very low background noise, makes our oligonucleotide carrying polypropylene tapes suitable for a variety of investigations with biomolecules. The surface immobilization of oligonucleotides through anchor groups stable to acid and ammonia treatment allowed multiple reuse of oligonucleotide strips for hybridization.

Alternatively, using an anchor group labile to ammoniacal treatment, we were able to detach the oligonucleotide prepared on the polypropylene surface and thus, perform an oligonucleotide synthesis on a microscale. This microsynthesis could be done using the

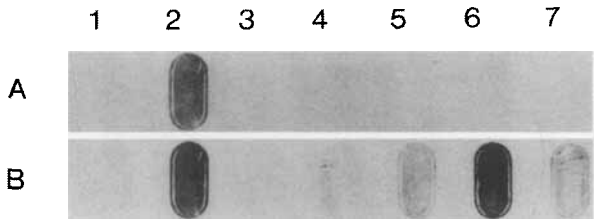


**FIG. 2** Fluorescent Polaroid image of different sequences with various mismatches (Table 1) (labeled target: same sequence as Panel 1 in Table 1)

routine elongation cycles and workup methods. In this way, oligonucleotides of length up to 200 bases were prepared in high purity. Further preparative and diagnostic applications of this system are in progress.



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