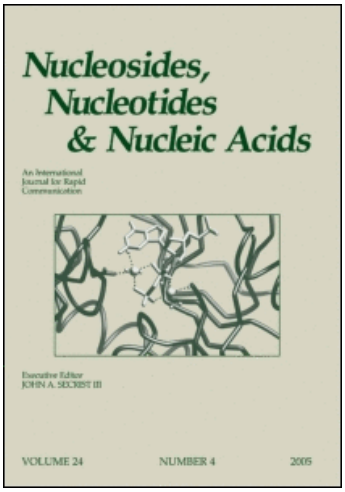

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A Rapid Method for the Preparation of a One Dimensional Sequence-Overlapping Oligonucleotide Library

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A RAPID METHOD FOR THE PREPARATION OF A ONE DIMENSIONAL SEQUENCE-OVERLAPPING OLIGONUCLEOTIDE LIBRARY

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ABSRACT: A new synthesis modul was constructed to prepare one-dimensional libraries of sequentally overlapping oligonucleotides on surface modified polypropylene tapes. The use of such a library for the diagnostic screening of a point mutation in the regulatory protein gene p53 is described.

INTRODUCTION

Oligonucleotide libraries have recently emerged as tools for a variety of molecular recognition processes, which are instrumental in nucleic acid sequence analysis, drug development and other areas of research. The term 'oligonucleotide library' relates to an array of different oligonucleotide sequences immobilized on the surface of a carrier matrix.

For hybridization purposes it is desirable to have as many different elements as possible on a small surface area. On the other hand, it is inevitable that the different elements are clearly discriminated, which requires a certain space between the desired elements. This is a particular problem when creating two-dimensional libraries. Two dimensional libraries, the currently prevailing format, have been prepared on glass plates[1,2] and silicon wafers[3]. Matson et al.[4,5] have described the functionalization of polypropylene surfaces with amino groups by plasma desorption for generating a two-dimensional oligonucleotide library. We have used a chemical method for the surface functionalization of polypropylene with hydroxyl groups, as described by Lee et al.[6].

Strips of such functionalized polypropylene were used in a special modular unit combined with a commercial synthesizer for the preparation of one-dimensional oligonucleotide libraries[7,8]. Here we describe a new type of synthesis module that creates a one-dimensional array of overlapping oligonucleotide sequences.

The principle of synthesizing an array of overlapping sequences is illustrated, for example, by scanning the simple word 'hybridization'. Scanning this word with all possibilities of nine characters leads to the combinations shown in Fig. 1.

MATERIALS AND METHODS

Preparation of the polypropylene film

The polypropylene sheets with size 29 cm x 21 cm and a thickness of 75μm contained no additives and were a gift from Lenzing AG, A-4860 Lenzing, Austria.

The preparation was done in the same manner as described by Lee et. al.[6] with slight modifications[8]. The film was oxidized with chromium(VI)oxide dissolved in acetic acid and acetic anhydride, then reduced in a solution of a borane/THF complex. To introduce OH-groups the film was treated with a mixture of H_2O_2/ 3M NaOH.

For these reactions the film was cut into tapes of 2cm x 29cm. The loading capacity of nucleotides has been determined by the absorption of the trityl release to be at least 5 nmol/cm^2.

Chemicals and materials

The chemicals for oligonucleotide synthesis were purchased from Eppendorf / Biotronik.

Polypropylene films 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. Radioactive labelling was done with γ-^{32}P ATP/ T4-polynucleotide kinase purchased from New England Biolabs.

Routine 0.2μmol standard cycles were run on different synthesis machines, in order to build up polypropylene-bound oligonucleotides. In our hands, the best yields were obtained, when combining our synthesis module with the model Ecosyn-D300 from Eppendorf / Biotronik.

Hybridization conditions

The samples we used to test the arrays were synthetic oligonucleotides, which were end labelled with (γ-^{32}P)ATP and T4 polynucleotide kinase under standard conditions.

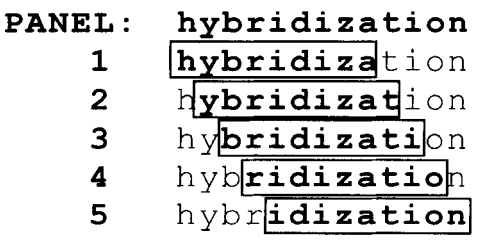

FIG. 1 This is an example for creating overlapping sequences. The word hybridization is scanned with a frame of nine characters. This frame is moved from the left to the right side and covers after five steps the whole word.

All hybridizations were done in plastic bags with a polypropylene net spacer with a mesh size of 1mm. This makes a good access of the labelled sample to the polypropylene strip. For the hybridization we used 5 x SSC with 0,01% SDS and 3 pmol of radiolabelled sample in a volume of 6ml. The hybridization was done without prehybridization and was repeated several times to see the reproducibility. The temperature range was from 4°C to 40°C. The polypropylene strips were washed in bidest water and in 75% formamide. The autoradiograms were done with films from AGFA (CURIX RP1.000G X-Ray film). The densitometric experiments were performed on a phosphorimager from Molecular Dynamics.

RESULTS

Synthesis module

A synthesis module for creating a library of oligonucleotides, shown in Fig. 2, was constructed from a teflon block. It can be combined with any commercial synthesizer. The block contains the parts *a-d*. The polypropylene strip is moved between the Teflon parts b and c; the block is pressed together by nuts and bolts and sealed in this way, using the polypropylene film as seal. With this reaction chamber including 15 panels (i.e. areas of oligonucleotide synthesis) the synthesized array will have elements containing pentadecanucleotides. The number of panels is the same as the length of individual oligonucleotide sequences within the array.

In Fig. 3 we show the process of creating an array with overlapping pentadecamers. In A1 the first nucleotide is coupled to the polypropylene strip. After the deprotection, the block is opened and the strip is moved exactly 5mm to the right side (A2). The oligonucleotides contained in the panel connections will not hybridize to a sample and the panels will clearly be separated. In Fig. 3,B we show the theoretical example of a

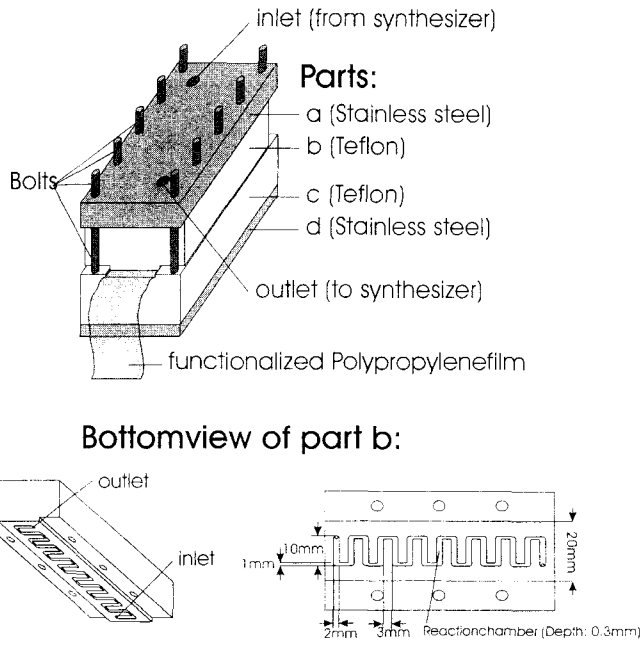

FIG. 2 Construction of the one dimensional synthesis module.

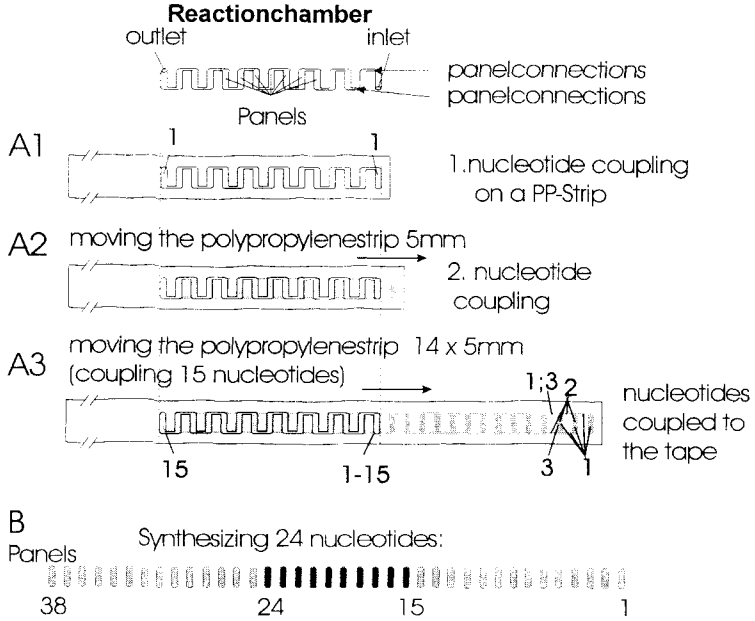

FIG. 3 Operation of the chamber as described in the text.

synthesis comprising 24 coupling steps. After 15 coupling steps the first 15-mer is created. This 15-mer encompasses the base positions 1 to 15.

After 24 coupling steps the last 15-mer at position 24 is created comprising the base positions 10 to 24. The panels 25 to 38 then are the shorter homologues of the last 15-mer produced. The coupled nucleotides are shown in the figure as numbers. After every coupling step the strip has to be translocated exactly 5 mm towards the right side.

By moving the strip exactly for this length, the panels on the tape always move from one reaction position to the next. The panel positions in the teflon block are connected alternatingly on the top and the bottom, thus creating a meander-shaped reaction chamber. With this special geometry the synthesis chemicals can always react with the film surface at each position of the panels. The panel connections on the top, however, are contacted with the chemicals only after translocation of 2 x 5mm. This is the same for the connections on the bottom. All connections will carry sequences differing significantly in length and composition from those within the panels, as shown in Fig. 3, A3.

To test the module and the herewith synthesized oligonucleotide library for diagnostic purposes, we prepared on the tape a nucleotide sequence of 39 bases corresponding to the sequence of the p53 gene from position 483 (1 = translation start) to position 521 (FIG. 4). In this segment of the gene there is a known base mutation leading to inhibition of control of cell proliferation and, thus, often observed in connection with tumors. The sequence on the polypropylene film is complementary to the wildtype. As samples we used two chemically synthesized oligonucleotides with 39 bases. We named the sequences 'wildtype' and 'mutant'.

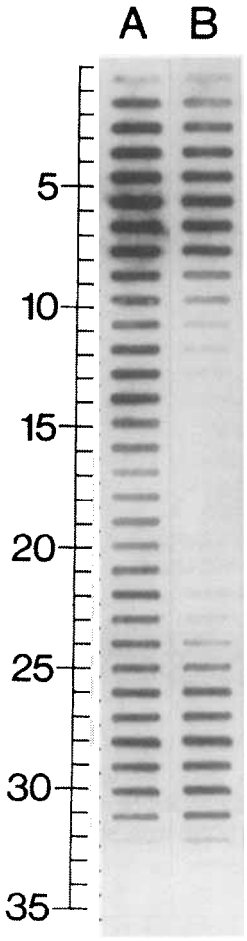

FIG. 4 Autoradiogram of sequences shown in Fig. 5. Probes: strip A: Sequence antisense to the sequence in Fig. 5 (39 nucleotides); strip B: The same antisense sequence with a single base mutation in the middle of the sequence at position 20 (cTc changes to cCc).

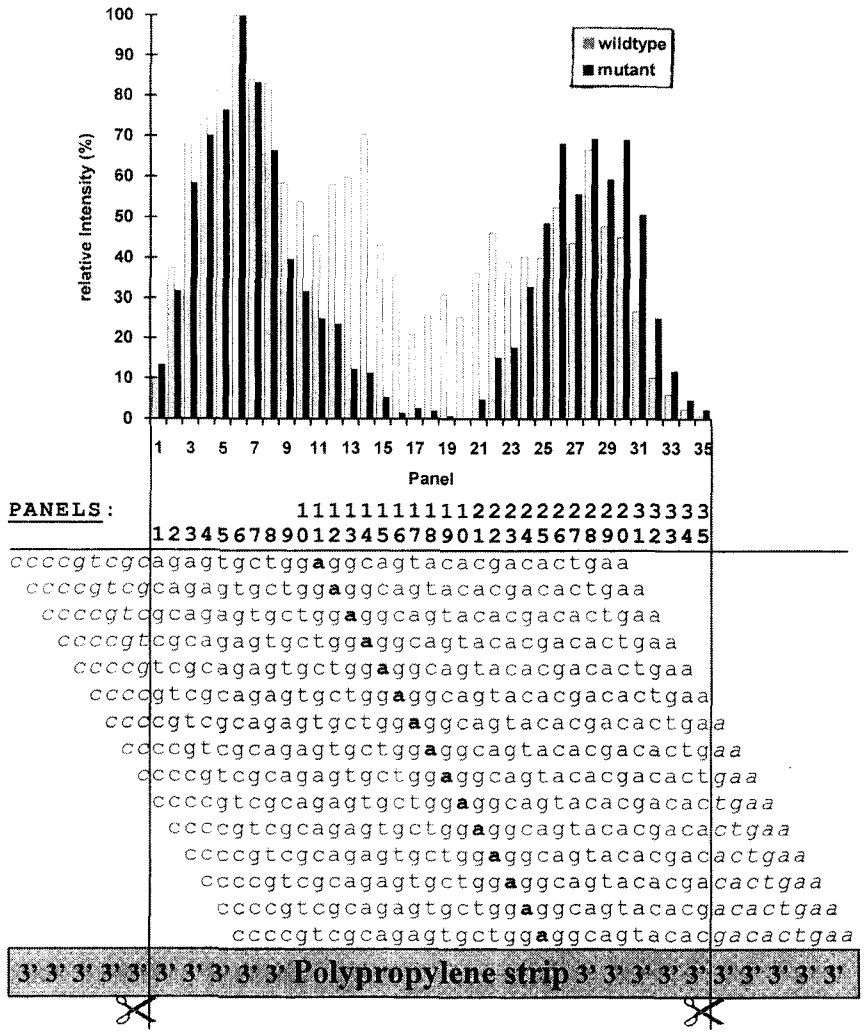

FIG. 5 Densitometric measurement of the autoradiogram shown in FIG. 4, with the synthesized sequences of the p53 gene. The first base is 483 nucleotides beyond the translation start (ATG). The sequence synthesized on the polypropylene strip (39 nucleotides) is:3' ccccgtcgcagagtgctggaggcagtacacgacactgaa 5'

The wildtype sequence 3' ttc agt gtc gtg tac tgc cTc cag cac tct gcg acg ggg 5' is totally complementary to the sequence attached to the tape. The mutant has one base change (from T to C) at the position 20. The sequence is 3' ttc agt gtc gtg tac tgc cCc cag cac tct gcg acg ggg 5'. The sequences synthesized onto the polypropylene tape are listed in FIG. 5. The strip was shortened after the synthesis by cutting off the panels corresponding to the sequences with nine bases are shorter ones. The remaining sequences start with a decamer and end with a decamer. This is indicated by the scissors shown beneath the polypropylene tape in FIG. 5.

CONCLUSION

With the method described here it is possible to create an array of overlapping sequences, where the relation between necessary coupling steps n and thus generated oligonucleotides m with the nucleotide length of 15 is: $m = n + 1 - 15$. The method leads to distinctly separated panels and high loading capacities for each panel.

The application of this library to the detection of gene mutations has been demonstrated. The main feature of this diagnostic application is, that it can be done at almost any ambient temperature, thus abolishing the requirement for temperature control. Other applications for such a scanning strip could be to find binding regions in DNA for proteins, such as enhancer or silencer. Studies on the behaviour of oligonucleotides with incorporated modified nucleotides can be done with this system. Only one coupling step with a modified nucleotide is necessary during the synthesis of such an array to introduce it at any given position, i.e. from 3' terminus to the 5' terminus of an oligonucleotide of certain length.

In this paper, we describe the preparation of scanning tapes by a semi-mechanized method. We are now in progress to a full automatization of this process. Therefore we have constructed a mechanical device to move the tape automatically. This set up is controlled by a micro controller and can be manually operated by a panel with three operator keys and also directly communicate with the DNA synthesizer Eppendorf/Biotronik D-300 Ecosyn. This completely automated system only requires the desired sequence to be programmed, and then the system moves the tape and synthesizes the library in almost any length.

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