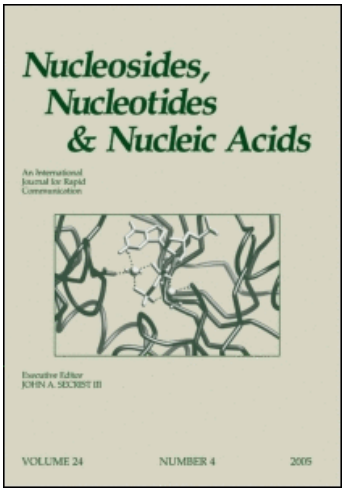

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Sequence Specific DNA Purification by Triplex Affinity Capture: Using Solid Support Linked Oligodeoxynucleotide

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To cite this Article Kumar, Ajay and Rustogi, Suchitra(1994) 'Sequence Specific DNA Purification by Triplex Affinity Capture: Using Solid Support Linked Oligodeoxynucleotide', Nucleosides, Nucleotides and Nucleic Acids, 13: 9, 1855 — 1860
To link to this Article: DOI: 10.1080/15257779408010668
URL: http://dx.doi.org/10.1080/15257779408010668

NUCLEOSIDES & NUCLEOTIDES, 13(9), 1855-1860 (1994)

Sequence Specific DNA Purification by Triplex Affinity Capture: Using Solid Support Linked Oligodeoxynucleotide

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ABSTRACT Sequence-specific DNA isolation by triplex affinity capture using solid support linked oligonucleotide has been described. It has been demonstrated that it is possible to separate oligonucletides differing in single point mutation by this method.

INTRODUCTION

Recently triplex DNA formation have been used for the isolation of specific DNA molecules from heterogeneous DNA mixtures(1,2). Such triplex mediated procedure seem to have some unique advantages over conventional hybridization based methods. In the methods developed so far target DNA is hybridized with a biotinylated oligonucleotide via intermolecular triplex formation, then picked up by streptavidine -coated magnetic beads or streptavidine - agarose column and finally recovered in double stranded form by elution with a mild alkaline buffer that destabilizes the triple-helix. We here, describe an alternative method for sequence specific DNA purification by triplex affinity capture using polymer support linked oligonucleotide . Our method does not require biotinylated oligonucleotide and

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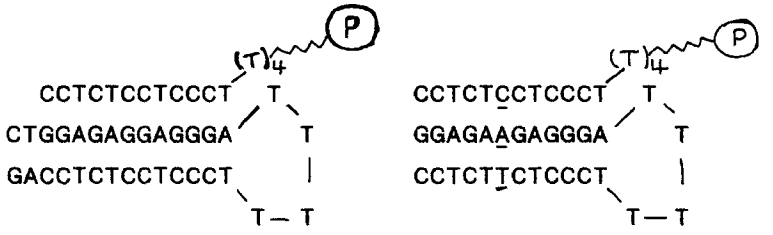
+ stayed as summer trainee from JNU.

immobilized streptavidine like the earlier developed methods. Our method is a one step hybridization method , unlike the earlier methods in which hybridization with the biotinylated oligonucleotde and then incubation with the immobilized streptavidine was carried out.

METHODS

Synthesis of Oligonucleotides

To demonstrate the capability of our method we synthesized two oligonucleotides 32 mer and 28 mer: d(CTGGAGAGGAGGG ATTTTTCCCTCCTCTCCAG),(target) and d(GGAGAAGAGGGATTTTTCCC TCTTCTCC), (mismatch control) differ in a single point mutation. The purified oligonucleotides were annealed by incubating the oligonucleotide solution in boiling water for 5 minutes and allowing to reach room temperature slowly. On annealing both the oligonucleotides form loop structure as shown below.



A oligonucleotide d(DMTrO-CCTCTCCTCCCTTTTT) which can form a triple helix with 32 mer (as shown above) was synthesised on to urethane linkage polystyrene support (3). The urethane linkage polymer support was synthesized as described by Sproat et.al.(3). The loading capicity of the support used was 41.0 μM/g (5). The oligonucleotide synthesized over urethane linkage polymer support was treated with 25% ammonia solution for 6 hrs to deblock the protecting groups from the oligonucleotide. Time course study (data not shown) of linkage stability revealed that 60% of the oligonucleotide remained linked to the polymer support after 6 hrs of

ammonia treatment. The support was filtered off , washed with water, methanol ,ether and dried in vaccum. Small amount (3 mg) of polymer support was treated with 2% dichloroacetic acid solution in dichloroethane. The orange color appeared due to $DMTr^+$ confirms the presence of oligonucleotide onto the polymer support. A oligonucleotide d(CCTCTCCTCCCTTTTT) was also synthesised , purified and phosphorylated by $\gamma^{32}P$ ATP using T 4 polynucleotide kinase.

Gel Shift

To check the authenticity of the synthesised oligonucleotides the gel shift analysis was carried out. The triplex formation was analyzed by constant temperature native gel (20% acrylamide ; acrylamide/bisacrylamide 19:1) at room temperature. The buffer system for the gel and sample was 1X NAE (100 mM sodium acetate/acetic acid/1 mM EDTA) pH 5. Gel samples were prepared by annealing mixtures of (32mer(500 nM) and 16 mer ^{32} P labeled (250 nM)) and (28 mer (500 nM) and 16 mer^{32} P labeled (250 nM)) from 70^{o}C down to 25^{o}C and loaded. The gel was run until the bromophenol migrated 7 cm and was then developed. The gel shift analysis shown in autoradiogram (Figure-1.) suggests that only 32mer forms triplex with 16 mer.

Triple helix Affinity Capture

For triplex affinity capture studies the oligonucletides 28 mer and 32 mer were mixed in equimolar amounts (0.5 OD :0.565 OD, 28:32). The solution containing mixture of the oligonucleotides was incubated with 30 mg of the polymer support in 100 ul of buffer A(2.0 M NaCl/0.2 M sodium acetate/ acetic acid pH ,4.5-5.5) at 50^{o} C for 2 hrs. After 2 hrs the polymer support was washed 5 times with 100 µl of buffer A. Finally the support was incubated with 100 µl of buffer B (1.0 M Tris. HCl, pH 9, 0.5 mM EDTA) for 20 min at 50^{o} C. Again the support was washed five times with 100µl of

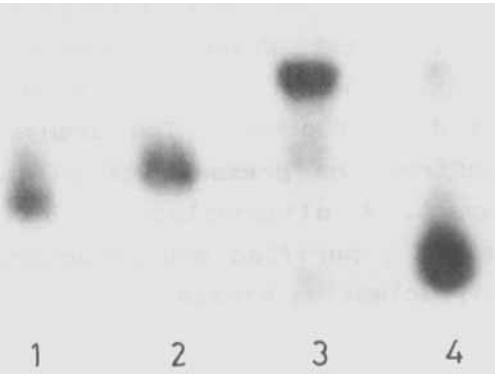


Figure-1.Autoradiogram: Lane 1,duplex 28 mer;Lane 2,duplex 32 mer;Lane 3,triplex of 32 mer and 16 mer;Lane 4,mixture of 28 mer and 16 mer (showing only 16 mer and no triplex formation).

buffer B. The OD of the washings of buffer A and B observed at 260 nm were quantitative. The triplex affinity capture experiment was repeated three times using the same polymer support. Finally the first washing of both the buffers every experiment were analyzed over FPLC using Mono Q column (Buffer, a: 0.4 M NaCL ; 10 mM NaOH, Buffer, b: 0.7 NaCL;10 mM NaOH), Gradient O to 100% b in 35 min., 100% b to 100% b for 10 min; 1.0 ml/min ; 0.5 cm/min.). The FPLC chromatograms are shown in Fig.-2. The equimolar mixture of two oligonucletides was also run on FPLC for comparison.

RESULTS

The gel shift analysis shows that the only 32 mer formed triplex with 16 mer,suggesting that the composition of the synthesised oligonucleotides were correct. The FPLC chromatogram demonstrates that 28 mer did not bind at all to the polymer support linked oligonucleotide and is eluted out in the buffer A. The 32 mer bound with affinity support and was eluted out when the triple helix hybrid was incubated for 20 min . at 50^{o} C in the alkaline buffer. The FPLC

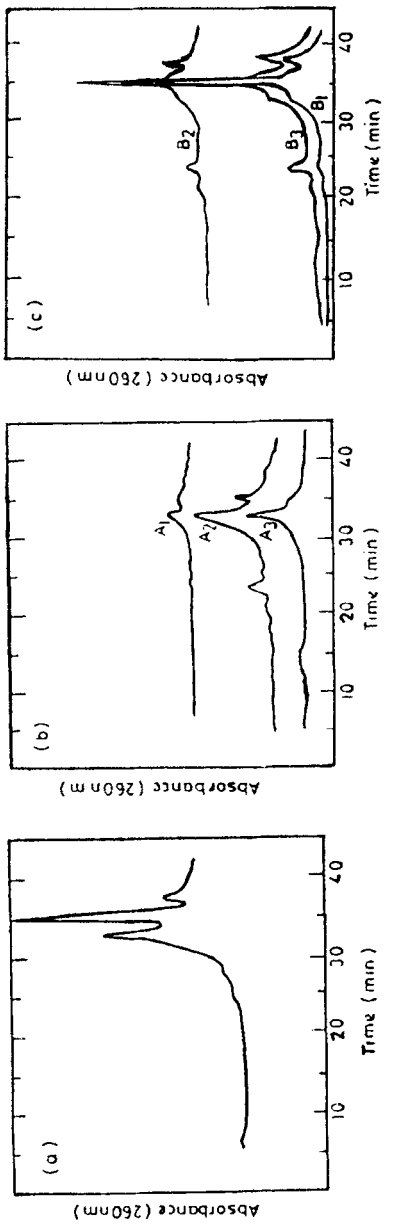


Figure-2:(a) FPLC profile of equimolar mixture of d(CTGGAGAGGAGGGATTTTCCCTCC TCTCCAG) and d(GGAGAAGAGGGATTTTCCCTCTTCTCC). (b) Washings with buffer A : peak A_1 , peak A_2 , peak A_3 of 1st , 2nd and 3rd hybridization experiments. (c) Washings with buffer B:peak B_1 ,peak B_2 ,peak B_3 of 1st ,2nd and 3rd hybridization experiments. Column , Mono Q , Flow rate 1 ml/ min.,Gradient 0-100% B in 35 min. Buffer A = 0.4 N NaCl, 10 mM NaOH. Buffer B = 0.7 N NaCl, 10 mM NaOH.

chromatogram clearly shows that no 28 mer was eluted along with 32 mer in buffer B. This suggests high sequence specifity of triplex affinity capture even if the oligonucletide is linked to support binding of oligonucleotide with support. The support after these experiments was still bearing the $DMTr^+$ group,and hence the oligonucleotide suggesting that affinity support can still be used for many times to capture the target oligonucleotide sequence. The oligonucleotide d(DMTrO-CCTCTCCTCCTTTTT) synthesised over CPG-3000^O A support containing a disulfide hexyl spacer (4) used for triplex affinity capture showed similar results suggesting that this support can also be used for triplex affinity capture. Further work for the isolation of specific sequence from complex genome mixture and genomic library using our method is in progress.

Conclusions A simple , one step method for the sequence specific DNA purification by triple helix affinity capture using reuseable solid support linked oligonucleotide is reported.

Acknowledgment : Acknowledgement is due to Dr.S.K.Basu, Director,NII for moral encouragement and support

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Received December 9, 1993
Accepted April 12, 1994