Volume 13 Number 20 1985 Nucleic Acids Research

Rapid transfer of DNA from agarose gels to nylon membranes

Ken C.Reed and David A.Mann

Department of Biochemistry, Faculty of Science, Australian National University, Canberra A.C.T. 2601, Australia

Received 3 September 1985; Accepted 26 September 1985

ABSTRACT

The unique properties of nylon membranes allow for dramatic improvement in the capillary transfer of DNA restriction fragments from agarose gels (Southern blotting). By using 0.4 M NaOH as the transfer solvent following a short pre-treatment of the gel in acid, DNA is depurinated during transfer. Fragments of all sizes are eluted and retained quantitatively by the membrane; furthermore, the alkaline solvent induces covalent fixation of DNA to the membrane. The saving in time and materials afforded by this simple modification is accompanied by a marked improvement in resolution and a ten-fold increase in sensitivity of subsequent hybridization analyses. In addition, we have found that nylon membrane completely retains native (and denatured) DNA in transfer solvents of low ionic strength (including distilled water), although quantitative elution of DNA from the gel is limited to fragments smaller than 4 Kb. This property can be utilized in the direct electrophoretic transfer of native restriction fragments from polyacrylamide gels. Exposure of DNA to ultraviolet light, either in the gel or following transfer to nylon membrane, reduces its ability to hybridize.

INTRODUCTION

The capillary transfer of DNA restriction fragments from an agarose gel to an appropriate membrane ('Southern blotting'; 1) is a technique fundamental to the analysis of genome organization and expression. In recent years, the availability of nylon membranes as matrices for binding nucleic acids has markedly enhanced the utility of both this and allied techniques in hybridization analyses. Relative to nitrocellulose, nylon membranes have greater mechanical strength, have a higher capacity for nucleic acids, are able to bind smaller oligonucleotides, and have stronger retention of bound nucleic acids permitting multiple re-probing of filters (refer to technical bulletins of suppliers). In addition, it has been reported recently that ultraviolet irradiation catalyzes the covalent attachment of nucleic acids to these membranes. They thus provide all the advantages of chemically-activated cellulose papers (2, 3) without any apparent disadvantages.

Nevertheless, it seemed to us that the full potential of nylon membranes is not realized in transfer protocols that are currently in use. These owe more to their historical succession from Southern's studies with nitrocellulose (1) than to intrinsic properties of the membranes themselves. For example, the fact that nylon membranes can be used in electrophoretic transfer argues that they bind nucleic acids efficiently in buffers of low ionic strength, in marked contrast to nitrocellulose. The use of low-salt buffers for capillary transfer would be expected to improve transfer efficiency, and would certainly be more convenient (in this context we note that Amersham, the suppliers of 'Hybond N' nylon membrane,

suggest the use of 25 mM phosphate for capillary transfer).

Indirect observations in our laboratory had suggested further that nylon membranes may be able to bind native (double-stranded) DNA, in addition to denatured (single-stranded) DNA. The potential saving in time and materials afforded by elimination of gel pre-treatment steps prompted us to investigate this question in more detail.

In the course of these studies we found that DNA is retained by nylon membrane when transferred in acid or alkaline solvents. Not only does this allow large fragments to be sheared by depurination during transfer, but alkaline solvents also promote covalent fixation of DNA to the membrane. The convenience of these direct transfer procedures is complemented by a marked improvement in the resolution and sensitivity of subsequent hybridization analyses.

MATERIALS AND METHODS

DNA Preparations.

Bovine genomic DNA was isolated from homogenates of frozen liver samples prepared in 10 volumes of ice-cold NTE (100 mM NaCl, 10 mM Tris-HCl (pH 7.5), 1 mM EDTA); nuclei were lysed by the addition of EDTA to 0.1 M and sarcosyl (sodium N-lauroyl sarcosinate; Sigma) to 2% (w/v), followed by incubation at 65^{o}C for 15 min. Solid CsCl was added at 1 g/ml, the suspension was made 0.7 mg/ml in ethidium bromide and centrifuged at 45,000 rpm in a Beckman Ti80 rotor at 25^{o}C for 60 h. The band of DNA was removed by side puncture, ethidium bromide extracted with n-butanol, and the sample dialyzed exhaustively against TE (10 mM Tris-HCl (pH 8.0), 1 mM EDTA). The final DNA solution was stored at 4^{o}C over a few drops of chloroform.

Human genomic DNA was isolated similarly from the Burkitt lymphoma-derived cell lines BJAB (female; provided by Dr. Barry Gorman, QIMR) and RAJI (male; ATCC).

Plasmid pSPIB3.8 is pSP64 (4) containing a 3.8 Kb Bam HI fragment of the human X chromosome (5). The 812 bp Eco RI/Bam HI fragment of this plasmid, which contains the promoter and first intron of the X-linked gene for phosphoglycerate kinase, was sub-cloned by digestion of pSPIB3.8 with Eco RI followed by re-circularization with T4 DNA ligase and transformation into HB101 (6). The resultant recombinant (pSPIB0.8) was amplified in liquid culture and the plasmid isolated and purified according to the method of Birnboim and Doly (7), with the inclusion of a final purification by isopycnic centrifugation in CsCl and ethidium bromide (8).

Bacteriophage Lambda DNA (cI*857* Sam*7*) was obtained from New England BioLabs.

Salmon sperm DNA (Sigma), used as the carrier in filter hybridizations, was dissolved in 0.2 M NaOH at 10 mg/ml and sheared by heating at 100^{o}C for 45 min. The solution was then chilled, neutralized with acetic acid and centrifuged to remove debris. DNA was recovered by ethanol precipitation and the pellet dissolved in TE and stored in small aliquots at -20^{o}C.

Restriction Endonuclease Digestions.

Samples of DNA were digested with the appropriate restriction endonuclease(s), obtained variously from Bethesda Research Laboratories, New England BioLabs, Boehringer, and Pharmacia-PL, under conditions recommended by the suppliers.

End-Labelling of DNA Digested with Restriction Endonuclease.

Bam HI-digested genomic DNA was incubated with the large (Klenow) fragment of DNA polymerase I (New England BioLabs) with 0.2 mM dGTP and [a-^{32}P]-dATP (Amersham) at room temperature for 30 min (8).

Agarose Gel Electrophoresis.

Gels were cast and run in the Pharmacia GNA-100 mini-gel apparatus, using either 5- or 8-tooth combs that form sample wells of 10 mm x 1 mm x 7 mm and 4.5 mm x 1 mm x 7 mm respectively. The gel volume was 60 ml, usually of 1% (w/v) agarose (Sigma Type I) in TAE buffer (40 mM Tris-acetate (pH 7.8), 20 mM sodium acetate, 2 mM EDTA) containing ethidium bromide (0.5 µg/ml); the gel dimensions were 100 x 80 x 7.5 mm. Electrophoresis was conducted at 125 mA/45 V (2.8 V/cm) for 100-200 min at room temperature.

Gel Pre-Treatment.

Following electrophoresis, each gel was photographed on a medium-wavelength (302 nm) ultraviolet transilluminator (Oliphant, Adelaide) then immediately subjected to one of the following pre-treatments (for treatments (A) and (B), the free-floating gel was agitated gently and continuously at room temperature):

(A) Acid/alkali/neutralization (depurination): the gel was treated sequentially with 2 volumes of 0.25 M HCl (2 x 10 min), 2 volumes of 0.5 M NaOH/1.5 M NaCl (2 x 15 min), and 2 volumes of 0.5 M Tris-HCl (pH 7.5)/1.5 M NaCl (2 x15 min) (9).

(B) Acid: the gel was treated with 2 volumes of 0.25 M HCl (2 x 10 min).

(C) Ultraviolet irradiation: the gel was placed 1 cm below the germicidal ultraviolet strip light (254 nm, 30 W) in a biosafety cabinet. Each side of the gel was exposed for half the total time indicated in the text, with the origin placed directly beneath the light to ensure that the largest DNA fragments received the greatest exposure (10).

(D) No pre-treatment.

Capillary Transfer to Nylon Membranes.

For overnight transfer, the pre-treated gel was placed on three sheets of saturated Whatman 3MM paper supported on an inverted gel casting tray in a small plastic box. The paper was the same width as the tray and had extended wicks dipping into a reservoir of solvent in the bottom of the box. A sheet of Zeta Probe nylon membrane (Bio-Rad), cut to the same dimensions as the gel and previously wetted in distilled water, was placed on the gel surface and this in turn was covered with eight sheets of 3MM paper and a stack of paper towel to the height of the box rim. Light pressure was applied to the stack by sealing the box with a snap-seal lid.

For shorter transfer periods, the protocol adopted was similar to that described by Wahl *et al.* (9). To effect a change of solvent, a glass plate was placed on top of the transfer assembly and the assembly inverted. The pad containing transfer solvent was carefully peeled off the base of the gel and replaced with a fresh pad saturated with the new solvent, the assembly was returned to its original orientation and transfer resumed.

Fixation of Transferred DNA.

Following the transfer of native DNA, the membrane was removed from the gel and placed, DNA surface uppermost, on a pad of 3MM paper saturated with 0.5 M NaOH/1.5 M NaCl for 5 min, then placed for 5 min on a second pad saturated with 0.5 M Tris-HCl (pH 7.5)/1.5 M NaCl (11).

These membranes, together with those to which denatured DNA had been transferred, were agitated briefly in 2 x SSC (0.3 M NaCl, 0.03 M trisodium citrate) to remove possible adherent gel fragments and, in the case of alkaline transfers, to neutralize the membranes. They were then blotted dry and subjected to one of the following fixation procedures:

(a) Baked in a vacuum oven at 80^{o}C for 2 h (1).

(b) Wrapped in Glad Wrap (equivalent to Saran Wrap) and placed, DNA surface down, on the ultraviolet transilluminator (refer above) for the times specified.

(c) Placed, DNA surface uppermost, on a clean sheet of 3MM on the work surface of the biosafety cabinet (refer above) and exposed to its ultraviolet light for the times specified. The light source was located 60 cm above the membrane.

Nick-Translation of DNA.

Probes for hybridization analyses (and, in the experiments of Figure 2, genomic DNA) were labelled with [a-^{32}P]-dCTP (Amersham) according to the principle of Rigby *et al.* (12), using a modification that ensures high efficiency of label incorporation (13).

Hybridization of DNA Bound to Nylon Membranes.

In most cases, the hybridization solution consisted of 1.5 x SSPE (0.27 M NaCl, 15 mM sodium phosphate (pH 7.7), 1.5 mM EDTA), 0.5% (w/v) BLOTTO ('Diploma' non-fat powdered milk; 14), and 1% (w/v) SDS. To ensure uniformity of hybridization conditions, all membranes prepared for a single comparative experiment were placed in a small plastic box with 1ml/4 cm^2 of hybridization solution containing heat-denatured, sheared salmon sperm DNA (0.5 mg/ml). A sheet of Glad Wrap was molded to the inside, in contact with the surface of the solution and overhanging the edges of the box, and the box closed with a snap-seal lid. Pre-hybridization was continued overnight at 68^{o}C with continuous agitation.

The radio-labelled probe was mixed with salmon sperm DNA in 0.2 M NaOH, heated at 100^{o}C for 10 min, chilled, and neutralized with acetic acid. The solution of denatured, sheared probe and carrier DNA (final concentration 0.5 mg/ml) was mixed with fresh hybridization solution (1 ml/8 cm^2 of membrane), the membranes were added and hybridization continued at 68^{o}C for up to 20 h. Subsequent studies have shown that the omission of carrier DNA from prehybridization and hybridization solutions has no adverse effects on either background or sensitivity under these conditions.

When probing for single-copy fragments of the PGK gene, prehybridization was carried out at 42^{o}C in plastic bags containing 10 ml of 4 x SSPE, 0.5% BLOTTO, 1% SDS, and 0.5 mg/ml salmon sperm DNA in 50% (v/v) formamide. Hybridization was performed under similar conditions, with the inclusion of 10% (w/v) dextran sulfate (9). It was found subsequently that in using dextran sulfate, carrier

DNA could be omitted from hybridization solutions but not from the prehybridizations.

Washing of Membranes and Autoradiography.

On completion of hybridization, the membranes were rinsed briefly in 2 x SSC/0.1% SDS then washed successively (for 15 min at room temperature with vigorous agitation) in 2 x SSC/0.1% SDS, 0.5 x SSC/0.1% SDS and 0.1 x SSC/0.1% SDS, with a final high-stringency wash in 0.1 x SSC/1% SDS at 50°C for 30 min. The membranes were blotted dry, wrapped in Glad Wrap and exposed to pre-flashed X-ray film (Fuji RX) with an intensifying screen (DuPont Cronex 'Lightning Plus') at -70°C (15).

Fixation and Drying of Gels.

On completion of transfer of radio-labelled restriction fragments, the gels were soaked in 7% (w/v) trichloroacetic acid for 30 min then dried by blotting with 3MM paper under a moderate weight (8). The dried gels and the membranes used in transfer were wrapped in Glad Wrap and autoradiographed at room temperature.

RESULTS

Retention of Native DNA by Nylon Membrane.

Initial experiments were designed simply to determine (i) whether Zeta Probe retains DNA at lower ionic strength than is recommended in standard protocols, and (ii) whether it binds native DNA with an efficiency comparable to that found with denatured DNA. Each of the 8 tracks of a gel contained

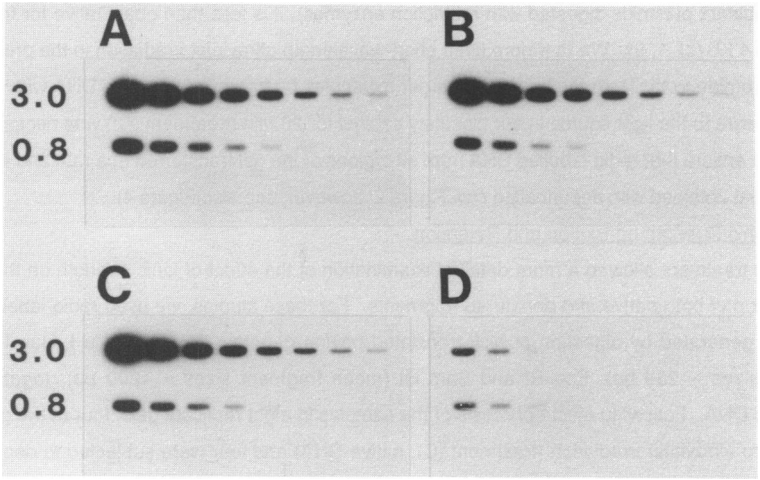

Figure 1. Transfer of native DNA to Zeta Probe. Bovine genomic DNA (0.8 µg, digested with Eco RI) was mixed with doubling dilutions of pSPIB0.8 (6.4-0.05 ng, digested with Bam HI and Eco RI) and electrophoresed on 1% agarose gels. The resolved fragments were transferred to Zeta Probe overnight, either directly ((c), (d)) or after first being subjected to acid depurination ((a), (b)); the transfer solvent was either 1 x SSC ((a), (c)) or 10 x SSC ((b), (d)). The membranes were rinsed (in (c) and (d), following denaturation), baked, and hybridized with nick-translated pSPIB0.8 (30 µCi/µg at 25 ng/ml) for 20 h, then washed and autoradiographed overnight. Numbers at the left refer to sizes (Kb) of the vector and insert fragments .

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.