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"Pocket Blotting": A Method for Transferring Nucleic Acids onto Nylon Membranes

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We have developed a fast and efficient method for transferring nucleic acids onto nylon membranes. This method requires less DNA for transfer; no decrease in efficiency is observed after successive probing, and several gels can be processed simultaneously. We believe that this technique is of general interest in routine analysis of multiple samples in population genetic studies or in diagnosis purposes. © 1991 Academic Press, Inc.

Transfer of nucleic acids from agarose gels to nitrocellulose or nylon membranes is a technique widely used in molecular biology. Several procedures already described include: (i) capillary blotting, originally developed by Southern (1): (ii) electroblotting (2): and more recently, (iii) vacuum blotting (3). This latter method offers some significant advantages: transfer is rapid and quantitative, with a high band resolution, but it does require an expensive specific device.

In this paper, we describe a very simple way of transferring nucleic acids from agarose gel to nylon membrane that combines both capillary transfer and vacuum blotting and avoids the requirement of a specific apparatus. It also allows simultaneous blotting of several gels of any size. We propose the name "pocket blotting" for this efficient, simple, and inexpensive method.

MATERIAL AND METHODS

Human DNA was prepared from peripheral blood samples. Cells were lysed in hypotonic buffer (4), essentially as described by Maniatis *et al.* (5). Digestions were performed overnight, with excess restriction enzymes (10 units/μg DNA). DNA samples were resolved electrophoretically in agarose (Sigma) gels, ranging from 0.7 to 2% in agarose concentration. After migration, gels were processed for DNA depurination and denaturation with alkali. In our method, the neutralization step was omitted, and the length of time for depurination and denaturation was slightly increased (cf. Results).

Pocket blotting transfer was performed in the following way. Using a solid support, (glass plate or perspex plate), we combined in the following order: (i) a pile of reusable wiping cloths (three to four times the thickness of the gel); (ii) two 3MM (Whatman) paper sheets wetted in denaturation buffer; (iii) a sheet of nylon membrane, cut to the exact size of the gel; and (iv) the agarose gel. This pile was then placed in a plastic bag ("destruction" bag purchased from Greiner, FRG) and the bag was heat-sealed on four sides, leaving only a small aperture in one corner. Then, by means of a water-driven pump, a vacuum was created within the bag and when it was completely achieved (after a few seconds), the open corner was heat-sealed under the vacuum pressure, forming an airtight container. Transfer was allowed to proceed for 90 to 120 min (depending on the agarose concentration) at room temperature, until the gel was almost completely dry. The membrane was then peeled from the gel and rinsed twice for 2 min in 2× SSPE (0.36 M NaCl, 20 mM sodium phosphate, pH 7.7, and 2 mM EDTA). Membranes were stored dried until the hybridization procedure.

For capillary blotting after electrophoresis agarose gels were placed in 0.25 M HCl twice for 10 min; the gels were rinsed in distilled water and were ready for transfer without any other treatment. Alkali blotting procedures were set up as described in Maniatis *et al.* (5), except that the transfer buffer was 0.4 M NaOH.

Probes were labeled by random oligonucleotide priming (6), using [α-^{32}P]dCTP (Amersham). Prehybridization and hybridization procedures depended on the type

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of nylon membranes used and were carried out according to the manufacturer's recommendations.

RESULTS

In a first set of experiments, we determined the best conditions for an efficient transfer onto nylon membranes and compared our results with those obtained with the conventional capillary blotting. λ phage DNA was digested with *Hind*III and the resulting fragments were end-labeled with Klenow polymerase in the presence of [α^{32}P]dCTP (5). Four sets of samples with two different concentrations (5000 and 10,000 cpm per well) were resolved electrophoretically in an 0.8% agarose gel. After migration, the DNA was depurinated within the gel with 0.25 N HCl, then denatured with 0.4 M NaOH (buffer mentioned for alkali blotting on Hybond N+ by Amersham). For pocket blotting we observed that increased times of depurination (2 times 20 min) and denaturation (2 times 25 min) resulted in a better transfer of the fragments from the gel to the membrane, without significant loss of short fragments (Fig. 1). For capillary blotting, we have strictly followed the instructions for gel treatment and transfer setting given by Amersham (see Material and Methods). As shown in Fig. 1, comparisons of our method (PB)[2] with capillary transfer (CB) carried out for varying times demonstrate clearly that our procedure is more effective than the conventional procedure in the same amount of time and at least as efficient as 4- or 16-h transfers, thus allowing substantial savings of time.

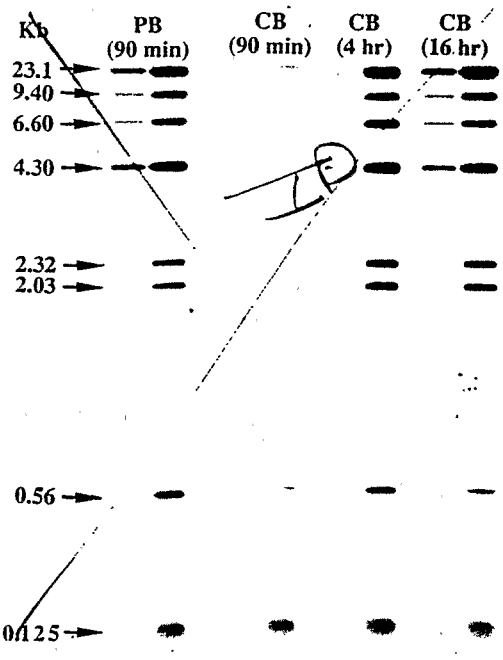

FIG. 1. Four sets of end-labeled λ *Hind*III (at two different concentrations, 5000 and 1000 cpm per well) were resolved electrophoretically in an 0.8% agarose gel. Each set was transferred onto Hybond N+ either by pocket blotting (PB) for 90 min or by capillary blotting (CB) for three different times: 90 min, 4 h, or 16 h; transfer buffer was 0.4 M NaOH. After transfer procedures, dried membranes were autoradiographed at room temperature for 4 h. In the left edge (1000 cpm) of the 4-h column CB DNA did not transfer well due to poor juxtaposition of the membrane and gel; the right edge is the correct result.

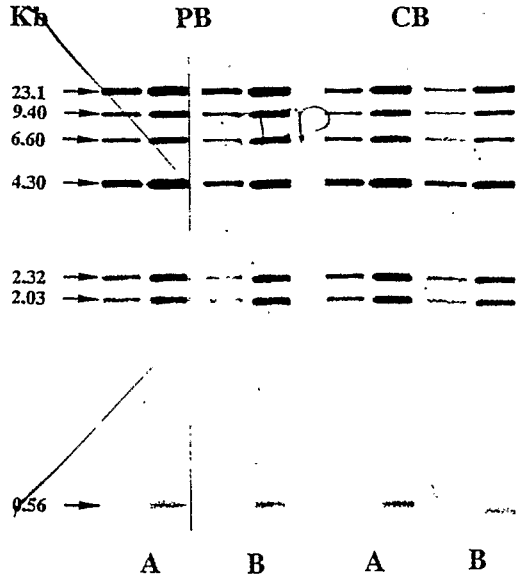

FIG. 2. Four sets of endlabeled λ *Hind*III (at two different concentrations, 5000 and 1000 cpm per well) were resolved electrophoretically in an 0.8% agarose gel. Each set was transferred onto Hybond N+ either by pocket blotting (PB: 90 min) or by capillary blotting (CB: 16 h) in two different buffers, 0.5 M NaOH + 1.5 M NaCl (A) and 0.4 M NaOH (B), and autoradiographed as in Fig. 1.

To improve the method, we tried different transfer buffers and finally found that (as shown in Fig. 2) the transfer obtained was better when high ionic strength (A: 0.5 M NaOH + 1.5 NaCl) was used in the denaturation buffer than when lower ionic strength (B: 0.4 M NaOH) was used. This is of particular interest when membranes must be processed through several successive cycles of hybridization with different probes.

Nitrocellulose membranes were also tested, but presented some inconveniences, e.g., a prerequisite step of neutralization of the gel, a significant loss of DNA during the transfer step, and the difficulty of handling and reutilization. For these reasons, the nylon membrane was preferred and is used currently in all our protocols.

Figure 3 shows the efficiency of our transferring technique with different concentrations of DNA Raoul (Appligène, France) molecular size markers (which have similar DNA concentrations within each of the 23 bands) resolved electrophoretically in a 1% agarose gel. Blots were transferred either onto a positively charged

[2] Abbreviations used: PB, pocket blotting; CB, capillary blotting.

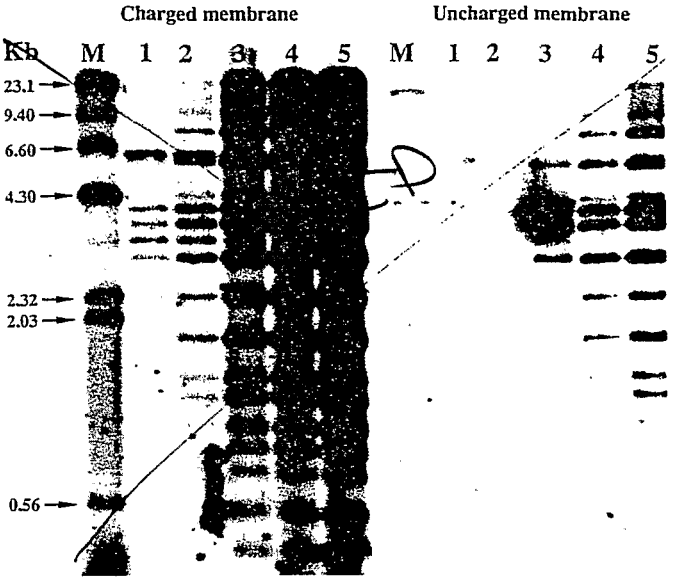

FIG. 3. Five different concentrations of Raoul DNA size marker (Appligène) were resolved electrophoretically in duplicate in 1% agarose gel: 1 = 10 pg, 2 = 20 pg, 3 = 50 pg, 4 = 100 pg, 5 = 200 pg. End-labeled λ *Hin*dIII (M) was used as internal control. After pocket blotting for 90 min (with 0.5 M NaOH + 1.5 M NaCl), onto either Zeta-probe (charged membrane) or Hybond N (uncharged membrane), nylons were briefly rinsed twice in 2× SSPE (0.36 M NaCl, 20 mM sodium phosphate, pH 7.7, and 2 mM EDTA). Prehybridization was carried out at 65°C for 2 h in 5× SSPE, 1% SDS, 0.5% nonfat milk, and 100 μg/ml sheared denatured salmon sperm DNA. Hybridization was performed (at 68°C for 16 h) in the same buffer supplemented with 10^6 cpm/ml of pBR322 labeled by oligopriming; membranes were washed 2× 15 min with 2× SSPE + 0.1% SDS and 2× 20 min with 0.2× SSPE + 0.1% SDS at 68°C. Dried membranes were autoradiographed for 20 h at −70°C with intensifying screens.

membrane or onto an uncharged nylon membrane. In both cases, we added end-labeled λ DNA *Hin*dIII fragments as an internal control for transfer. After pocket blotting, membranes were hybridized with labeled pBR322, washed at low ionic strength, and autoradiographed for 20 h. The sensitivity of the detection method and the efficiency of transfer were estimated from the DNA concentration and the number of counts hybridized in each individual band. A good efficiency was obtained for fragments ranging from 30,000 to 300 bp, and around 0.1 pg DNA per band can be detected on charged membranes (Fig. 3). We also observed that our technique gave a much better recovery when a positively charged membrane was used in place of an uncharged one. This was possibly due to some loss of DNA material during the transfer and/or hybridization step, as suggested by the control λ DNA marker (Fig. 3, lane 1). Several nylon membranes were tested (GeneScreen, NEN; Hybond N, Amersham; and Compass, Genofit), and better results were always obtained with charged membranes (Zeta-probe, Bio-Rad; or Hybond N+, Amersham).

Another example of both the sensitivity and the efficiency of our blotting technique is shown in Fig. 4: 1 μg (instead of 10 μg, as usually loaded in conventional techniques) of different individual human samples resolved electrophoretically and pocket-blotted was hybridized with a probe originating from the 3′ end region of the LDL receptor gene (7). The characteristic polymorphic bands (representing at most 0.5 pg each) (8) were clearly visible on the autoradiogram (40-h exposure at −70°C with Kodak intensifying screens). Furthermore, an interesting rapid application of our pocket blotting technique can be made in the genetic analysis of populations and clinical diagnosis by restriction fragment length polymorphism (RFLP) of human polygenic disease. As shown in Fig. 4, the use of a minigel and a rapid hybridization procedure (RSH Multiprime, Amersham) reduces the entire protocol, from the electrophoretic step to the autoradiography, to one working day. Pocket blotting was also found to be very efficient when nonradioactive probes were used (9).

DISCUSSION

In this paper, we describe a simple procedure for transferring nucleic acids from agarose gel to nylon membrane. Although the vacuum blotting technique, which has been recently described (3), seems to be slightly faster, our pocket blotting method presents several advantages over other methods of DNA transfer: (i) Gels of any dimension (from mini to maxi gels: 20 × 25 cm) and of any pore size (agarose concentration ranging from 0.7 to 2.0%) can be easily processed, at low cost, and with no expensive commercial device. (ii) Our method is fast enough that the entire procedure can be completed within one working day. (iii) Its efficiency

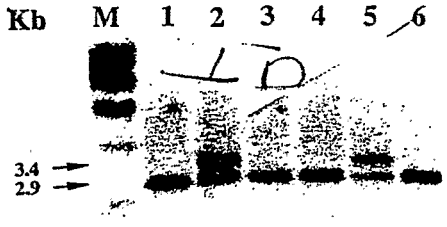

FIG. 4. Six individual human DNAs (1 μg each) previously digested with *Pst*I were resolved electrophoretically for 1 h in 0.8% agarose minigel and subsequently pocket blotted for 90 min on Hybond N+ membrane. Prehybridization and hybridization were carried out (for 15 and 120 min, respectively) with labeled probe (specific activity of about 10^9 cpm/μg) from the 3′ end LDL receptor gene by using a rapid hybridization kit (RHS Multiprime, Amersham). For the washing conditions we followed the manufacturer's instructions. Dried membranes were autoradiographed for 40 h at −70°C with intensifying screens.

allows us to save substantial amounts of biological sample: less DNA is required for transfer, and the blots can be reused several times for successive probing, without significant decrease in signals. (iv) There is no theoretical limit to the number of gels being pocket-blotted at a time, in contrast with other commercially available equipments.

We have studied over several hundred human DNA samples with different probes without any failure. Our technique seems therefore to be perfectly adapted to routine analysis of multiple samples. Modifications to make this pocket blotting suitable for RNA and pulsed-field gel (PFGE) transfers are currently being investigated.

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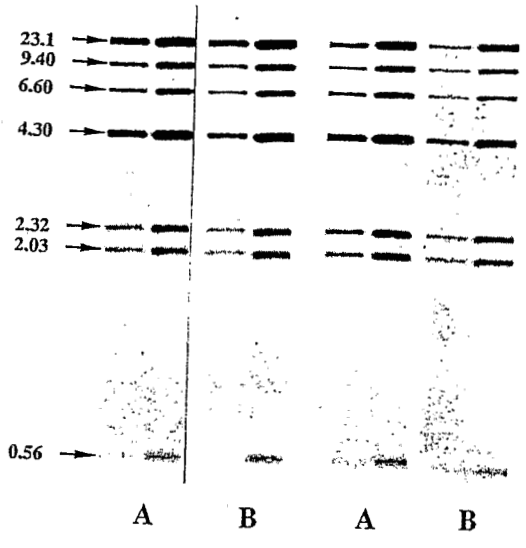

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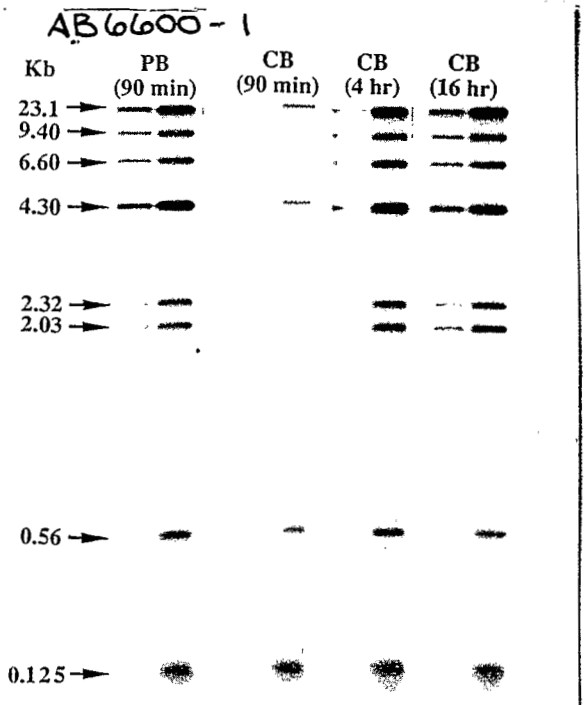

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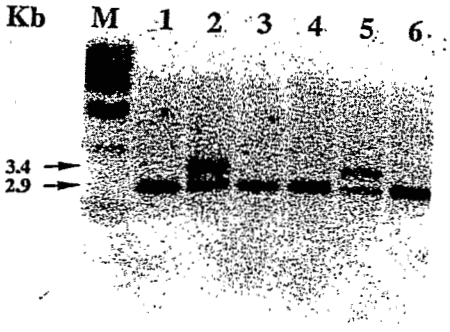

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