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ALKALINE TRANSFER OF DNA TO PLASTIC MEMBRANE

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SUMMARY: DNA forms a stable complex with Gene Screen Plus membrane at alkaline pH. Based on this, a method of alkaline transfer of DNA from agarose gel to Gene Screen Plus membrane was elaborated. The procedure entails the use of 0.4 M NaOH for both, the DNA denaturation and DNA transfer steps. The alkaline transfer offers a higher hybridization efficiency and simplifies the transfer procedure as compared with the standard method of DNA transfer at neutral pH. In addition, it can be used to remove RNA contamination from the transferred DNA

Since the elaboration by Southern (1), the method of DNA transfer from agarose gels to nitrocellulose membrane and subsequent hybridization of the membrane bound DNA with a radioactive probe, has become a major tool in molecular biology. Soon, other media, such as DBM paper (2,3) or APT paper (4) which bind DNA more firmly than nitrocellulose by the formation of covalent linkages with DNA, were adopted for the DNA transfer. Recently, Gene Screen Plus (GSP), a plastic membrane, was introduced for DNA transfer. The GSP membrane binds DNA firmly enough to be reused in several hybridization rounds, while providing low background, and is easy to handle (5). In this report, we show that GSP membrane can bind DNA at alkaline pH. On this basis, we have elaborated a method of alkaline transfer of DNA from agarose gel to GSP membrane. Due to the alkaline conditions during the transfer step, DNA binds to the membrane in the completely denatured form. As a result, the efficiency of hybridization with the DNA transferred by the alkaline method is higher than with DNA transferred by the standard method.

Abbreviation used: GSP, Gene Screen Plus

0006-291X/84 $1.50

MATERIALS AND METHODS

Materials were purchased from the following sources: Gene Screen Plus from New England Nuclear; nitrocellulose BA 85 from Schleicher and Schuell, Inc.; nick-translation kit from Bethesda Research Laboratory; agarose from International Biotechnologies, Inc.; dextran sulfate from Pharmacia; X-ray film OSRAY RPJ from Agfa-Gevaert.

Solutions: Transfer solution (TS): 0.4 M NaOH, 0.6 M NaCl; Neutralizing solution (NS): 0.5 M Tris-HCl, pH 7.5, 1 M NaCl; Washing solution (WS): 0.15 M NaCl, 10 mM Na phosphate, pH 6.5, 1 mM EDTA, 0.5% SDS; SSC - 0.15 M NaCl, 15 mM Na citrate.

Nick-Translation: Plasmid p 35-αLA with cDNA insert encoding rat α-lactalbumin (6) was labeled by nick-translation with [^{32}P] dCTP to about 10^9 dpm per µg DNA. Nick-translated DNA was passed through a Sepharose 2B column and precipitated with ethanol.

Hybridization: Hybridization of DNA bound to GSP membrane was performed according to the manufacturers protocol with minor modifications. Briefly, prehybridization was carried out in a solution containing 1 M NaCl, 50 mM Tris-HCl pH 7.5 and 1% SDS for 2 h at 65°. For hybridization , the above solution was supplemented with 10% dextran sulfate, sonicated salmon sperm DNA (100 µg/ml) and [^{32}P] p35-αLA DNA (10 ng/ml). The [^{32}P] probe and salmon sperm DNA were boiled for 10 min., added to the hybridization solution, and the mixture pipeted into the hybridization bag containing the membrane. Hybridization was carried out for 12 h at 65°.

Hybridization of nitrocellulose-bound DNA was carried out according to Southern (1) with 10% dextran sulfate.

Following hybridization, membranes were washed twice for 10 min. at room temperature, twice for 25 min. at 65° with WS, and twice for 25 min. at room temperature with 0.1 x WS.

RESULTS AND DISCUSSION

The binding of DNA to GSP membrane at alkaline pH was tested using nick-translated plasmid p35-αLA. [^{32}P] p35-αLA DNA was denatured in TS for 30 min. at room temperature, then 5 µl aliquots were spotted onto GSP membrane. To mimic transfer conditions, the membrane was placed in TS and incubated with gentle agitation for 23 h at room temperature. Fig. 1 shows that out of the initial amount of radioactivity, 82% and 78% remained on the membrane after 2 h and 23 h of incubation, respectively. This indicates that GSP membrane is able to attract DNA even at alkaline pH. GSP membrane binds DNA fragments longer than 75 base pairs (5). Therefore, the radioactivity released during the first two hours of incubation may be the result of the release of small DNA fragments generated during nick-translation as well as the release of contaminating [^{32}P] phosphate. The results show that the

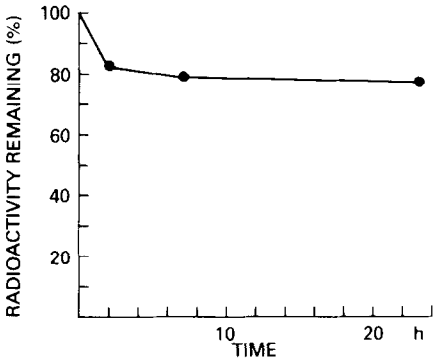

Figure 1: Stability of DNA binding to GSP membrane in alkaline solution. Nick-translated p35-αLA DNA was denatured in TS for 5 min. at room temperature, and 5 µl aliquots containing 0.4 ng DNA (64 000 Čerenkov cpm) were spotted on GSP membrane 1 x 1 cm squares. The squares were immediately placed in scintillation vials containing 2 ml TS and incubated at room temperature for 23 h with gently agitation. At 2, 7, and 23 h of incubation TS was removed from the scintillation vials and the radioactivity remaining on GSP membrane was counted by Čerenkov radiation. Each point is an average of 5 determination.

DNA - GSP complex is stable for at least 23 h when exposed to 0.4 M NaOH. This allows the transfer of DNA from agarose gel to GSP membrane under denaturing alkaline conditions. Thus the denaturation of DNA, which is done prior to the DNA transfer using the Southern procedure, can now be combined with the transfer step using this new procedure.

The procedure was tested with rat liver DNA digested with EcoRl restriction enzyme. The digest was electrophoresed in a 1% agarose gel and transferred to nitrocellulose or GSP membrane (Fig. 2). Parts 1 and 2 of the gel were used to transfer DNA to nitrocellulose and GSP membrane, respectively, by the standard procedure at neutral pH (1). Part 3 of the gel was used to transfer DNA to GSP membrane by elution at alkaline pH with TS. Judged from the elution of λ DNA fragments, the alkaline transfer is slightly more efficient than the standard procedure. Fragments up to 9.3 kb were completely eluted, and 70% - 80% of the 23 kb fragment was eluted during 10 h alkaline transfer from a 3 mm agarose gel (data not shown).

Following alkaline transfer, the GSP membrane was incubated in NS for 15 min., dried at 55° for 15 min., and used for hybridization. The membrane can also be dried at room temperature. Fig. 2B shows the hybridization patterns of the α-lactalbumin gene in DNA transferred by the alkaline

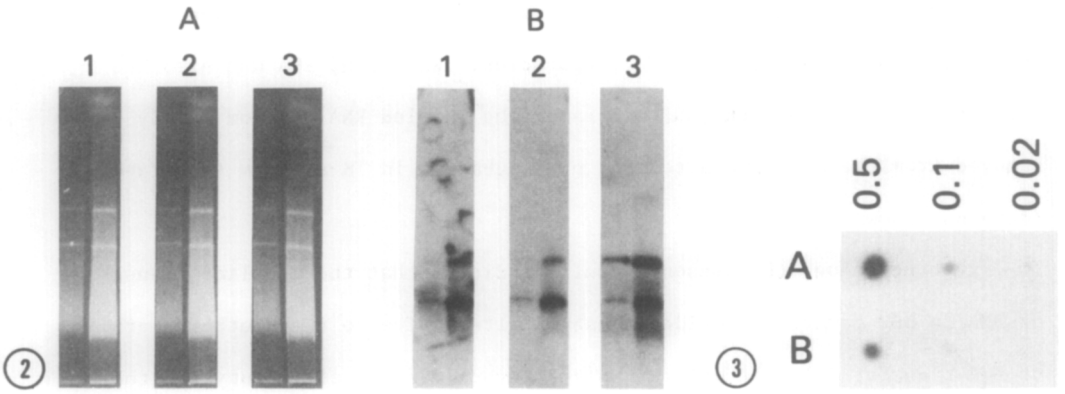

Figure 2: Comparison of hybridization efficiency of DNA transferred from agarose gel by the alkaline and Southern methods. Rat liver DNA digested with EcoR1 (5 µg and 1.25 µg) and Hae III digested λ DNA were eletrophoresed in 1% agarose mini gel (8). After electrophoresis, the gel was stained with ethidium bromide (A) and cut into three parts which were used for DNA transfer by the Southern method with 10 x SSC, to nitrocellulose (part 1), and to GSP membrane (part 2) or by the alkaline method with TS to GSP membrane (part 3). The membranes were hybridized with nick-translated p35-αLA DNA (1.45 x 10^6 Čerenkov cpm), washed and autoradiographed as described in Methods (B).

Figure 3: Comparison of dot-blot hybridization of DNA applied to GSP membrane in TS or SSC. (A) Rat liver DNA was denatured in TS for 5 min. at room temperature and aliquots containing 0.5, 0.1, and 0.02 µg DNA were spotted onto GSP membrane. The membrane was neutralized for 15 min. in NS, dried at 55° for 15 min. and used for hybridization. (B) The same amounts of DNA were spotted on GSP membrane in 10 x SSC. The membrane was dried at 55° for 15 min., incubated in TS for 5 min. and neutralized in NS for 15 min. at room temperature. Hybridization with nick-translated p35-αLA DNA (1.5 x 10^6 Čerenkov cpm) was carried out as described in Methods.

and standard methods. In each case two bands of DNA with an apparent length of 6 kb and 2.5 kb can be seen. A third 0.9 kb DNA band is best visualized after alkaline transfer to the GSP membrane. In this instance, the hybridization signal is weak because only 87 nucleotides hybridize with the cDNA probe (7). Based on the comparison of the intensity of bands, it can be concluded that the DNA eluted from agarose gel by the alkaline solution gives the highest efficiency of hybridization.

The DNA binding at alkaline pH can also be employed for dot blot analysis. Fig. 3 shows the results of hybridization of DNA samples applied to GSP membrane in TS (A) or in 10 x SSC (B). The latter samples were denatured in TS after binding to the membrane. By comparison of the dot intensities it is clear that the hybridization of DNA samples applied to GSP membrane in TS is 2-3 times more efficient than samples applied in SSC.

In addition, the alkaline transfer can be used as an effective step in the removal of RNA from a DNA preparation. When [^{3}H] RNA was adsorbed together with DNA onto the GSP membrane, the labeled RNA was completely removed from the membrane after 5 h of incubation in TS at room temperature (data not shown).

In conclusion, the present results indicate that the alkaline transfer of DNA to GSP membrane provides a useful alternative to the Southern method of DNA transfer (1). It offers a higher hybridization efficiency and the transfer procedure is simpler compared to the standard method. It has an added advantage in that it can be used as a last step in RNA removal from the DNA transferred to GSP membrane. Preliminary results indicate that another plastic membrane, Biodyne A (ICN Pharmaceuticals) can also be used for the alkaline transfer of DNA.

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