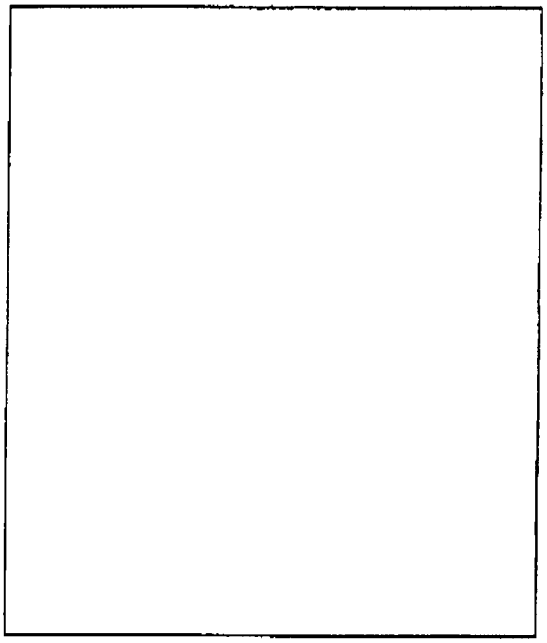
Procedures for Nucleic Acid Transfer to BIOTRANS™ Nylon Membranes



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1. Introduction

1.1

BIOTRANS™ membranes are reinforced nylon 66 membranes for use in DNA, RNA and protein transfers. The physical characteristics of BIOTRANS membranes make them especially useful as a transfer medium. BIOTRANS membranes provide excellent band resolution due to their uniform and carefully controlled pore rating. The membranes are inherently hydrophilic and therefore do not require prewetting prior to use. BIOTRANS membranes are also heat resistant and solvent-resistant and will not shrink, crack or tear thus allowing multiple hybridization cycles to be performed.

1.2 Regular BIOTRANS Membranes (0.2 & 1.2 µm pore sizes)

BIOTRANS is an amphoteric nylon membrane with asurface chemistry comprising 50% amino and 50% carboxyl groups. This provides a hydrophilic membrane with an isoelectric point at pH 6.5. BIOTRANS membranes have a high binding capacity for biomolecules in transfer applications and offer greater sensitivity, improved nucleic acid retention and superior handling characteristics over traditional nitrocellulose.

1.3 BIOTRANS(+) Membranes (0.45 µm pore size)

BIOTRANS(+) has a pore surface populated by a high density of quarternary ammonium groups which make it strongly cationic. The positive surface charge is maintained in the range pH 3 to over pH 10 and promotes strong ionic binding of negatively charged proteins and nucleic acids. BIOTRANS(+) membrane is ideally suited to

new rapid transfer techniques for nucleic acids which provide excellent levels of sensitivity (e.g. Alkaline Transfer Procedure). In addition, the membrane's immediate immobilization characteristics make it suitable for prolonged transfer procedures without the risk of nucleic acid diffusion from the membrane.

1.4 Application Table

Procedure	0.2 µm	1.2 µm	0.45µm
Southern Transfer	+	+	+++
Improved Southern Transfer	-	-	++++
Alkaline Transfer	-	-	+++
Vacuum Tansfer	+	-	+++
Northern Transfer	+	-	+++
Electro-Transfer	+++	-	+++
Nucleic Acid Dot Blots	+	+	+++
Serum Nucleic Acid Dot Blots	-	+++	-
Colony & Plaque Lifts	+	+++	-
Replica Plating	+++	+	-
Western Transfer	+	-	+++

1.5 Membrane Handling

BIOTRANS membranes are naturally hydrophilic and do not require pre-wetting before use. The membranes are mechanically very strong and resistant to tearing or cracking making removal from the agarose gel particularly easy. BIOTRANS membranes should be handled using gloves or forceps to prevent membrane

contamination. Either scissors or a sharp scalpel must be used to cut the membrane.

2. Nucleic Acid Transfer Procedures

2.1 Solutions

2.1.1	Depurination Solution	: 0.25M Hydrochloric Acid
2.1.2	High Salt Denaturation Solution	:0.5M Sodium Hydroxyde 1.5M Sodium Chloride
2.1.3	Alkaline Denaturation Solution (used as transfer buffer)	:0.4M Sodium Hydroxyde
2.1.4	Neutralizing Solution	: 0.5M Tris/HCl pH 7.4 1.5M Sodium Chloride
2.1.5	20 x SSC	: 3M Sodium Chloride 0.3M Sodium Citrate
2.1.6	20 x Electrotransfer Buffer	: 0.06M Citric Acid 0.08M Disodium Hydrogen Phosphate
2.1.7	Digestion Solution (Plaque & Colony Lifts)	: 50mM Tris/HCl pH 7.6, 0.1% (w/v) SDS (Sodium Dodecyl Sulfate) 50mM Sodium Chloride 100 µg/ml Proteinase K
2.1.8	Non-homologous DNA	:10 mg/ml Salmon Sperm DNA Sonicate for 30 minutes then boil for 30 minutes Store at -20°C.

2.1.9	100 x Denhardt's	:2.0% (w/v) "Ficoll" (400,000MW) 2.0% (w/v) Polyvinylpyrolidine (360,00MW) 2.0% (w/v) Bovine Serum Albumin
2.1.10	20 x SSPE	:3.6M Sodium Chloride 0.2M Sodium Phosphate buffer pH 7.7 0.02M EDTA
2.1.11	10% SDS	:10% (w/v) SDS in distilled water
2.1.12	Formamide	: deionized using a mixed bed ion exchange resin

2.2 Nucleic Acid Dot Blots

The application of nucleic acid in solution directly onto a transfer membrane is termed a dot blot. Both BIOTRANS and BIOTRANS(+) membranes may be used for dot blots. It is recommended that both DNA and RNA dot blots be fixed on BIOTRANS membranes by UV irradiation, although baking can also be employed. DNA *loaded* in alkali containig buffers should only be fixed by baking and *not* exposed to UV radiation. With BIOTRANS(+) membranes, DNA dot blots need no fixation providing the DNA has been exposed to alkali on the membrane. RNA dot blots on BIOTRANS(+) membrane should be fixed either by baking or UV irradiation.

2.2.1 DNA dot blots

2.2.1.1 Solubilize the DNA in *either* 6 x SSC by heating to 95°C for 10 minutes then chilling in ice *or* by dissolving in High Salt Denaturing Solution (Solution 2.1.2) for 5 minutes prior to blotting. DNA dissolved in

6 x SSC may be used for dot blots on either BIOTRANS or BIOTRANS(+); DNA dissolved in High Salt Denaturing Solution is recommended for dot blots on BIOTRANS(+) membrane but can also be used for regular BIOTRANS.

2.2.1.2 Spot the dissolved DNA onto the appropriate BIOTRANS membrane in a volume less than or equal to 1.5 µl. If necessary the spot can be dried and multiple pipettings performed until the desired amount of DNA has been applied.

2.2.1.3 For DNA dissolved in High Salt Denaturation Solution spotted on either BIOTRANS or BIOTRANS(+) membrane, proceed directly to 2.2.1.4.

For DNA dissolved in 6 x SSC and spotted onto either BIOTRANS or BIOTRANS(+) membrane, place the membrane on filter paper saturated with High Salt Denaturation Solution (Solution 2.1.2) for 5 minutes. Remove the membrane and place on filter paper saturated with Neutralizing Solution (Solution 2.1.4) for 2 minutes; proceed to step 2.2.1.4.

2.2.1.4 Place the membrane on filter paper saturated with 2 x SSC for 5minutes.

2.2.1.5 For BIOTRANS(+) membrane either air dry (the membrane may be stored overnight at 4°C) or proceed directly with the wet membrane to Section 2.2.1.6.

For BIOTRANS membrane it is recommended that the DNA be fixed on the damp or dry membrane by UV irradiation, although in many applications baking will lead to satisfactory results. For DNA loaded in High Salt Denaturation Solution onto BIOTRANS, baking will give the best result (see Section 2.7 for fixation procedures).

2.2.1.6 Refer to Hybridization Procedure (Section 3).

2.2.2 RNA Dot Blots

NOTE: When working with RNA it is important to keep the equipment and reagent solutions free from RNAse and contamination. Gloves should be worn at all times.

2.2.2.1 Spot the RNA in a solution of 5 x SSPE and 0.1 mg/ml Salmon sperm DNA at a volume of no more than 1.5 µl. If necessary, the spot can be dried and multiple pipettings performed until the desired amount of RNA has been applied.

2.2.2.2 Fix the RNA dot blots by baking or UV irradiation (see Section 2.7 for fixation procedures).

2.2.2.3 Refer to Hybridization Procedure (Section 3).

2.3 Capillary Transfer Procedures for BIOTRANS and BIOTRANS(+) Membranes

2.3.1 Conditions for electrophoresis

Electrophorese in an appropriate agarose gel to separate the DNA fragments. Use a sample buffer containing bromophenol blue.[1]

2.3.2 Depurination Procedures

It is often necessary to reduce the size of the DNA fragments in the gel after electrophoresis to facilitate their transfer to the membrane.[2] There are two approaches to this: the first is to depurinate the DNA by soaking the gel in 0.25M Hydrochloric acid (HCl) followed by alkali treatment; alternatively, the DNA can be fragmented in the gel by UV irradiation in the presence of ethidium bromide. Both methods can give variable results if not closely controlled

Whatever method is employed, it is important to perform optimization experiments to determine treatment times and the effect on subsequent transfer and hybridiza-

tion in the DNA/probe system under investigation. In this example the acid treatment is the preferred method.

2.3.3 Transfer Conditions

For conventional Southern Transfer to BIOTRANS membrane the gel is neutralized after denaturation using Neutralizing Solution 2.1.4 and transferred in 20 x SSC.

The Improved Procedure for Transfer to BIOTRANS(+) membrane omits neutralization after denaturation and the transfer is performed in 20 x SSC.

The Alkaline Transfer Procedure for BIOTRANS(+) membrane uses only 0.4M NaOH as the transfer solution, which results in denaturation during transfer. The gel is not neutralized.

2.3.4

Place two reservoirs containing the appriate transfer solution (Solution 2.1.5: 20 x SSC for conventional and Improved Southern Transfer; Solution 2.1.3: Alkaline Denaturation Solution for Alkaline Transfer) side by side and span with a glass plate.

2.3.5

Place a sheet of dry filter paper from one reservoir, over the glass plate, to the adjacent reservoir. Saturate the filter paper with the appropriate transfer solution. For small DNA fragments (< 10kb) proceed to Section 2.3.7.

2.3.6 For large DNA fragments

After electrophoresis soak the gel in 2 gel volumes of 0.25M HCl with gentle agitation until the bromophenol blue marker dye goes yellow. Place the gel in a further 2 gel volumes of fresh 0.25M HCl for 10 minutes.

2.3.7

For Alkaline Transfer proceed to Section 2.3.8

For Improved and conventional Southern Transfer place the gel in 2 gel volumes of High Salt Denaturing Solution (Solution 2.1.2) until the marker dye turns back to its original color.

For Improved Southern Transfer proceed directly to Section 2.3.8. For conventional Southern Transfer place the gel in 2 gel volumes Neutralzing Solution (Solution 2.1.4) for 10 minutes and then proceed to Section 2.3.8.

2.3.8

Place the electrophoresis gel on the paper/glass support and surround with strips of waterproof material such as plastic film to ensure that the transfer solution goes through the gel and not around it.

2.3.9

Carefully place a piece of the appropriate BIOTRANS membrane on top of and completely covering the gel surface. Roll a clean pipette over the membrane to remove trapped air bubles (BIOTRANS membranes need not be pre-wetted and will wet instantly on contact with the gel).

2.3.10

Place two sheets of filter paper prewetted in transfer solution over the membrane; roll with a clean pipette to remove trapped air bubbles. Cover with a 7 to 8 cm stack of paper towels, a glass plate and a 1 kg weight. Carefully inspect that the paper towels to ensure that they do not touch the gel.

2.3.11

Allow the DNA transfer from the gel to the membrane to proceed for up to 12 hours or overnight.

NOTE: Transfer of partially cleaved DNA fragments may be complete in as little as 2

hours, transfer time is reduced by changing the paper towels once during transfer.

2.3.12 Remove the membrane from the gel surface

For conventional Southern and Improved Transfer do not rinse the membrane. The preferred method of fixation for conventional Southern Transfer to BIOTRANS is UV irradiation. The preferred method of fixation for Improved Transfer to BIOTRANS(+) membrane is baking for 15 minutes at 80°C and then rinsing in 2 x SSC (other methods of fixation can be employed, see Section 2.7 for fixation procedures).

For Alkaline Transfer to BIOTRANS(+) membrane, rinse the membrane in 2 x SSC for 5 minutes and either proceed directly to hybridization with the wet membrane or air dry (the membrane may then be stored overnight at 4°C).

2.3.13

Refer to Hybridization Procedure (Section 3).

2.4 Vacuum Blotting Procedure

2.4.1

Vacuum blotting systems employ a low pressure vacuum to transfer nucleic acids from the electrophoresis gel to the transfer membrane. Transfer can be completed in less than one hour with very high transfer efficiency. Because of the short transfer time there is less diffusion giving sharper, more focussed bands. Both BIOTRANS and BIOTRANS(+) membranes may be used in vacuum blotting; in the following example BIOTRANS(+) has been employed with the Improved High Salt Transfer procedure.

NOTE: This procedure should be carried out in conjunction with the manufacturer's

instructions for the use of a given vacuum blotting apparatus.

2.4.2

Electrophorese in an appropriate agarose gel to separate the DNA fragments. Use a sample buffer containing bromophenol blue.

2.4.3

Assemble the vacuum transfer apparatus ensuring that the mask is cut so that the electrophoresis gel overlaps the window by approximately 5 mm.

2.4.4

Cut the BIOTRANS(+) membrane to the appropriate size. Place the dry sheet of BIOTRANS(+) so that it is exactly positioned on the window of the mask.

2.4.5 Depurination Procedure

Note the comments made on depurination in Section 2.3.2.

Acid depurination may be performed outside the vacuum transfer apparatus using the procedure outlined in Sections 2.3.6 and 2.3.7 for the Improved Southern Transfer procedure with High Salt Denaturation Solution. In our hands depurination and denaturation outside the vacuum transfer apparatus have given the best sensitivity with BIOTRANS(+) in the Improved Procedure.

After denaturation in High Salt Solution (do not neutralize the gel), transfer the gel directly to the BIOTRANS(+) membrane in the vacuum transfer apparatus. Be careful to accurately position the gel and avoid trapping air bubbles between the gel and the membrane. Ensure that the mask is clamped down then turn on the vacuum pump to immobilize the gel. Without waiting for the vacuum to reach working level, pour Depurination Solution (Solution 2.1.1)

onto the center of the gel. Use sufficient solution to completely cover the gel and leave for 4 minutes at a vacuum of 40 cm H_2O.

Remove all the Depurination Solution by aspirating with a pipette and tilting the apparatus to allow any excess fluid to flow from the concave center of the agarose gel.

Pour High Salt Denaturation Solution (Solution 2.1.2) onto the centre of the gel using sufficient solution to completely cover the gel and leave for 3 minutes. Remove all the solution as above.

2.4.6

Do not add neutralization solution to the gel. Pour on 20 x SSC transfer buffer (Solution 2.1.5) to completely cover the gel. Ensure that during the subsequent transfer time (40-120 minutes) the gel remains completely covered with transfer buffer.

2.4.7

Remove all the transfer buffer as above and with the vacuum still on, carefully peel off the gel while leaving the BIOTRANS(+) membrane still in place. Turn off the vacuum and remove the membrane. Do not rinse the membrane. The preferred method of fixing is by baking for 15 minutes at 80°C and then rinsing in 2 x SSC.

2.4.8

Refer to Hybridization Procedure (Section 3).

2.5 Northern Transfer Procedures

The following procedures are suitable for both BIOTRANS 0.45µm and BIOTRANS(+) 0.2µm. BIOTRANS(+) is recommended for rapid transfer techniques such as vacuum transfer and electrotransfer.

NOTE: When working with RNA it is important to keep the equipment and reagent solutions free from RNAse contamination. Gloves should be worn at all times. It is recommended that 1.0% SDS be incorporated in all solutions except the 20 x SSC transfer buffer.

2.5.1 Conditions for electrophoresis

Electrophorese formamide denatured RNA in an appropriate formaldehyde agarose gel.[4]

2.5.2 Transfer Procedures

2.5.2.1 Capilary Transfer of RNA

Place the two resrvoirs containing 20 x SSC transfer buffer side by side and span with a glass plate. Place a sheet of dry filter paper from one reservoir, over the glass plate, to the adjacent reservoir. Saturate the filter paper with 20 x SSC. Follow Sections 2.3.8 to 2.3.11 for conventional transfer.

NOTE: Overnight transfer is recommended for RNA to ensure complete recovery of higher molecular weight RNA from the gel.

After transfer is complete, remove the membrane from the gel surface. Do not rinse the membrane. With BIOTRANS the preferred method of fixing the RNA is by UV irradiation (see Section 2.7 for fixation procedures).

2.5.2.2 Vacuum Transfer of RNA

Refer to the general notes on the Vacuum Blotting Procedure given in Section 2.4.1. Assemble the vacuum apparatus as detailed in Sections 2.4.2 and 2.4.3. After electrophoresis carefully slide the gel from the glass support plate onto the BIOTRANS membrane in the vacuum transfer apparatus. Take care to accurately position the gel and avoid trapping air bubbles be-

tween the gel and the membrane. Ensure that the mask is clamped down and then turn on the vacuum pump to immobilize the gel. Without waiting for the vacuum to reach working level, pour 20 x SSC Transfer Buffer (Solution 2.1.5) onto the center of the gel. Use sufficient solution to completely cover the gel. Stabilize the vacuum at 40 cm H_2O. Ensure that during the subsequent transfer time (40-120 minutes), the gel remains completely covered with transfer buffer.

Remove all the transfer buffer by aspirating with a pipette and tilting the apparatus to allow any excess fluid to flow from the concave center of the agarose gel. With the vacuum still on, carefully peel off the gel while leaving the BIOTRANS membrane still in place. Turn off the vacuum and remove the membrane. Do not rinse the membrane. For BIOTRANS the preferred method of fixing the RNA is by UV irradiation of the damp or dry membrane. For BIOTRANS(+) membrane the RNA may be fixed by baking or UV irradiation (see Section 2.7 for fixation procedures).

2.5.2.3 Electrotransfer of RNA

NOTE: This procedure should be carried out in conjunction with the manufacturer's instructions for the use of a given electrotransfer apparatus.

Cut the BIOTRANS membrane to the exact size of the gel. Place the gel on two sheets of filter paper which have been pre-wetted with 1 x Electrotransfer Buffer (prepared by diluting Solution 2.1.6). Place the dry BIOTRANS membrane onto the gel surface taking care not to trap any air bubbles. Cover the BIOTRANS membrane with two sheets of filter paper pre-wetted with 1 x Electrotransfer Buffer. Place the assembly (filter paper, gel, BIOTRANS, filter paper) between two SCOTCH-BRITE pads and secure between two plastic grids to complete the module.

Fill the electrotransfer apparatus with 1 x Electrotransfer Buffer and insert the completed gel membrane module into the apparatus with the BIOTRANS membrane positioned between the gel and the anode. Where appropriate connect the heat exchanger to the tap water supply or cooling source.

Transfer the RNA from the agarose gel to the BIOTRANS membrane by applying a constant current for 3 hours (e.g. 1 amp at 90V). After transfer is complete remove the membrane from the module. Do not rinse the BIOTRANS membrane. For BIOTRANS membrane the preferred method of fixing the RNA is by UV irradiation of the damp or dry membrane. For BIOTRANS(+) membrane the RNA may be fixed by baking or UV irradiation (see Section 2.7 for fixation procedures).

2.5.2.4 Refer to the hybridization procedure in Section 3.

2.6 Colony Lifts, Plaque Lifts and Replica Plating on BIOTRANS Membranes

It is recommended that 1.2 µm BIOTRANS BE USED for Colony and Plaque lifts and 0.2 µm BIOTRANS for Replica Plating. To avoid contamination, the membranes should be pretreated in the following way:

Boil the membranes in 1 mM EDTA for 5 minutes; thoroughly rinse in double distilled water; interleaf the membranes with chromatic filter paper and wrap in aluminum foil; autoclave for 20 minutes and vacuum dry.

2.6.1 Colony and Plaque Lifts on BIOTRANS

The petri dish, with colonies or clearly visible plaques, should be precooled in a +4°C refrigerator for 30 minutes. Using aseptic technique, carefully lay the BIOTRANS 1.2 µm membrane onto the agar surface. Make location marks by piercing the membrane with a red hot needle.

Carefully remove the membrane disc in a single movement and place on filter paper saturated with High Salt Denaturation Solution (Solution 2.1.2) *with the colony or plaque side uppermost.* Leave for 5 minutes, then remove the membrane disc and place briefly on dry filter paper to remove excess fluid. Neutralize by placing on filter paper saturated in Neutralizing Solution (Solution 2.1.4) for 5 minutes. Fixation may be accomplished by UV irradiation or baking, (see Section 2.7 for fixation procedures) then rinse in 2 x SSC.

2.6.2 Replica Plating on BIOTRANS

Bacterial colonies containing plasmids and cosmids can be directly plated on BIOTRANS 0.2 µm membrane. The membranes must be pretreated and autoclaved as above to avoid contamination.

Using aseptic technique, carefully lay the BIOTRANS membrane disc onto the surface of the agar in the petri dish, ensuring that there are no air bubbles trapped between the membrane and the agar. Prepare an appropriate dilution of the bacterial suspension and directly plate onto the membrane (use 0.5 ml of suspension for 82 mm discs and 1.2 ml for 132 mm discs).

After a suitable incubation time at 37°C, very small colonies (<0.5 mm) should be visible. Remove the membrane disc and place *colony side up* on a piece of sterile

filter paper mounted on a glass plate. Take a fresh sterile BIOTRANS membrane disc and position this exactly over the first, laying it down in a single movement. Take care not to realign the filters after they have made contact, or the colonies will be blurred. Cover with a second piece of sterile filterpaper and a glass plate. Apply strong hand pressure on the upper glass plate, then remove it and the filter paper. Using a red hot needle, make location marks by piercing the two membranes while stuck together.

Carefully separate the membranes and place each respective membrane *colony side up* on a fresh agar petri dish. The original colonies regrow on the first membrane disc; this plate is kept in a refrigerator as the master plate.

The colonies on the second membrane (the replica) usually take slightly longer to grow (4-6 hours). Remove the membrane from the agar plate once very small colonies can clearly be seen. Denature and neutralize the replica membrane using the method given in Section 2.6.1 above. Fixation may be by UV irradiation or baking (see Section 2.7 for fixation procedures).

NOTE: After fixation, proteins are removed by digestion for 6 to 12 hours at 37°C in Digestion Solution (Solution 2.1.7). The yellow colored colony spots will disappear during this treatment. Rinse the membrane disc in 2 x SSC.

2.6.3

Refer to Hybridization Procedure (Section 3).

2.7 Fixation Procedures

The two main fixation methods are oven baking at 80°C and UV irradiation. These

fixation procedures are not required when alkaline transfer procedures for DNA are employed with with BIOTRANS(+) membranes; under these alkaline conditions the negatively charged DNA forms a highly stable complex with the positively charged membrane. Similarly, alkaline dot blots on BIOTRANS(+) do not require any further fixation.

2.7.1 Baking at 80°C

This method is recommended for all DNA transfer techniques, including dot blots with BIOTRANS membranes. A vacuum oven is not required - a conventional convection oven or a laboratory oven with a fan to circulate air is perfectly adequate. After transfer, the membrane should be baked at 80°C for 15 minutes and then rinsed in 2 x SSC before proceeding with hybridization.

2.7.2 UV Irradiation

This method of fixation is preferred for all DNA and RNA transfer techniques to BIOTRANS membranes. In addition UV irradiation is also the recommended method for RNA fixation to BIOTRANS(+) membrane, particularly when reprobing (baking at 80°C has also given good results).

It is important to determine the optimum irradiation time and distance for a given system.

Typical irradiation times are between 30 seconds and 5 minutes; a typical irradiation distance is 15 cm. After transfer the dry or damp membrane is irradiated for the optimum time/distance (the membrane can be air dried prior to irradiation at either room temperature for 60 minutes or at 80°C for 15 minutes). If a transilluminator is employed, the damp or dry membrane is wrapped in UV transparent Saran Wrap to

protect the surface of the membrane when it is laid face down on the transilluminator plate. After irradiation, the membrane is briefly rinsed in 2 x SSC before proceeding with the hybridization.

3.0 Hybridization Procedures[5,6]

This section covers prehybridization and hybridization procedures for Southern and Northern transfers including dot blots and colony and plaque transfers.

3.1 Solutions

3.1.1 Stock DNA Solution

Denature nonhomologous DNA (10 mg/ml Salmon or Herring sperm DNA in water) by sonicating for 30 minutes and then boiling for 30 minutes. Store frozen in small aliquots at -20°C.

3.1.2 Hybridization Solution: Southerns

Denature an aliquot of nonhomologous DNA (Stock Solution 3.1.1) by heating to 100°C for 10 minutes, followed by snap cooling on ice. Add the Hybridization Solution until a final concentration of 100 µg/ml is reached. The DNA Hybridization Solution is composed of: 5 x Denhardt's Solution (2.1.9), 0.5 x SSC (2.1.5) and 0.1% (w/v) SDS (2.1.11).

NOTE: Denhardt's Solution may not be required in some systems; for these applications the use of 0.1% SDS solution has been found adequate.

3.1.3 Hybridization Solution: Northerns

Denature an aliquot of nonhomologous DNA (Stock Solution 3.1.1) by heating to 100°C for 10 minutes followed by snap cooling on ice. Add the RNA Hybridization Solution until a final concentration of 100 µg/ml is reached. The RNA Hybridization

Solution is composed of: 50% deionized formamide (2.1.12), 5 x SSPE (2.1.10), 5 x Denhardt's (2.1.9), 0.1% (w/v) SDS (2.1.11).

3.1.4 Rinse Solution

2 x SSC.

3.1.5 Wash Buffer

2 x SSC, 0.1 % SDS.

NOTE: This should be optimized for a given probe system; 0.1% SDS at 50-65°C is recommended for 100% homologous probe systems.

3.1.6 Preparation of the probe

The exact method will depend on the nature of the probe and the existing laboratory procedure; radiolabelled probes[6,7] can be denatured by heat alone (100°C for 10 minutes in the presence of 100 µg/ml nonhomologous DNA and then snap cooled on ice) or by alkali denaturation by adding 1/10 volume 1M NaOH to the probe in the presence of 100 µg/ml nonhomogolous DNA and heating at 65°C for 10 minutes; neutralize the DNA solution by the addition of 1/10 volume 1M HCl.

NOTE: Biotinylated probes must *not* be subjected to alkaline denaturation since this will cleave the biotin from the probe.

3.2 Prehybridization

3.2.1

Seal the membrane in a plastic bag with 2 to 4 ml of appropriate Hybridization Solution per 100 cm^2 of membrane (Working Solution 3.1.2 for Southerns and 3.1.3 for Northerns).

3.2.2

Incubate the bag at 65°C for Southerns and 42°C for Northerns for 15 minutes to 1 hour.

3.3 Hybridization

3.3.1

Open the bag containing the membrane and remove excess Hybridization Solution. Roll a pipette over the bag in order to remove as much of the solution as possible.

3.3.2

Add the denatured labelled probe to an appropriate volume of fresh Hybridization Solution (Working Solution 3.1.2 for Southerns and 3.1.3 for Northerns). To calculate this volume use 2 ml per 100 cm^2 of membrane.

3.3.3

Add the appropriate Hybridization Solution containing the labelled probe to the membrane and reseal the bag.

3.3.4

For Southern Transfers immediately immerse the bag at 65°C for the duration of hybridization (2 hours to overnight).

For Northern Transfers immerse the bag at 42°C for the duration of hybridization (4 hours to overnight).

3.4 Washing

3.4.1

After hybridization, remove the membrane from the plastic bag; rinse briefly in Rinse Solution (Working Solution 3.1.4).

3.4.2

Place the membrane in a fresh plastic bag.

3.4.3

Add 250 ml of Wash Buffer at room temperature (Working Solution 3.1.5) per 100 cm^2 of membrane.

3.4.4

Agitate the bag vigorously (200 rpm) for 20 minutes at room temperature, then discard the buffer.

3.4.5

Repeat steps 3.4.3 and 3.4.4 two times.

3.4.6

The membrane is now ready for autoradiography (or detection of biotylated probe with an avidin or streptavidin conjugate).

3.5 Rehybridization

Both BIOTRANS and BIOTRANS(+) are easily reprobed. For rehybridization we recommend UV irradiation as the method to fix DNA and RNA to BIOTRANS membrane and RNA to BIOTRANS(+) membrane; DNA should be fixed to BIOTRANS(+) by baking at 80°C. The preferred stripping method is boiling in 0.1 % aqueous SDS.

The procedures given below are intended for radiolabelled probes. Special conditions are required for biotinylated probes[8] which can be succesfully reprobed on nylon membranes. Refer to specific manufacturer's protocols for reprobing using non-radioactive probe systems.

NOTE: It is important that the membrane is not allowed to dry before stripping of the probe.

3.5.1

Pour boiling 0.1 % aqueous SDS solution onto the membrane and shake for a few minutes. Discard the solution and immediately add fresh bollig 0.1 % SDS solution. Allow to cool to 40°C or room temperature.

NOTE: Certain systems may require more rigorous treatment to remove the probe; boiling in 0.1 % SDS may be extended for up to 30 minutes.

3.5.2

If in doubt as to the successful removal of the probe, autoradiograph the membrane at this stage.

3.5.3

Prehybridize the membrane again using the procedure in 3.2 above before reprobing.

3.6 Alternative Stripping Agents

A variety of stripping methods may be employed as an alternative to boiling 0.1 % SDS; these are as follows:

3.6.1 Alkali Stripping (this must not be used for RNA transfers)

A 0.4M NaOH solution at 45°C is added to the wet membrane for 30 minutes. The membranes are washed at room temperature with 0.1 x SSCand 0.1 % (w/v) SDS for 15 minutes followed by 0.2M Tris/HCl pH 7.4 for 15 minutes. Check for probe removal and prehybridize as in Sections 3.5.2 and 3.5.3 respectively.

3.6.2 Low Molarity Tris (RNA Transfers)

Prepare the buffer by making up 4 ml 0.5M EDTA, 2 ml 50 x Denhardt's and 5 ml 1M Tris/HCl pH 7.6 to 1 liter with distilled water. This solution is added to the wet membrane at 68°C for two hours. The membranes are washed at room with 0.1 x SSC and 0.1 % (w/v) SDS for 15 minutes followed by 0.2M Tris/HCl pH 7.4 for 15 minutes. Check for probe removal and prehybridize as in sections 3.5.2 and 3.5.3 respectively.

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