Colony and Plaque Lifts onto Whatman™ Membranes

User Manual

Codes:	10-4011-16	PROTRAN BA85 82 mm 50/PK
	10-4011-24	PROTRAN BA85 132 mm 25/PK
	10-4011-47	PROTRAN BA85 137 mm 25/PK
	10-4011-64	PROTRAN BA85 87 mm 50/PK
	10-4013-16	PROTRAN BA83 82 mm 50/PK
	10-4025-06	PROTRAN BA85 25 mm 100/PK
	10-4025-25	PROTRAN BA85 132 mm 50/PK
	10-4025-48	PROTRAN BA85 137 mm 50/PK
	10-4025-78	PROTRAN BA85 25 mm 1000/PK
	10-4025-79	PROTRAN BA85 82.5 mm 50/PK
	10-4053-16	PROTRAN BA85/20 82 mm 50/PK
	10-4391-16	OPITRAN BAS85 82 mm 50/PK

Page finder

1. Legal

First published April 2014.

http://www.gelifesciences.com

GE Healthcare UK Limited.
Amersham Place, Little Chalfont,
Buckinghamshire, HP7 9NA UK

2. Colony Lifts onto Whatman Membranes

Colony hybridization, originally described by Grunstein and Hogness (3), is a rapid and effective technique for detecting recombinant sequences isolated directly from cells grown on transfer membranes. The sensitivity of the assay depends on factors such as colony size and density on the plate, the number and complexity of the target sequence and the type of probe used, in addition to the properties of the solid phase. Several modifications of the original procedure have been developed to increase the reproducibility and accuracy of the assay (1–6). The solid support used for this technique should be compatible with cell growth (i.e., containing no extractables or other contaminants), and should have sufficiently high surface binding capacity to prevent lateral migration of the cellular material during the transfer and immobilization steps of the procedure. Whatman Protran NC (BA85) Nitrocellulose Membranes are the standard for this technique; Optitran BA-S Supported NC, Nytran™ SuPerCharge and Nytran N Nylon Membranes may be used if reprobing is necessary.

2.1. Membrane Preparation

In general, membranes may be used directly from the package; always wear gloves when handling transfer membranes.

If sterilization of the membrane is desired, place membrane between 2 sheets of Whatman 3MM filter paper and wrap in foil; autoclave on liquid cycle for 15 minutes.

Note: In most cases it is not necessary to prewet the membrane for this procedure. Whatman membranes contain no extractables (such as Triton™ X-100).

2.2. Transfer of Colonies

Mark the membrane and the plate on which the colonies have been grown in order to orient them for later identification of the positive colonies. A Whatman Membrane Marking Pen, a needle dipped in India ink, or a soft lead pencil may be used for this purpose.

2.2.1. Growth of colonies on membrane from liquid culture

Place membrane on an agar plate containing the appropriate media. Remove all air bubbles with a sterile bent glass rod; allow membrane to wet thoroughly. Place 200–400 µl of cells from an exponential phase culture and spread evenly over membrane with a glass rod. Incubate membranes with colonies on an agar plate with the appropriate media at 35°C – 37°C until colonies are 0.1–0.5 mm in diameter. Dilute culture to achieve the optimal density of colonies on the membrane without risk of overlapping or smearing of the signal.

2.2.2. Replication of colonies on transfer membrane

Place 1–2 sheets of Whatman 3MM paper on a firm, level surface. With flat forceps, grasp the edge of the membrane and lift it from the surface of the plate, placing it on top of the filter paper. Place a new membrane on top of the first one (on which the colonies have been grown) and apply even pressure with a glass plate or velvet replication device. Carefully separate the two membranes without smearing the colonies.

2.3. Amplification of Plasmid

To amplify plasmids, incubate the membrane on an agar plate containing 170–250 µg/ml chloramphenicol at 37°C for approximately 12 hours.

2.4. Isolation of DNA

A. Place membrane with colonies facing up on top of 2 pieces of Whatman 3MM filter paper saturated with 0.5 N NaOH, 1.5 M NaCl, for 5 minutes.

B. Neutralize filter by placing it on 2 sheets of Whatman 3MM paper saturated with 1 M Tris-HCl, pH 8.0, for 5 minutes. Then incubate membrane on another set of filter papers saturated with 0.1 M Tris-HCl, pH 7.5/2X SSC for 5 minutes.

C. Wash membrane briefly in 2X SSC.

2.5. Immobilization of DNA

Dry membranes thoroughly by baking at 80°C for 30 minutes. A vacuum oven must be used with nitrocellulose membranes; however, this is not necessary with Nytran Membranes. Nucleic acids may be covalently linked to Nytran Membranes by UV crosslinking. Expose membrane to a UV source of 254 mm. Total exposure should be approximately 120 mJ for damp membranes.

2.6. Washing and Storage

A. Wet membrane in 1X TBS.

B. Using Kimwipes™ wetted in 1X TBS, gently wipe the colony side of the filters to remove colony debris. Transfer filters into 1X TBS + 200 µg/ml proteinase K and incubate for 1 hour at 37°C.

C. Rinse in an excess of 1X TBSs for 5 minutes at room temperature and proceed to hybridization. Alternatively, filters may be air dried and stored dry at 4°C prior to use.

(continue with step 4. page 6)

3. Plaque Lifts onto Whatman Membranes

Plaque Lifts, or the direct transfer and probing of phage onto solid support media, provide a rapid means of detecting target sequences without prior purification of the nucleic acid (8). The sensitivity of the assay depends on several factors, including plaque size and density, the number and complexity of the target sequence, and the type of probe, in addition to the properties of the solid phase. Whatman Protran NC (BA85) Nitrocellulose Membranes are the standard for this technique; Optitran BA-S Supported NC, Nytran SuPerCharge and Nytran N Nylon Membranes may be used if reprobing is necessary.

3.1. Membrane Preparation

Membranes may be used from the package; sterilization is not necessary. If sterilization is desired, place membrane between two sheets of GB003 paper, wrap in foil and autoclave on the liquid cycle for 15 minutes.

3.2. Cell Infection

Plate cells with phage in soft agarose with the appropriate medium (hard agar may stick to the membrane). Plaques should not be confluent. Incubate plates at 37°C until plaques are approximately 0.2 mm in diameter, then place the plate at 4°C for at least 15 minutes to set the upper layer of agarose.

3.3. Transfer of Phage

Place membrane on the surface of the infected plate so that the entire membrane is in contact with the plate. Mark the plate and the membrane to orient the replicate. Allow phage to transfer for approximately 5 minutes. If the same plate is used for additional transfers, the exposure time should be increased.

Note: Washing filter briefly in 5X SSC, then blotting on filter paper, may aid transfer.

Remove air bubbles from beneath the membrane. Do not move the membrane during transfer or smearing of the phage may result.

3.4. Isolation of Viral DNA

A. Place membrane plaques facing up on one sheet of GB003 paper saturated with 0.5 N NaOH/1.5 M NaCl for 5 minutes at room temperature.

B. Transfer membranes to one sheet of GB003 paper saturated with 1 M Tris-HCl, pH 8.0, for 5 minutes.

C. Place filters on a third filter paper sheet saturated with 0.1 M Tris-HCl, pH 7.5, 2X SSC, for 5 minutes.

3.5. Immobilization of DNA

Dry membrane by baking at 80°C for approximately 1 hour. A vacuum oven must be used with nitrocellulose filters; this is not necessary for Nytran SuPerCharge, however. Nucleic acids may be immobilized by UV cross-linking. Expose membrane to a UV source of 254 nm. Total exposure should be approximately 120 mJ for damp membranes.

3.6. Washing and Storage

Wet filters in 1x TBS. Using soft cellulose cloth, such as Kimwipes wetted in 1x TBS, gently wipe the plaque side of the filters to remove cellular debris. Transfer filters into 1x TBS + 200 µg/ml proteinase K, and incubate for 1 hour at 37°C. Wiping filters and treating with proteinase K removes the cellular debris and eliminates background. It is important to perform these steps after baking the membrane; otherwise "streaking" of plaques may be observed upon detection. Rinse in an excess of 1x TBS for 5 minutes at room temperature and proceed to hybridization. Alternatively, filters may be air-dried and stored at this point at –18°C prior to use.

4. Hybridization of DNA

Carry out all steps with approximately 5–8 ml of solution per filter, agitating throughout the procedure. Add an additional 2 ml of solution for added filters.

A. Prehybridization

Prehybridize for 1 hour 42°C in 50% deionized formamide, 5X SSPE, 1–3X Denhardt's solution, 0.1–0.5% SDS, 100–200 µg/ml fragmented denatured low molecular weight DNA.

B. Hybridization

Probe preparation: denature probe by adding 0.1 vol of 1 N NaOH for 5 minutes. (Alternately, denature probe by heating for 5 minutes at 100°C, then placing immediately on ice. This method may result in some renaturation of the probe.) Add a small amount of the prehybridization buffer to the probe.

Add approximately 1–5X 10^6 cpm/ml of probe, or approximately 5–20 ng/ml.

Add probe of the hybridization solution and incubate for 12–20 hours at 42–45°C. (Incubate at 60–65°C if formamide is not used).

Note: Other blocking agents as heparin (9), and gelatin (10) may be used instead of Denhardt's reagent. The hybridization conditions used with colony and plaque lifts may be slightly different than those used with other transfer procedures. A wide range of conditions for hybridizations with colony/plaque lifts has been described.

C. Washes

Wash filters 2–3 times in 2X SSC/0.2% at 65°C for 30 minutes each. Additional washes in 1X SSC/0.1% SDS may be added to eliminate nonspecific binding if necessary. For a more stringent probe removal, wash in 0.1 X SSPE/0.1% SDS for 30 minutes to 1 hour at 65°C. Lower washing temperatures may be used.

5. Alternative Probes

5.1. Oligonucleotide Probes

A. Hybridize at the T_H of the hybridization buffer without formamide.

B. Carry out washes as direct at room temperature and perform a final 5 minute wash at the T_H of the hybrid.

Note: Oligonucleotide probes should not bind to the membrane under the conditions given. Denhardt's reagent and the heterologous DNA may be eliminated from the buffer if background binding is not problematic. 0.5% Non idet P40 may be used instead of SDS.

The hybridization temperature (T_H) for oligonucleotide probes (14–20 bp) is 5° less than the temperature at which 50% of the hybrids formed between the oligonucleotides and the bound DNA dissociates (T_m), determined by the length and base composition of the oligomer (11): $T_H = T_m$ - 5°C = 2°C (#A:T bp) + 4°C (#G:C bp) - 5°C.

5.2. RNA Probes

A. Hybridize as indicated for DNA probes.

B. Background binding of the probe may be reduced by first washing the filer in 2X SSC (immediately after hybridization), then incubating the filter at 37°C for 15 minutes in 2X SSC/1 µg/ml RNase A.

C. Wash membrane as indicated for DNA probes. The highest washing temperature (65°C) may be necessary when Nytran Membranes are used.

Note: Care should be taken to prevent RNase contamination of all materials and reagents used with these probes (12). SDS will act as RNase inhibitor. Blocking agents such as nonfat dry milk solutions that may contain RNase should not be used.

6. Detection

When using isotopic probes, wrap membrane in plastic food wrap while still slightly damp, place in an imaging cassette with X-ray film and an intensifying screen. Place at -70°C for 1–10 days.
For colorimetric detection of streptavidin alkaline phosphatase conjugates, prepare BCIP/NBT substrate and incubate filters at room temperature until sufficient color development has occurred (20 minutes to overnight). Stop the reaction by washing a number of times in deionized water. Alternatively, alkaline phosphatase conjugates may be detected via chemiluminescence or Horse Radish Peroxidase (HRP). Follow manufacturer's instructions for the specific substrate used.

6.1. Probe Removal

Probe will remain on filter permanently if the membrane is dried; store lift only after probe has been removed.

6.2. Removal of DNA Probes

Heat 0.1X SSPEE or SSC/0.1% SDS to boiling, remove from heat and add membrane for 15 minutes; repeat. Expose membrane to film to check for complete removal of the probe. Dry and store.

6.3. Removal of RNA Probes

Incubate lift in 50% formamide/2X SSC at 42°C. Increase temperature up to 65°C until probe is completely removed. The actual incubation temperature necessary will depend on the thermal stability of the hybrid, based on the base composition and the length of the probe. These probes are more difficult to remove from nylon membranes.

7. Immunoscreening of Expression Libraries

In addition to probing with DNA probes, plaques can also be screened for expressed proteins (12, 13). The recommended protocol involves use of IPTG to increase the amount of recombinant protein by inducing expression from the lac promoter which directs to expression.

7.1. Procedure

Many cloning projects involve the expression of cloned genes from a wide variety of expression vectors. While vectors, hosts and induction conditions may be very different in each cloning system, the treatment of colony or plaque lifts from such libraries is very similar (12, 13). Whatman Protran BA85 nitrocellulose membranes are the standard for this technique. Opitran BA-S 85 reinforced nitrocellulose may be advantageous if a higher mechanical strength is required.

A. Mark the membranes (sterilized by autoclaving at 121°C, 1 bar, 15 minutes if necessary) and plates for orientation. Induce expression, cell lysis, etc, as appropriate for your expression system (e.g. 12, 13) Immediately after the lift wash the membranes in TBS-Tween (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05 % Tween™ 20) to remove any remnants of agar.

B. Block the membranes in TBS-Tween with 1% casein (or other blocking agent) for 30 minutes. Use 7.5 ml for each 82 mm membrane, or 15 ml for each 132 mm membrane.

C. Incubate membranes in TBS-Tween plus the primary antibody at a concentration of 0.5–10 µg/ml (or a dilution used in western blot detection) for 2–24 hours at 4°C.

D. Wash the membranes 3 times, 5–10 minutes each TBS-Tween.

E. Incubate membranes in the appropriate secondary conjugate for 30 minutes at room temperature.

F. Wash membranes as in step D.

G. Detect as specified for colony and plaque lifts (Section 6, Page 8)

8. Screening of Yeast Libraries for ß-galactosidase Activity (15)

The recommended membrane for this purpose is Opitran BA-S due to its high mechanical stability.

8.1. Procedure

A. Mark membrane as decribed in Section 2.2 for proper orientation and identification and autoclave required number of Opitran BA-S 85 for 15 minutes at 121°C at 1 bar.

B. Place sterile membrane without smearing for 0.5–1 minute on the yeast colonies.

C. Using blunt-ended forceps, put the membrane colony-side up for 5 seconds (no more than 10 seconds) onto a 1–2 cm high layer of liquid nitrogen.

D. For color development put the membrane colony-side up into a petri-dish containing a piece of 3MM soaked with Z-buffer (60 mM Na_2HPO_4,, 40 nM Na_2PO_4 10 mM KCl, 1 mM $MgS0_4$, 40 mM b-mercaptoethanol, pH 7.0. containing 1 mg/ml X-GAL).

9. References

1. Grunstein, M and J. Wallis. in *Methods of Enzymology*. **Vol. 68** (Wu. R. ed.). Academic Press, New York pp. 379–389 (1979).
2. Hanahan, D and M. Meselson in *Methods of Enzymology*. **Vol. 100** (Wu. R. ed.). Academic Press, New York, pp. 333–389. (1983).
3. Grunstein, M and D.S Hogness *Proc. Natl. Acad. Sci. USA*. **Vol. 72.** Academic Press, New York, pp. 3961–3965 (1975).
4. Maas, R *Plasmid.* 10, pp. 296–298 (1983).
5. Maas. R *Colony Hybridization Method.* New York University, New York, NY. Pat, No 4,533,628. (Aug 6 1985).
6. Haas, M.J. and D.J. Fleming. *Nucl. Acids Res.* 14: p. 3976 (1986).
7. Gergen, J.P., R.H. Stern and P.C. Wensink. *Nucl Acids Res.* 7: pp. 2115–2134. (1979).
8. Benton, W.D and R.W. Davis. *Science.* 196: pp. 180–182. (1977).
9. Amasino, R.M. *Anal. Biochem.* 152: pp. 304–307 (1986).
10. Renart, J., J. Reiser and G.R. Stark. *Proc. Natl. Acad. Sci. USA*. 76, 3116–3120. (1979).
11. Suggs, S.V., T. Hirose, T. Miyake, E.H. Kawashime, M.J. Johnson, K. Itakura and R.B. Wallace. *ICN-UCLA Symp. Dev. Biol. Using Purified Genes*. (D.D. Brown ed). Academic Press, New York. 23: pp. 683–693 (1981).
12. Sambrook, J, E.F. Fritsch and T. Maniatis. *Molecular Cloning: A Laboratory Manual*, **Second Edition,** Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1989).
13. St. John, P. in *Current Protocols in Molecular Biology*. (Asubel, *et al* eds). Greene Publishing Assoc, and Whiley-Interscience. 6.71–6.74 (1990).
14. Dubitsky, A and J. Defilia *BioTechniques*. 19 (2): 210–212 (1995).
15. Breeden, L. and K. Nasmyth. *Cold Spring Harbor Symp. Quant. Biol.* L: 643–650 (1985).

10. Related products

Article number	Catalogue Number	Item Description
28414963	10-4011-16	PROTRAN BA85 82 MM 50/PK
28414968	10-4011-24	PROTRAN BA85 132 MM 25/PK
28414973	10-4011-47	PROTRAN BA85 137 MM 25/PK
28414975	10-4011-64	PROTRAN BA85 87 MM 50/PK
28415007	10-4013-16	PROTRAN BA83 82 MM 50/PK
28415097	10-4025-06	PROTRAN BA85 25 MM 100/PK
28415099	10-4025-25	PROTRAN BA85 132 MM 50/PK
28415102	10-4025-48	PROTRAN BA85 137 MM 50/PK
28415104	10-4025-78	PROTRAN BA85 25 MM 1000/PK
28415106	10-4025-79	PROTRAN BA85 82.5 MM 50/PK
28415159	10-4053-16	PROTRAN BA85/20 82 MM 50/PK
28415478	10-4391-16	OPTITRAN BAS85 82 MM 50/PK

Page intentionally left blank

GE Healthcare offices:

GE Healthcare Bio-Sciences AB
Björkgatan 30, 751 84 Uppsala,
Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg,
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327,
Piscataway, NJ 08855-1327,
USA

GE Healthcare Japan Corporation
Sanken Bldg. 3-25-1, Hyakunincho,
Shinjuku-ku, Tokyo 169-0073,
Japan

For your local office contact information, visit
www.gelifesciences.com/contact

GE Healthcare UK Limited
Amersham Place
Little Chalfont, Buckinghamshire,
HP7 9NA, UK

http://www.gelifesciences.com

imagination at work

29-1042-16 AA 04-2014