Protocol

Southern Blotting. Dot Blotting

This protocol is for the Southern Blotting. Dot Blotting

Required solutions

1. Denaturation solution: 1.5 M NaCl, 0.5 M NaOH.
2. Neutralization solution: 1.5 M NaCl, 0.5 M Tris-HCl (pH 7.2), 1 mM EDTA.
3. 20X SSC, pH 7.0 (blotting buffer): 3 M NaCl, 0.3 M sodium citrate.
4. 100X Denhardt\'s solution: 2% (w/v) BSA, 2% (w/v) Ficoll™, 2% (w/v) PVP (polyvinylpyrrolidone).
5. Pre-hybridization solution: 6X SSC, 5X Denhardt's solution, 50% formamide, 0.5% SDS.

Electrophoresis

Load genomic DNA probes along with the marker (e.g. DNA Markers for Genomic DNA analysis) on 0.7% agarose gel (20 cm length). Run for 18 hours at 3 V/cm in 1X TAE buffer.

Southern Blotting

1. Rinse the gel in deionized water, add Denaturation solution and shake for 30 min at room temperature. Rinse the gel in deionized water and add Neutralization solution. Shake for 15 min at room temperature. Repeat neutralization procedure.
2. Fill the glass dish with 20X SSC blotting buffer. Make a platform and cover it with a sheet of Whatman™ 3 MM filter paper, saturated with the blotting buffer (see picture below).
3. Place the gel upside down on the filter and avoid trapping air bubbles beneath it.
4. Cut a sheet of SensiBlot™ Plus Nylon Membrane to match the size of the gel and place it on the top of the gel. Avoid trapping air bubbles beneath the membrane.
5. Cut 2-3 sheets of Whatman™ 3 MM filter paper to the size, wet with blotting buffer and place on the top of the membrane.
6. Place a stack of absorbent paper towels on top of the 3 MM paper, place a glass plate on the top of the paper towels and put a 0.5 kg weight on the top.
7. Allow upward capillary transfer of DNA at room temperature for 18 hours.
8. Wash the membrane in 2X SSC buffer to remove any residual agarose, dry at room temperature and fix for 2 min under UV-light.

Dot Blotting

1. Prepare several dilutions of the labeled probe (from 1 ng/µl to 10 fg/µl) and spot 1 µl of each dilution onto a nylon membrane strip.
2. Air-dry the spotted membrane at room temperature for 30-45 minutes or at 80°C for 10 minutes.
3. Place the membrane on a UV trans-illuminator (spotted side down) and cross link the probe to the membrane for 1-5 minutes.

Note
The spotted membrane can be stored at 4°C or at room temperature in a plastic bag until needed.

Generation of Labeled Probes

Two labeled probes are prepared using Biotin DecaLabel™ DNA Labeling Kit, DecaLabel™ DNA Labeling Kit or using protocol for random-primed labeling.

1. Hybridization probe for the genomic DNA (test probe).
2. Hybridization probe for visualization of DNA Marker (e.g. DNA Markers for Genomic DNA analysis). 50 ng of marker is sufficient for generation of radioactively labeled probe for 3-5 hybridization reactions.

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Hybridization

1. Prepare 30 ml of the pre-hybridization solution.
2. Denature sonicated salmon sperm DNA solution (10 mg/ml) by heating at 100°C for 5 min. Chill on ice and add to the pre-hybridization solution to a final concentration of 50-100 µg/ml.
3. Place the membrane into the hybridization container, add pre-hybridization solution with the denatured salmon sperm DNA (0.2 ml/cm^2 of membrane) and pre-hybridize for 2 hours at 42°C with shaking.
4. Prepare the hybridization solution:
   - mix the two prepared probes: labeled probe for the DNA marker and probe for genomic DNA. Denature by heating at 100°C for 5 min and chill immediately on ice.
5. Add the following amounts of the probe mixture to the pre-hybridization solution:
   - to 10 ng/ml (⅕ of probe mix) if specific activity is 108 dpm/µg,
   - to 2 ng/ml (1/25 of probe mix) if specific activity is 109 dpm/µg,
   - to 25-100 ng/ml if non-radioactively labeled probes.
6. Discard the pre-hybridization solution (from step 3) and add the prepared hybridization solution to the hybridization bag (60 µl/cm^2). Incubate for at least 12 hours at 42°C.
7. Carry out the following washes of the membrane:
   - twice in 2X SSC + 0.1% SDS for 10 min at room temperature,
   - twice in 0.1X SSC + 0.1% SDS for 10 min at 65°C (for high stringency).
8. Dry the membrane using sheets of Whatman™ 3 MM paper.

Autoradiography

Wrap the dried membrane with Saran Wrap™ and expose to a phosphoimager or a film with an intensifying screen.

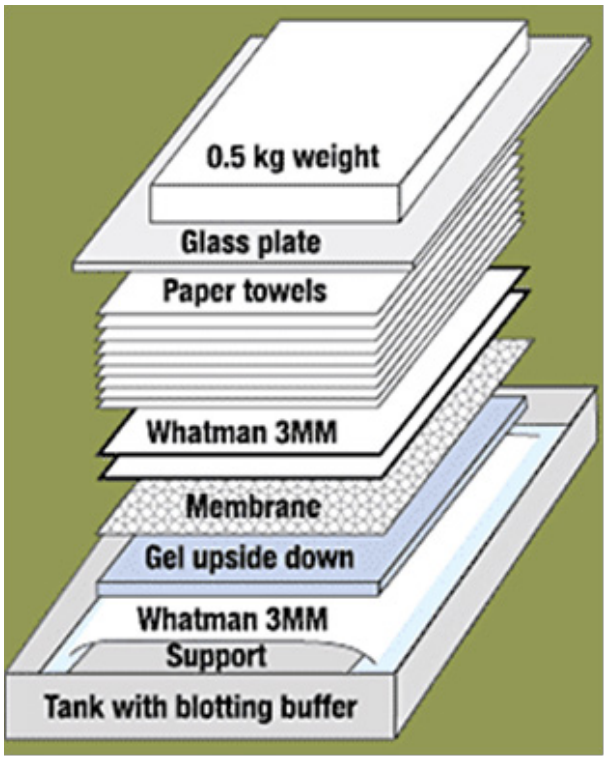

Figure. Upward capillary transfer of DNA from agarose gels.

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