Southern Blotting

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Abstract

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Protocol

Step 1.
Digest 10 µg of genomic DNA overnight with desired restriction enzymes for each Southern blot probe. (10 µg per 1 well on gel)

Step 2.
After digest add 6x loading dye, incubate 3-5 min at 55-65°C

Step 3.
Run 0.75 % agarose gel (70 V **without EtHBr**), approximately 3-3.5 hours while bromophenol reach the end of the gel

Step 4.
Stain the gel later because EtHBr influences DNA migration, 15-20min. (0.5l + 20ul-30ul EtBr, the solution should slightly change the colour). If the gel is big put it on the plastic bag or food film – it will be easier to take it out without damage

Step 5.
Document gel with fluorescent marker. Place ruler alongside the gel in order to estimate distance of DNA band migration directly from photo.

Step 6.
Cut part with the gel pockets and unused areas of the gel, trim 1 angle

Transfer

Step 7.
Presoak gel in 0.125 M HCl (not too old) for max 10 min at RT (depurination for more efficient transfer) // pH 1; Bromphenol has to turn yellow-grey; Orange G stays the same

Transfer

Step 8.
Rinse 3 times in dH2O, 5 min, change dH2O 3 times

Transfer

Step 9.

Denature for 30 min at RT
// pH 14; Orange G turns red-brown; gel shrinks

Transfer

Step 10.

Rinse 3 times in dH2O, 5 min, change dH2O 3 times

Transfer

Step 11.

Neutralize for 30 min at RT,
// pH 7-8, Orange G turns yellow again

Transfer

Step 12.

Rinse 3 times in dH2O, 5 min, change dH2O 3 times

Transfer

Step 13.

Keep gel in 20x SSC

Step 14.

Cut Zeta-Probe blotting membrane of exactly the same size as the gel. Trim 1 angle of the membrane as in the gel and mark 3 parts of the membrane with 2 small cuts, put membrane in dH2O // one side at a time and transfer wet membrane into 20x SSC for at least 5 min

Step 15.

repare Whatman filter paper: 1filter paper should be +2 cm for each size to douse it into the 20xSSC buffer; 2 1filter paper should have the same size as glass. Pre-wet Whatman filter paper in 20x SSC.

Blotting

Step 16.

Invert the gel, DNA part should be close to the membrane

Step 17.

Make sure there is no bubbles between the membrane and the gel

Step 18.

Cover the first layer filter paper with food film to isolate it from napkins. Make sure there is no short-cut between the filter paper and the

Step 19.

Step 20.

Step 21.
Cross-link DNA using Auto crosslink option // membrane with DNA should face upward

Step 22.

Step 23.

Radioactive DNA Labeling (Thermo Scientific DecaLabel DNA Labeling Kit, #K0622)

Step 24.
Radioactive decay calculator (Phosphorus32-alpha)
1) Add the following components into 1.5 ml microcentrifuge tube:

DNA template	100 ng
Decanucleotide in 5X Reaction Buffer	10 µl
Water, nuclease-free	X µl (depends on V of[α- 32 P]-dATP)

Vortex the tube and spin down in a microcentrifuge for 3-5 s. Incubate the tube in a boiling water bath for 5-10 min and cool on ice. Spin down briefly.
2) Add the following components to the same tube:

Mix A	3 µl
[α-32 P]-dATP (minimum 16.5 µCi per reaction)	X µl
Klenow fragment, Exo – (5 u)	1 µl

Total V of reaction should be 50 µl. Vortex the tube and spin down briefly. Incubate for 5 min at 37°C.
3) Add 4 µl of dNTP Mix and incubate for 5 min at 37°C.
4) Purify probe using the Illustra G-50 column

Probe purification (Illustra G-50 columns, GE Healthcare, #28-9034-08)

Step 25.

Prehybridization (from Zeta-Probe membrane protocol)

Step 26.

1) Preheat Ultrahyb at 60°C and keep it until prehyb step.
2) Put blotted Zeta-Probe membrane inside a 50 ml falcon tube DNA side facing inwards.
2) Pipet 6-10 ml of hybridization solution inside the falcon tube:
0.5 M Na_2HPO_4, pH 7.2
7% (w/v) SDS

Put 50 ml falcon tube to big glass hybridization tube.
3) Incubate in hybridization oven at 60°C for 1 hour.

Hybridization (from Zeta-Probe membrane protocol)

Step 27.

1) Add the denatured probe. Hybridize overnight (16 hours) at 60°C with agitation.
2) Carefully pour the hybridization solution with labeled probe to 15 ml falcon tube. It can be reused during 1 week. Keep it at -20°C in the radioactive material container.
Note: At no stage, before washing should the membranes be permitted to dry

Washing (from Zeta-Probe membrane protocol)

Step 28.

1) Wash the membrane at 60°C: rinse 1 time (in 5 ml) and wash 2 times (in 10 ml) for 10 min each, in the following:
1x SSC
0.1% (w/v) SDS
The first wash should be conducted at room temperature; the second and third washes should be conducted in the hybridization oven.
2) Wash the membrane at 60°C, 2 times for 30 min each, in the following:
0.1x SSC
0.1% (w/v) SDS
These washes should be conducted in the hybridization oven.
3) After washing, the blotted membranes are ready for autoradiography. Put moist membrane on filter paper wetted in MQ and put them in a sealable plastic bag.
4) Keep the screen under the light for 20-30 min.
Start exposure in Fuji Imaging phosphor screen (Art. No. 28956475, BAS-MS 2025, 20x25 cm).
Scan phosphor screen in 4 hours in P-imager (Amersham, GE Healthcare, Typhoon 9400),.