SOP: Genomic Southern Blot Analysis

By

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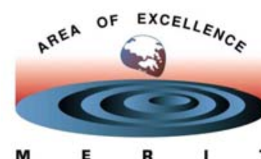

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PART A: Restriction digestion and gel electrophoresis

1. Digest 5 µg genomic DNA overnight (performed in an air incubator or thermal cycler) with ca. 50 U (or max. 2.5 µl) appropriate restriction enzymes. If possible, chose the enzymes which do not cut inside your probe. Examples of cheap and efficient 6-bp cutters are *Bam*HI, *Eco*RI, *Hind*III and *Sac*I.

10× Reaction buffer	2.5 µl
Genomic DNA in water	5 µg
Restriction enzyme	50 U (or max. 2.5 µl)
0.1 M spermidine (optional)	1 µl
DNase-free distilled water	make to 25 µl

Comments:
(1) Spermidine (final conc. = 4 mM) can be added to enhance the digestion. However, it should be noted that (1) concentrations > 4 mM will inhibit the reaction, and (2) DNA cannot be ethanol precipitated after exposure to spermidine.
(2) DNA quality critically determines the degree of complete digestion. Qiagen Genomic-tip 100/G (Cat# 13343) can be used to extract clean and high-yield genomic DNA from animal tissues. Qiagen DNeasy Tissue Kit (Cat# 69504) is spin column-based, however, DNA is often extracted in low yield with this kit.
(3) Do not dissolve genomic DNA in TE buffer. The EDTA in TE buffer can inhibit restriction enzyme activities.

2. The next morning run 1/10 (2.5 µl) of the reaction on a 0.8% agarose minigel to check digestion. Completely digested DNA should appear as an even smear (Fig. 1). Often there are brighter bands within the smear, these are repetitious sequences, not artefacts.

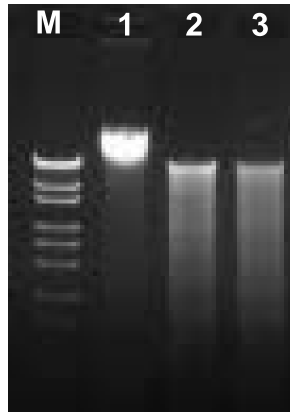

Figure 1. Digestion of genomic DNA. 1. Undigested genomic DNA, 2. Fairly digested genomic DNA, 3. Completely digested genomic DNA

3. If partial digestion has occurred, add another dose of enzyme and increase the reaction to 30 µl with fresh buffer and water.

O/N reaction	22.5 µl (vortex for 20 sec)
10× Reaction buffer	0.75 µl
Restriction enzyme	50 U (or max. 2.5 µl)
DNase-free distilled water	make to 30 µl

Incubate for 6 more hours.

4. Mix the reaction thoroughly with appropriate volume of DNA loading dye (e.g. 6× MassRuler Loading Dye Solution, Fermentus Cat# R0621). Load the entire reaction in parallel with a high-ranged DNA ladder (e.g. MassRuler DNA Ladder Mix, ready-to-use, Fermentas Cat# SM0403) onto a 0.8% 1× TAE buffered agarose gel (bubble-free, ethidium bromide-free).

5. Run the gel overnight at 30 V.

6. The following morning, stain the gel in ethidium bromide (0.5 µg ml^{-1}) for ca. 30 min. View the gel under UV. The DNA should have run 2/3 to 3/4 of the length of the gel.

7. Photograph the gel with a fluorescent ruler alongside the marker lane.

8. Trim gel by cutting off excess agaroase (un-used lanes).

9. Rinse with distilled water and proceed to PART B.

PART B: Gel treatment

Perform the procedures shown in the following flowchart:

Acid treatment (depurination)
0.125 M HCl (~500 ml)
5 min, RT, gentle shaking

Rinse with 3D-H_2O

Alkali treatment (denaturation)
1.5 M NaCl, 0.5 M NaOH
30–45 min, RT, gentle shaking

Rinse with 3D-H_2O

Neutralization
1.5 M NaCl, 0.5 M Tris-HCl, pH 7.2
15 min, RT, gentle shaking

Rinse with 3D-H_2O

PART C: Capillary Blotting

1. A typical setup for capillary blotting is described as in Figure 2. Note: Always use gloves when handling the nylon membrane.

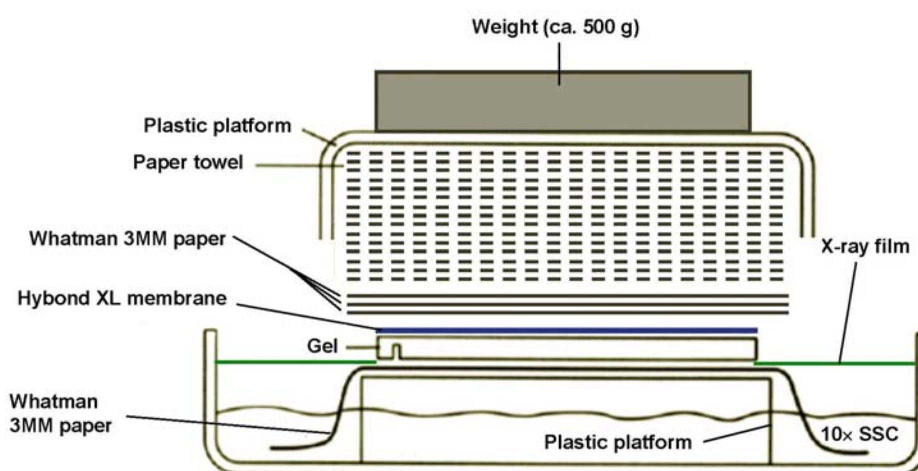

Figure 2. Setup for capillary blotting

2. Fill the reservoir with an appropriate volume (~500 ml) of 10× SSC (transfer solution).

3. Place the gel platform at the reservoir center, cover the platform surface with a Whatman 3MM paper (prewetted with 10× SSC), and dip the ends into the buffer.

4. Measure the gel size and cut a piece of Hybond XL membrane (Amersham) to the exact size (or a bit larger than the gel). Wet the membrane briefly in distilled water and then 10× SSC for 5 min.

5. Invert the neutralized gel and place it on the buffer-saturated 3MM paper on the level platform. Remove any air bubbles trapped.

6. Surround the gel with X-ray film strips to prevent by-passing of the transfer solution and buffer evaporation.

7. Place the wetted membrane on top of the gel. Gently roll a 10-ml glass pipette over the gel surface and squeeze the bubbles out to the sides of the gel.

8. Mark the well positions on membrane with pencil. Cut off the wells with a scalpel at this point.

9. Snip off the top right-hand corner of the membrane to mark orientation.

10. Cut 3 sheets of 3MM papers to the same size as the membrane. Wet them briefly with 10× SSC and place them on top of the membrane. Avoid bubble formation.

11. Stack paper towel (of the same size as the membrane) to a thickness of 5 cm on top of the 3MM papers. Put another plastic platform and a weight of ca. 500 g on the top.

12. Leave the setup at RT overnight.

13. Disassemble the blot. Carefully peel off the membrane with forceps and place in 2× SSC to remove any agarose debris.

14. View the gel under UV to check that most of the DNA has been transferred.

15. Pat dry the membrane between 2 Whatman 3MM paper sheets. Fix the DNA by drying the membrane in air for 30 min and then baking at 80°C for 2 h. Alternatively, UV-crosslink the DNA with an appropriate irradiation program.

16. This blot can now be stored by sealing either in a plastic bag or Saran wrap at RT until required.

PART D: Probe labeling and purification

(1) **Recommended kit for ^{32}P-based probe labeling:**
Ready-to-Go DNA labeling beads (-dCTP), GE
(http://www.jp.amershambiosciences.com/tech_support/manual/pdf/nadna/74003992aa.pdf)

(2) **Recommended kit for probe cleanup:**
QIAquick Nucleotide Removal Kit, Qiagen
(http://www1.qiagen.com/literature/handbooks/PDF/DNACleanupAndConcentration/QQ_Spin/1021422_HBQQSpin_072002WW.pdf)
Protocol printed in pages 21–22.

(3) **Specific activity measurement:**
After purification (eluted in 50 µl Buffer EB), add 5 µl of the radiolablled probe to 5 ml of Ready Safe Liquid Scintillation Cocktail (Beckman) in a LS vial. Count cpm with a LS6000 Scintillation Counter (Beckman).

PART E: Hybridization and autoradiography

1. Warm the ExpressHyb Solution (Clontech Cat# 636831 http://www.clontech.com/clontech/techinfo/manuals/PDF/PT1190-1.pdf) at 60°C and stir well to completely dissolve any precipitate. Comment: For daily storage, the solution can be placed at 30°C in an air incubator.

2. Prehybridize the membrane with 10–20 ml ExpressHyb Solution at 60°C for 30 min to 1 h in a hybridization column rotating in a hybridization oven.

3. Denature the radioactively labeled DNA probe (1×10^6 cpm per ml hybridization buffer) at 95–100°C for 2–5 min with a thermal cycler installed in radioisotope room. Then chill quickly on ice.

4. Replace the ExpressHyb Solution with the fresh solution containing the radiolabeled DNA probe. Remove all air bubbles from the container, and make sure that the ExpressHyb Solution is evenly distributed over the entire blot.

5. Incubate with continuous rolling at 60°C for 1 h.

6. Rinse the blot thrice (30–40 min each) in Wash Solution 1 (2× SSC, 0.05% SDS) at RT with continuous shaking.

7. Wash the blot twice (20 min each) in Wash Solution 2 (0.1× SSC, 0.1% SDS) at 50°C with continuous shaking. (Replace with 1× SSC, 0.1% SDS if a heterlogous probe is used.)

8. Remove the blot with forceps and shake off excess wash solution.
Note: Do not blot-dry the membrane. If the membrane is allowed to dry even partially, subsequent removal of the probe from the membrane may be difficult.

9. Immediately cover the blot with plastic wrap.

10. Expose to x-ray film at –70°C with two intensifying screens for 1–2 days or a phosphor screen (Kodak-K) at RT for 1–2 days (captured using the Molecular Imager FX System, BioRad).

11. Probe removal (stripping)
    i. Heat the sterile H_2O/0.5% SDS solution to 90–100°C.
    ii. Remove plastic wrap from blot and immediately place in the heated solution. Make sure that exposure to air is minimal.
    iii. Incubate for 10 min, shaking frequently.
    iv. Allow the H_2O to cool for 10 min before removing the blot.
    v. Remove the blot and air-dry until it is dry enough to be slipped into a plastic bag. The membrane can be stored at –20°C until needed.