6. Lyse the bacteria and bind the liberated DNA to the nitrocellulose filter by whichever of the two following procedures you find more convenient. Try to avoid (1) getting any of the solutions on the upper surface of the filter, and (2) trapping air bubbles under the filter.

Binding Liberated DNA—Procedure I

1. Cut four pieces of Whatman 3MM paper so that they fit neatly on the bottom of four Pyrex baking dishes (20 cm × 20 cm). Saturate one piece of paper with 10% SDS. Pour off any excess liquid.

2. Using blunt-ended forceps (e.g., Millipore forceps), peel the nitrocellulose filter from the plate and place it, colony side up, on the SDS-impregnated 3MM paper for 3 minutes. This treatment, which is optional, seems to result in a sharper hybridization signal. It probably works by limiting the diffusion of the plasmid DNA during denaturation and neutralization (E. F. Fritsch and P. Boyer, unpubl.).

3. Transfer the filter to the second sheet of 3MM paper that has been saturated with denaturing solution (0.5 M NaOH, 1.5 M NaCl). Leave the filter for 5 minutes. When transferring filters from one Pyrex tray to another, use the edge of the first tray as a scraper to remove as much fluid as possible from the underside of the filter.

4. Transfer the filter to the third sheet of 3MM paper that has been saturated with neutralizing solution (1.5 M NaCl, 0.5 M Tris · Cl [pH 8.0]). Leave the filter for 5 minutes.

5. Lay the filter, colony side up, on a sheet of dry 3MM paper. Allow to dry at room temperature for 30–60 minutes.

6. Sandwich the filter between two sheets of dry 3MM paper. Bake for 2 hours at 80°C in a vacuum oven.

7. Hybridize the filter to a ^{32}P-labeled probe as described on page 320. 324

Binding Liberated DNA—Procedure II[1]

1. Make a puddle (0.75 ml) of 0.5 M NaOH on a piece of Saran Wrap. Place the filter on the puddle, stretching the Saran Wrap so that the filter wets evenly. Leave for 2–3 minutes.

2. Blot the filter on a dry paper towel and repeat step 1, using a fresh piece of Saran Wrap and fresh 0.5 M NaOH.

3. Blot the filter and transfer to a fresh piece of Saran Wrap with 0.75 ml of 1 M Tris · Cl (pH 7.4). After 5 minutes, blot the filter dry and repeat.

4. Blot the filter and transfer to Saran Wrap with 0.75 ml of a solution of 1.5 M NaCl and 0.5 M Tris · Cl (pH 7.4). After 5 minutes, blot and transfer the filter to a piece of dry 3MM paper. Allow the filter to dry at room temperature for 30–60 minutes.

5. Sandwich the filter between two sheets of 3MM paper. Bake for 2 hours at 80°C in a vacuum oven.

6. Hybridize the filter to a ^{32}P-labeled probe as described on page 324.

Note

Any filters not used immediately in hybridization reactions should be wrapped loosely in aluminum foil and stored under vacuum at room temperature.

[1]D. Hanahan (unpubl.).