Immobilization of Bacteriophage λ Plaques on Nitrocellulose Filters or Nylon Membranes

This method is modified from that of Benton and Davis (1977).

1. Mix aliquots of a packaging reaction or bacteriophage λ stock containing no more than 50,000 bacteriophage particles in a volume of 50 μl or less with 0.3 ml of plating bacteria (see page 2.60). Incubate the infected bacteria for 20 minutes at 37°C.

2. Add to each aliquot 6.5 ml of molten (47°C) top agarose (0.7%) and pour onto a 150-mm agar plate. The plates must be dry; otherwise, the layer of top agarose will peel off when the filter is removed. Usually, 2-day-old plates that have been dried for several additional hours at 37°C with the lids slightly open work well. In humid weather, however, incubation for 1 day or more at 41°C may be necessary.

   Be sure to use top agarose rather than top agar, since agar peels off even more easily than agarose.

3. Incubate the plate containing the infected bacteria at 37°C until the plaques reach a diameter of approximately 1.5 mm and are just beginning to make contact with one another (10–12 hours). The plate should not show confluent lysis.

4. Chill the plates at 4°C for at least 1 hour to allow the top agarose to harden.

5. Number nitrocellulose filters (Millipore HATF or HAWP, Schleicher and Schuell BA85, or equivalent) or nylon membranes (Schleicher and Schuell, Nytran, or equivalent) with a soft-lead pencil or a ballpoint pen.

   If the filters will be hybridized to a number of different probes, it is better to use nylon membranes rather than nitrocellulose filters. Nylon membranes are more durable than nitrocellulose filters and withstand repeated exposure to extremes of temperature far better. Nylon membranes are also preferred when hybridization is to be carried out in solvents containing tetramethylammonium chloride (see Chapter 11). However, different brands of nylon membranes vary in their ability to bind DNA, in the ease with which they can be stripped of radiolabeled probes, and in the degree to which they distort during repeated rounds of stripping and rehybridization (Brandhian 1987). It would therefore be sensible to check the properties of nylon membranes obtained from different manufacturers before attempting large-scale screening of bacteriophage libraries. Follow the manufacturer's recommendations closely, since they have presumably been shown to lead to optimal results. The protocol given below works well with all commercial brands of nitrocellulose filters.

6. Remove the plates from the cold room or refrigerator, and, at room temperature, place a dry circular nitrocellulose filter gently onto the surface of the top agarose so that it comes into direct contact with the plaques. Be careful not to trap air bubbles. The filter should be handled with gloved hands; finger oils prevent wetting of the filter and affect transfer of DNA. Mark the filter in three or more asymmetric locations

by stabbing through it and into the agar beneath with an 18-gauge needle attached to a syringe containing waterproof black drawing ink.

Once in contact with the top agarose, the filter wets very rapidly and transfer of bacteriophage DNA occurs quickly. Therefore, do not move the filter once contact with the plate is made. The easiest way to place the filter on the plate is to hold it by its edges, bending it slightly so that the middle of the filter makes contact with the center of the plate. Wetting action then pulls the filter onto the plate. Make certain that the keying marks are asymmetrically placed and that both the filter and the plate are marked. There must be enough ink on the plate to be easily visible when a second filter is in place. Large blotches of ink, however, are undesirable.

7. After 30–60 seconds, use blunt-ended forceps (e.g., Millipore forceps) to peel off the first filter and immerse it, DNA side up, in a shallow tray of denaturing solution (0.5 N NaOH, 1.5 M NaCl) for 1–5 minutes. Transfer the filter into neutralizing solution (1.5 M NaCl, 0.5 M Tris · Cl [pH 7.4]) for 5 minutes. Rinse the filter in 2 × SSC, and place it, DNA side up, on paper towels to dry.

If any top agarose peels off the plate and adheres to the filter, remove it by gently agitating the filter in denaturing solution.

G. Struhl (unpubl.) has developed the following procedure to fix bacteriophage λ DNA to nitrocellulose filters that avoids treating filters with alkali and neutralizing solution. This can save time when dealing with large numbers of filters.

a. After removing the filters from the top agarose, place them, DNA side up, on paper towels for 5–10 minutes.
b. When the edges of the filters begin to curl, place them in stacks of ten interleaved with circular Whatman 3MM papers. Place a few 3MM papers on the top and bottom of the stack.
c. Place the stacks on a small platform (e.g., the bottom of a Pyrex dish) in an autoclave. Expose to "streaming steam" for 3 minutes (i.e., 100°C—avoid superheated steam).
d. Transfer the stack of filter papers and nitrocellulose filters to a vacuum oven. Bake for 2 hours at 80°C while drawing a vacuum continuously. Any papers that stick to the nitrocellulose filters can be removed by soaking in 2 × SSC before prehybridizing.

When many filters are to be processed at one time, an alternate procedure is to transfer them from the surfaces of the agarose plates to a large piece of 3MM paper soaked in denaturing solution. Plastic cafeteria trays can be used to hold the 3MM paper. After 2 minutes, quickly transfer the filters to a fresh piece of 3MM paper soaked in neutralizing solution. When transferring the filters, use the edge of the first cafeteria tray to remove as much fluid as possible from the underside of the filters. After 3 minutes, transfer the filters to a third sheet of 3MM paper that has been soaked in neutralizing solution. After 3 minutes, rinse the filters briefly in 2 × SSC and place them on paper towels to dry.

8. Place a second dry filter onto the same plate and mark it with waterproof black drawing ink at the same locations as in step 6. After 1–2 minutes, peel off the filter, denature the DNA, and neutralize as described in step 7.

Generally, the first filter is left in contact with the plaques for 30–60 seconds and subsequent filters are left in contact about 30–60 seconds longer, or until the filter is completely wet. As many as seven replicas have been prepared from a single plate (Benton and Davis 1977), but the strength of the hybridization signal decreases significantly after the third filter.

9. After all of the filters are dry (usually 30–60 minutes at room temperature), sandwich them between sheets of 3MM paper. Fix the DNA to the filters by baking for 30 minutes to 2 hours at 80°C in a vacuum oven.

Overbaking can cause the filters to become brittle. Filters that have not been completely neutralized turn yellow or brown during baking and chip very easily. The background of nonspecific hybridization also increases dramatically.

Baking at 80°C is not necessary to fix DNA to some types of nylon membranes (see manufacturer's instructions).

10. Hybridize the DNA immobilized on the filters to a ^{32}P-labeled probe as described on pages 2.114–2.117.

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