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A Simple, Efficient PCR Technique for Characterizing Bacteriophage Plaques

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Many molecular cloning strategies involve the identification of bacteriophage plaques or bacterial colonies containing a DNA sequence of interest after phage infection or plasmid transformation. The polymerase chain reaction (PCR)[1,2] could provide a much simpler and faster way of identifying plaques relative to the conventional techniques (e.g., filter hybridization). This could be done with either extracted DNA or DNA picked up through a pipette tip.[3,4] The former requires extra effort for preparing DNA samples, especially when many samples are to be screened. The latter method works well for bacterial colonies but has not given consistent results with phage plaques. Looking for a better alternative, we have been using small pieces of nucleic acid transfer membrane to pick up DNA from bacterial plaques and transfer directly to PCRs. The same procedure works well with both plaques and colonies. In addition, the plaques were preserved for subsequent experiments. A recent report presented a similar method using a special type of film.[5] Because the membranes we used are readily available in any molecular biology laboratory, we report here a simple PCR procedure for identifying positive plaques and colonies.

MATERIALS AND METHODS

An EMBL3 clone harboring an *Arabidopsis thaliana* L. genomic fragment containing a gene preferentially expressed at low temperature[6] was used. PCR using two primers (left, 5′-TACTCGTGGCACCACACTCC-3′; right, 5′-TGCAGCATCCTTGGCCTTGT-3′) detected a 395-bp sequence within the fragment. Infection of *Escherichia coli* strain NM538 and plating were carried out as described.[7]

Six different membranes were arbitrarily chosen, as they were already available in the laboratory (Table 1). They were supplied by Schleicher & Schuell (Keene, NH), BioRad Laboratories (Richmond, CA), Promega (Madison, WI), and Amersham Canada (Oakville, Ontario). Small membrane disks were cut out from a larger piece using a glass Pasteur pipette (punching a hole by twisting the pipette tip with the membrane on a soft support, e.g., paper towel). The cut membrane disks had a diameter of 1.2–1.3 mm and thus an area of 1.1–1.3 mm^2. The disks were used because they were uniform and of known area. A membrane disk was applied to a phage plaque or a bacterial colony, as in filter hybridization, and lifted after about 15 sec. The disc was used directly in a 50-μl PCR without further treatment. For testing the effect of excess membrane materials on PCR, two parallel reactions were performed and the results were compared. The first reaction contained one membrane disk lifted from a plaque. The second contained one membrane disk lifted from a plaque (thus containing similar amount of DNA as the first reaction) and two additional blank (that is not applied to a plaque) membrane disks. Care was taken to use plaques of a similar size for these reactions. To determine the effect of multiple lifting on sequence amplification, 10 membrane disks of Schleicher & Schuell nitrocellulose and nylon membranes were lifted consecutively from the same plaques. To determine the minimum number of cycles required to amplify the sequence from one membrane disk to a clearly visible band, a set of PCRs with the following number of cycles were carried out: 25, 23, 21, 19, 15, 13, 11, and 9. Plaques of the same apparent size were used. For all these reactions, the last cycle was followed by a 3-min incubation at 72°C for extension.

TABLE 1 Different Membranes Used in the Experiments

Manufacturer	Membrane type	Brand
Schleicher & Schuell	nitrocellulose	BA-S NC
Schleicher & Schuell	nylon	Nytran
BioRad	nitrocellulose	Trans-Blot
BioRad	nylon	Zeta Probe
Promega	nitrocellulose	
Amersham	nylon	Hybond

Parallel experiments were performed using *E. coli* colonies. The transformed strain αH5 (GIBCO BRL Canada, Burlington, Ontario) had pGEM3Zf(+) with an insert (2.2 kb) containing the same sequence as the above.

All PCRs were run in 0.5-ml microtubes containing 50-μl reaction mixture consisting of PCR buffer (10 mM Tris, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, and 3 mM dithiothreitol with pH 8.4), 200 μM each of dNTPs, 0.25 μM each of the two primers, and 0.75 unit of *Taq* DNA polymerase (GIBCO BRL). After

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adding the membrane disks lifted from the plaques or colonies, the samples were heated at 94°C for 50 sec and then run for 25 thermal cycles in a Perkin-Elmer 4800 heating block unless stated otherwise. The cycling temperatures were 94°C for 50 sec, 57°C for 50 sec, and 72°C for 50 sec. The last cycle had an additional 3 min at 72°C for chain extension. An 8.5-μl aliquot of each reaction mixture was electrophoresed on 14-well, 0.9% agarose gels and DNA was visualized with 0.5 μg ml^{-1} ethidium bromide.

RESULTS AND DISCUSSION

Type of Membrane

Membranes, as listed in Table 1, were tested to determine the effect of different types and brands and also the effect of excess amount of membrane on PCRs. The results after 25 cycles are shown in Figure 1. When only one membrane disk was used, all samples produced the expected band with similar fluorescence intensity. When three membrane disks (one lifted from the plaque and two blank, with a total area of about 3.6 mm^2) were used, the band was visible in reactions with all three nylon membranes and the Promega nitrocellulose membrane, although in each case with reduced fluorescence intensity. These results show that when excess membrane material is present, nitrocellulose appears to be more inhibitory than the nylon membranes, although this depended on the brand. However, because a single nitrocellulose membrane disk was as effective as a single nylon membrane disk (also shown later), the inhibitory effect of excessive nitrocellulose membrane should not be a concern for normal screening. Actually only a portion of a single membrane disk is needed, if smaller pieces can be conveniently cut. Since all six different membranes tested showed positive results, nitrocellulose and nylon membranes from other manufacturers should probably work equally effectively. Small membrane pieces could be cut by other convenient tools and used in the procedure as long as they do not exceed the 1.3 mm^2/50 μl ratio tested here.

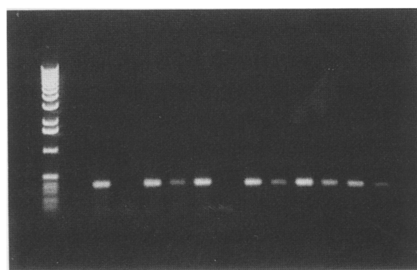

FIGURE 1 Testing different membranes for fast PCR identification of phage plaques. The membrane disks were lifted from different plaques of similar sizes and used in 50-μl PCRs. After 25 cycles, 8.5 μl of the reaction mixture was separated on a 0.9% agarose gel by eletrophoresis at 75 volts for 30 min. From *left* to *right*: (lane standard) 1 kb ladder (BRL); (lane *1*) negative control (blank Schleicher & Schuell cellulose); (lanes *2*, *4*, *6*, *8*, *10*, and *12*) amplified DNA from PCRs containing one membrane disk lifted from a plaque; (lanes *3*, *5*, *7*, *9*, *11*, and *13*) PCRs containing one membrane disk lifted from a plaque and two additional blank membrane disks. (Lanes *2–3*) Schleicher & Schuell nitrocellulose; (lanes *4–5*) Schleicher & Schuell nylon; (lanes *6–7*) BioRad nitrocellulose; (lanes *8–9*) BioRad nylon; (lanes *10–11*) Promega nitrocellulose; (lanes *12–13*) Amersham nylon.

Longer sequences can also be amplified from the membranes. For example, a 2.4-kb DNA segment of the same genomic clone was amplified from plaque DNA (data not shown), showing that the technique can be applied to complete genes as well as fragments.

Multiple Probing of the Same Plaque

Results from multiple nitrocellulose disks show that consecutive lifting from the same plaque up to the tenth disk produced the same intensity band (Fig. 2). Similar results were obtained with nylon membrane (data not shown). These results might be useful when multiple PCRs are to be performed for different DNA sequences. They suggest either that there was no reduction in the amount of DNA picked up by consecutive lifting of membrane disks or that the amount from each disk was large enough so that DNA synthesis had reached a plateau before the twenty-fifth cycle. In the latter case, the slight reduction in the amount of DNA picked up with each lifting would not be reflected in the final amount of DNA.

Number of PCR cycles

The results demonstrate that for both nitrocellulose and nylon membranes a prominent band was visible after 15 cycles and the maximum was reached after about 21 cycles (Fig. 3). They suggest that a PCR with more than 15 cycles will be sufficient to identify positive plaques using this method. To verify this and also to determine whether this was true with variable sizes of plaques, a 16-cycle PCR was run using membrane disks lifted from plaques of various sizes

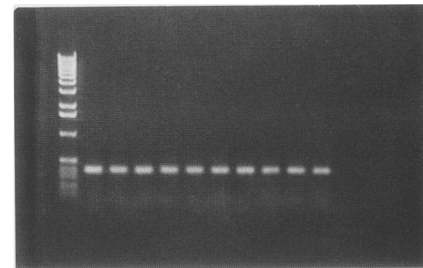

FIGURE 2 PCRs using multiple membrane disks lifted from the same plaque. Only the results with Schleicher & Schuell nitrocellulose membrane are shown here. Results using the nylon membrane are the same. PCR and electrophoresis conditions are as in Fig. 1. (Lane standard) 1-kb ladder; (lanes *1–10*) the first and the tenth membrane disk lifted from the same plaque, respectively.

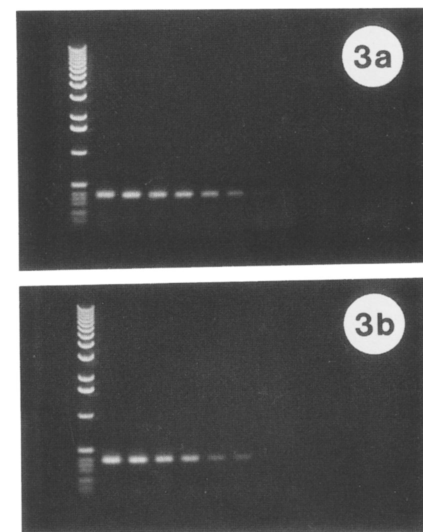

FIGURE 3 Determining minimum number of PCR cycles required to detect a specific DNA sequence using membrane disks. PCR and electrophoresis are as in Fig. 1. (*a*) PCRs using Schleicher & Schuell nitrocellulose membrane; (*b*) PCRs using Schleicher & Schuell nylon membrane. (Lane standard) 1-kb ladder. Following are the lane numbers and the corresponding number of cycles: (*1*) 25; (*2*) 23; (*3*) 21; (*4*) 19; (*5*) 17; (*6*) 15; (*7*) 13; (*8*) 11.

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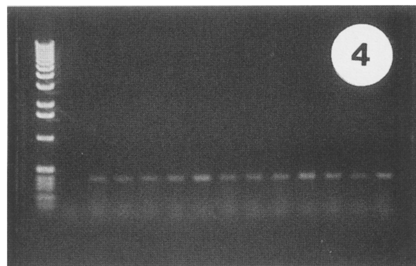

FIGURE 4 PCRs of 16 cycles using membrane disks (Schleicher & Schuell nitrocellulose) to identify the DNA sequence insert in phage plaques of various sizes. The difference in the band intensity reflects the difference in plaque size. (Lane standard) 1-kb ladder; (lane *1*) negative control (membrane disk lifted from a negative plaque); (lanes *2–13*) PCRs from 12 different plaques.

(some were among the smallest visible on a plate). A band was indeed present for all the plaques (Fig. 4). The difference in the band intensity was related to the difference of plaque size.

Bacterial Colonies

Parallel experiments with bacterial colonies had similar results. When the minimum number of PCR cycles was determined, it was shown that the colonies required about 13 cycles to amplify the DNA to the same extent (data not shown). It should be pointed out that unlike other similar procedures for bacterial colonies,[3,8] the present technique does not require any pretreatment prior to PCR.

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