High-Yield Method for Isolation of λ DNA

BioTechniques 23:598-600 (October 1997)

Isolation of bacteriophage λ DNA is among the most common procedures used by those working with recombinant DNA methodologies. Although there are many protocols for λ DNA isolation (1,2,4), some are cumbersome and time-consuming, and others result in poor DNA yield. Among the most important parameters for successful phage DNA isolation is estimating the correct phage:host cell ratio for infection (3). If this ratio is too high, lysis occurs too quickly, before the phage population builds to an optimal level. If the ratio is too low, the host bacteria will outgrow the phage. In both cases, the result is a poor DNA yield. With the method described here, we have found it is not necessary to titrate the phage. An innovation of this procedure is supplementation of the LB growth medium with glycerol. Although the exact reason that this improves the yield is not known, the glycerol may serve as an additional carbon source for the host cells.

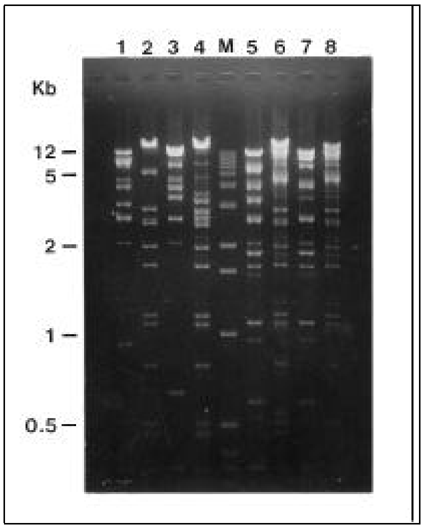

Figure 1. Restriction digestion of phage DNA from a λEMBL3 genomic library and a λZAP cDNA library prepared by the protocol described here. Lanes 1 and 2: clone A from genomic library. Lanes 3 and 4: clone B from genomic library. Lanes 5 and 6: clone C from cDNA library. Lanes 7 and 8: clone D from cDNA library. Lanes 1, 3, 5 and 7 were digested with *Cla*I. Lanes 2, 4, 6 and 8 were digested with *Pst*I. M denotes the 1-kb DNA ladder (Life Technologies, Gaithersburg, MD, USA).

Table 1. Protocol for DNA Isolation

1. Add 0.5 mL chloroform to the lysed culture medium and continue shaking for 30 min.
2. Transfer culture to a 50-mL centrifuge tube and spin at 4000× *g*, 4°C for 15 min to pellet the host cells.
3. Transfer the supernatant to a fresh tube. Add 2.8 g NaCl and shake for 5 min until dissolved. Add 5 g polyethylene glycol (PEG; mol wt = 8000) (To facilitate dissolution, grind PEG with a mortar and pestle before adding) and mix gently for 30 min at room temperature until PEG has dissolved. Incubate on ice for 1 h.
4. Pellet the phage by centrifugation at 4000× *g* for 15 min at 4°C. Discard the supernatant and leave the tube inverted on a paper towel for 15 min. Remove residual PEG from the inside of the tube with a laboratory wipe. Add 1 mL SM buffer to the phage pellet and resuspend the pellet by moving the pipet through the pellet while dispensing. (Once resuspended, 100 µL of this phage suspension can be stored at 4°C as a stock.)
5. Transfer the suspended phage to a 1.5-mL microcentrifuge tube and add DNase I to 20 µg/mL and RNase A to 50 µg/mL and incubate at 37°C for 30 min.
6. Centrifuge at 2000× *g* for 5 min and transfer the supernatant to a fresh tube. Add sodium dodecyl sulfate (SDS) to 0.5%, EDTA to 10 mM and proteinase K to 50 µg/mL. Incubate at 37°C for 30 min.
7. Extract the solution once with phenol/chloroform (1:1) and twice with chloroform/isoamyl alcohol (24:1), removing the supernatant to a fresh tube each time. Add 0.5 vol of 7.5 M ammonium acetate and 2.5 vol of absolute ethanol. Mix completely but gently and incubate 10 min at -20°C. Centrifuge for 10 min at 10 000× *g* at 4°C to pellet the phage DNA.
8. Rinse the DNA pellet with 70% ethanol and air-dry. Resuspend in 500 µL sterile, distilled water or TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). It may be necessary to allow the pellet to resuspend overnight at 4°C. Before using, incubate the DNA for 30 min at 37°C and mix gently with a pipet tip to ensure complete resuspension. Concentrations of 1–2 µg/µL DNA are routinely obtained.

The following protocol is fast, relatively easy and reproducible. From a 50-mL culture, we routinely obtain 1.0–2.0 mg of high-quality phage DNA.

On day 1, the first step is preparation of host cells. Inoculate a single colony of appropriate host bacteria into 50 mL LB medium supplemented with maltose and magnesium sulfate (1% tryptone, 0.5% yeast extract, 1% NaCl, 10 mM $MgSO_4$, 0.2% maltose). Incubate for 4–6 h at 37°C with vigorous shaking; the optical density $(OD)_{600}$ reaches, but does not exceed, 0.3. Collect the cells by centrifugation at 4000× *g*, 4°C for 10 min. Resuspend in ice-cold 10 mM $MgSO_4$ to a final OD_{600} of 0.8–1.0.

Next is preparation of phage stock. Using a sterile toothpick, remove a single plaque along with the top agar and a small amount of surrounding bacterial lawn from a phage library and place into 1 mL sterile SM buffer (0.1 M NaCl, 0.1% $MgSO_4$, 0.05 M Tris-HCl, pH 7.5, 0.01% gelatin [type A300; Sigma Chemical, St. Louis, Mo, USA]) in a microcentrifuge tube. Incubate at 37°C with shaking at 225 rpm for at least 4 h.

The third step is inoculation. Add 100 µL phage stock to 0.5 mL host bacteria and incubate at 37°C for 20 min. Add this to 50 mL LB medium supplemented with 0.3% glycerol and 10 mM $MgSO_4$ and grow at 37°C with shaking at 280–300 rpm. Incubate the culture overnight (14–16 h).

On day 2, examine the culture for bacterial debris, indicating lysis. If lysis has not occurred, continue growing for 1–2 h more. If lysis does not occur after the additional time, dilute the culture 1:3 with fresh LB in a new flask and continue to grow until lysis occurs (usually 3–4 h). Once lysis occurs, proceed with the DNA isolation. The protocol for DNA isolation is outlined in Table 1.

This protocol was used to isolate phage DNA from two different libraries and phage vectors. The first was a λEMBL3 genomic library of the house fly, *Musca domestica*. The second was a λZAP® (Stratagene, La Jolla, CA, USA) cDNA library of *Drosophila virilis*. An aliquot of 2 μL resuspended DNA was used per individual restriction digest. As shown in Figure 1, this small volume was sufficient for restriction mapping and Southern hybridization.

The effect of glycerol during cell-phage growth was compared. Identical 50-mL cultures were prepared for inoculation with phage isolated from the same purified plaque. One flask contained LB with 0.3% glycerol as decribed above, and the second contained LB without glycerol. Following lysis and DNA isolation, total DNA yield was compared. Total DNA yield from the glycerol-supplemented LB was 1.0–2.0 mg while the yield for LB only was 0.125–0.25 mg. Thus, glycerol seems to have a dramatic effect on the DNA yield from this protocol.

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Received 18 February 1997; accepted 14 April 1997.

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Efficient Recovery of Plasmid DNA from *Erwinia herbicola* with High Nuclease Activity

BioTechniques 23:600-603 (October 1997)

An efficient method is described for the isolation and purification of plasmid DNA from *Erwinia herbicola* (a bacterium with high nuclease activity), for which none of the standard methods yielded plasmid DNA in desired amounts. The procedure adopted here is based on elimination of the endogenous nuclease activity using guanidinium thiocyanate. The DNA preparation does not need a separate purification step. The method precludes the use of RNase, protease and phenol. The plasmid DNA preparation obtained by this method is of high purity suitable for restriction digestion, cloning, hybridization, preparation of DNA probes and sequencing.

E. herbicola (Catalog No. 21998; ATCC, Rockville, MD, USA) is an industrially important organism because of its ability to produce keto-sugar acids as intermediates of ascorbic acid. As a result, the organism is a good candidate for genetic manipulations. We studied the organism for the presence of plasmid DNA using several standard methods from Sambrook et al. (11) such as the alkaline lysis method (2), the sodium dodecyl sulfate (SDS) lysis method (6), the boiling method (7) etc. The gel picture revealed that the bacteria harbored the plasmid DNA, but the yields were invariably very low, and the isolated DNA was susceptible to degradation within a day, even when preserved at -20°C. Apparently, it shows that the organism possesses high endogenous nuclease activity.

Various other methods described for the isolation of plasmid DNA from prokaryotic organisms were also tried (1,3–5,9). However, plasmid DNA could not be recovered easily and efficiently in reasonably large quantities from this bacteria. Several modifications in the known standard procedures were also unable to increase plasmid DNA yield or inhibit its degradation.

Guanidinium thiocyanate, a strong protein denaturant, has been success-