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COMMUNICATION

Rapid Preparation of Lambda Phage DNA

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A rapid procedure for the isolation of λ phage DNA is described. The method combines the advantages of direct lysate ultracentrifugation with phage purification on a DEAE-celluiose minicolumn. It can be applied to either liquid or plate lysates on a small or medium scale. The isolated DNA is suitable for restriction and direct sequencing. ©1992 Wiley-Liss, Inc.

RATIONALE AND DESIGN

The most commonly used procedures for λ phage DNA isolation include nuclease digestion of cellular nucleic acids, precipitation of phage by polyethylene glycol, digestion of protein, and finally phenol/chloroform extraction of DNA (Sambrook et al., 1989). These procedures can introduce enzyme-inhibiting impurities (Brzezinski et al., 1984; Schweizer, 1988). To overcome this problem, two main approaches are used: A) purification of intact phage particles, prior to isolation of DNA, by means of Biogel (Sain and Erdei, 1981), Sepharose (Brzezinski et al., 1984), DEAE-cellulose (Helms et al., 1985, 1987; Manfioletti and Schneider, 1988; Reddy et al., 1988; Xu, 1986), ammonium sulfate precipitation (Verma, 1989), or CsCl gradient centrifugation (Sambrook et al., 1989); B) purification of phage DNA by chromatography (Ivanov and Gigova, 1985; Schweizer, 1988). The first approach is rather cumbersome, time-consuming, and expensive. The second method suffers from poor DNA yield and its use is often limited by clogging and overloading of the column.

In the method reported here phage virions are precipitated from the lysate by a brief ultracentrifugation (Dumanski et al., 1988; Grossberger, 1987; Schweizer, 1988) and then purified on DEAE-cellulose minicolumns. This procedure avoids laborious and expensive steps such as CsCl centrifugation and hazardous phenol extraction, is relatively rapid, and yields DNA suitable for restriction analysis or sequencing.

The procedure described is applicable to either plate or liquid lysates. Our experience as well as previous findings indicate that the plate lysates provide greater phage yield more consistently (Helms et al., 1985) and the sequencing quality of the isolated DNA is better (Steffens and Gross, 1989).

The most common critical step in λ DNA isolation is the phage growth, which depends dramatically on the initial phage input, i.e., pfu plated. For three different λgt11 clones and a λgt10 clone we observed a maximum phage yield when the phage input was between 10^5 to 10^6 pfu/plate (Fig. 1). Higher phage input decreased the yield (Figure 1), contrasting with a previous recommendation to use a minimum of 10^7 pfu/plate (Helms et al., 1987). The reasons for the strong dependence of phage yield on phage input may be that, in the case of low input, most cells enter stationary phase before infection, giving a non-cleared culture, whereas in the case of too high an input, cells are lysed at an early stage of growth (Helms et al., 1987).

We checked the yield of virions after DEAE-cellulose chromatography by determining the total pfu in fractions collected from the column. Results in Table 1 show nearly quantitative recovery of the virions from the column. This indicates that ion exchange chromatography apparently does not injure the infective ability of the phage particles as previously suspected (Reddy et al., 1988).

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Poor phage binding and column clogging has been reported as a persistent failure of the DEAE-cellulose minicolumn method (White and Rosenzweig, 1989). We also failed to retain the virions from untreated lysate on the DE-52 column (see Table 1), even after the recommended lysate dilution (Helms et al., 1987). Moreover, the columns were frequently clogged.

To overcome these problems we concentrate the virions by a brief ultracentrifugation, and then load them on the column. With this procedure we observed no significant loss of phage in flow through during loading (<2%) and no column clogging even when a single small column was loaded with phage concentrated from 8 plate lysates. Thus, this method allows a medium scale preparation (up to 100 µg DNA) on a single small column, saving time and resin in comparison to the use of one column per plate lysate (Helms et al., 1985; White and Rosenzweig, 1989) or big columns (Brzezinski et al., 1984; Reddy et al., 1988). Moreover, ultracentrifugation also removes the soluble substances, including enzyme-inhibiting polyanions, which stay in the supernatant.

The present method purifies the phage DNA from all chromosomal DNA and the bulk of the cellular RNA. The RNase digestion step may be skipped. Traces of RNA, if present, do not interfere with DNA restriction. But to avoid RNA completely (important for direct sequencing) the RNase digestion step is required.

The proteinase K digestion step is necessary to destroy the nucleases, thus to protect the DNA after denaturation of the virions. The enzyme concentration and incubation time were optimized empirically, and they are much below those recommended previously; 25 µg/ml for 15 min instead of 1,000 µg/ml or even more for 120 min (Dumanski et al., 1988; Grossberger, 1987; White and Rosenzweig, 1989).

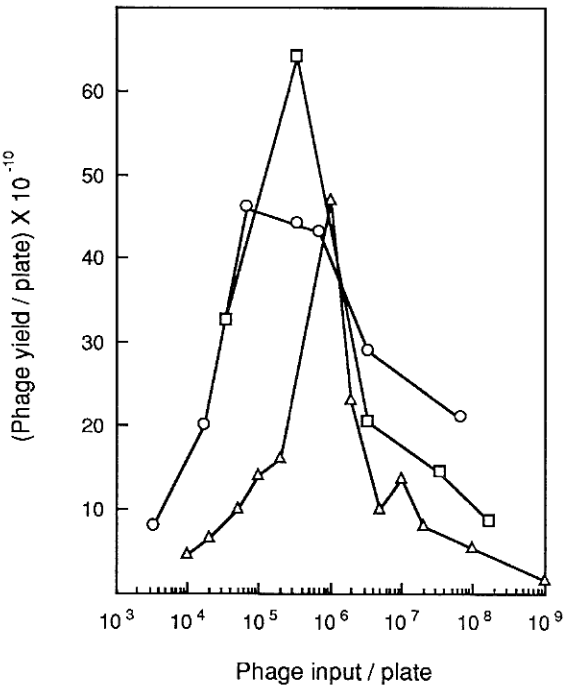

Figure 1. The dependence of phage titer in plate lysate on the phage input/plate. The three symbols represent different λgt11 clones.

Together with the omission of the usually adopted DNase treatment (Manfioletti and Schneider, 1988; Malik et al., 1990), this greatly shortens the procedure. The whole protocol, from infection to pure DNA, may be accomplished in 24 h.

The phage DNA obtained could be cut with all the restriction enzymes tested, and the cDNA inserts of recombinant phage DNAs could be amplified by the polymerase chain reaction and directly sequenced.

TABLE 1. Phage Yield at Successive Steps of Isolation*

	Total pfu x 10^{-7}			
	Experiment 1		Experiment 2	
	w/o spin	w/spin	w/o spin	w/spin
In 5 ml of crude lysate	12,200	12,200	9,250	9,250
After ultracentrifugation				
In supernatant	—	48	—	8
In 3 ml of resuspended pellet	—	10,200	—	9,300
In DE-52 column fractions				
Loading and wash buffer	9,400	19	9,070	106
1 ml of elution buffer	880	765	100	225
0.6 ml of elution buffer	360	5,100	310	7,000
1 ml of elution buffer	—	450	—	875

*In each experiment combined plate lysates were divided into equal volumes. w/o spin: The plate lysate was directly loaded onto the DE-52 minicolumn according to Helms et al. (1985). w/spin: After ultracentrifugation of the plate lysate, the pellet was dissolved in Tris buffer and loaded onto the column. The pfu was determined by plating bacteria infected with 1 µl of each fraction.

METHODOLOGY

Phage Lysate Preparation

1. Inoculate the growth medium (LB broth, 0.2% maltose, 10 mM $MgCl_2$) with a single colony of host bacteria (we used Y1090) and incubate with vigorous shaking for 4–5 h at 37°C.
2. Infect 0.2 ml of fresh culture with 10^5–10^6 plaque forming units (pfu) of phage for 15 min at 37°C.
3. Mix the infected bacteria with 3 ml of melted top agar (LB broth, 10 mM $MgCl_2$, 0.7% bactoagar) kept at 50°C, and pour on a 90 mm LB agar plate.
4. Incubate the plates at 37°C overnight. Chill the plates for 1 h ar 4°C, and then overlay with 5 ml of cold SM buffer (100 mM NaCl, 8 mM $MgSO_4$, 50 mM Tris, pH 7.5). Shake the plates gently for 2 h or leave without shaking at 4°C overnight.
5. Pipet off the lysate and centrifuge at 12,000*g* for 10 min to remove cell debris and agar particles.

Phage Precipitation by Ultracentrifugation

1. Centrifuge the lysate at 100,000*g* for 30 min at 4°C.
2. Suspend the pellet by gentle Vortex mixing in 3 ml of 10 mM Tris, pH 8.

Phage Purification on DEAE-Cellulose Column

1. Load the phage suspension onto the DEAE-cellulose column (2 ml preswollen Whatman DE-52 in a Bio-Rad plastic minicolumn) equilibrated with 10 mM Tris, pH 8.
2. Wash the column with 3 ml of wash buffer (5 mM $Mg(CH_3COO)_2$, 30 mM CH_3COONa, and 10 mM Tris, pH 8) and discard the runthrough.
3. Load 1 ml of elution buffer (50 mM $Mg(CH_3COO)_2$, 10 mM Tris, pH 8) and discard this runthrough as well.
4. Load a final 0.6 ml of elution buffer and collect the phage enriched fraction in a microcentrifuge tube.

Enzymatic Treatment, Phage Lysis, and DNA Concentration

1. Add 2 µl of 10 mg/ml Ribonuclease A and incubate for 30 min at 37°C.
2. Add 15 µl of 1 mg/ml Proteinase K and 25 µl of 10% SDS. and incubate for 15 min at 37°C.
3. Add 100 µl of 3 M CH_3COOK, heat 15 min at 88°C, and cool on ice.
4. Centrifuge at 12,000*g* for 15 min at 4°C.
5. Transfer the supernatant to a new tube and mix with 700 µl isopropanol. Keep at –20°C for 1 h.
6. Precipitate the DNA at 12,000*g* for 15 min at 4°C. Replace the supernatant with cold 70% ethanol and centrifuge for 5 min again.
7. Dry the DNA pellet under vacuum and resuspend in 20–40 µl of 10 mM Tris, pH 8.

Plate growth of phage infected bacteria

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Phage isolation from plate lysate by centrifugation

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Phage purification on DEAE-cellulose column

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DNA release by protein digestion

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Ethanol precipitation

Flowchart.

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LITERATURE CITED

Brzezinski, R., Vezina, G., Blakeley, S., and Thirion, J.-P. (1984), Removal of restriction enzyme inhibitors from lambda phage preparations. *Gene Anal. Technol.* **1**: 67–69.

Dumanski, J.P., Ruttledge, M., and Datta, S. (1988), Rapid minipreparations of bacteriophage λ DNA. *Nucleic Acids Res.* **16**: 9044.

Grossberger, D. (1987), Minipreps of DNA from bacteriophage lambda. *Nucleic Acids Res.* **15**: 6737.

Helms, C., Graham, M.Y., Dutchik, J.E., and Olson, M.V. (1985), A new method for purifying lambda DNA from phage lysates. *DNA* **4**: 39–49.

Helms, C., Dutchik, J.E., and Olson, M.V. (1987), A λ DNA protocol based on purification of phage on DEAE-cellulose. *Methods Enzymol.* **153**: 69–82.

Ivanov, I., and Gigova, L. (1985), Isolation of phage λ DNA by hydroxylapatite chromatography. *Anal. Biochem.* **146**: 389–392.

Malik, A.N., McLean, P.M., Roberts, A., Barnett, P.S., Demaine, A.G., Banga, J.P., and McGregor, A.M. (1990), A simple high yield method for the preparation of lambda gt10 DNA suitable for subcloning, amplification and direct sequencing. *Nucleic Acids Res.* **18**: 4031.

Manfioletti, G., and Schneider, C. (1988), A new and fast method for preparing high quality lambda DNA suitable for sequencing. *Nucleic Acids Res.* **16**: 2873–2884.

Reddy, K.J., Kuwabara, T., and Sherman, L.A. (1988), A simple and efficient procedure for the isolation of high-quality phage λ DNA using a DEAE-cellulose column. *Anal. Biochem.* **168**: 324–331.

Sain, B., and Erdei, S. (1981), Bacteriophage purification by gel chromatography. *Anal. Biochem.* **110**: 128–130.

Sambrook, J., Fritsch, E.F., and Maniatis, T., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989), pp. 2.60–2.81.

Schweizer, H.P. (1988), An improved procedure for the rapid purification of bacteriophage lambda DNA from small phage lysates. *DNA Prot. Eng. Tech.* **1**: 35–36.

Steffens, D.L., and Gross, R.W. (1989), Sequencing of cloned DNA using bacteriophage λ gt11 templates. *BioTechniques* **7**: 674–677.

Verma, M. (1989), Use of ammonium sulfate precipitation and guanidine isothiocyanate lysis to isolate lambda DNA. *BioTechniques* **7**: 230–232.

White, B.A., and Rosenzweig, S. (1989), A reliable method for the purification of bacteriophage DNA. *BioTechniques* **7**: 694.

Xu, S.-Y. (1986), A rapid method for preparing phage λ DNA from agar plate lysates. *Gene Anal. Technol.* **3**: 90–91.