Large- and Small-Scale Preparation of Bacterio-
phage λ Lysate and DNA


At present, there are many vectors such as plasmids, cosmid, bacteriophage λ and λ-derived vectors available for cDNA or genomic DNA cloning. Among these vectors, λ-derived vectors are the most popular for library construction and gene cloning. They permit researchers to screen large libraries and clone genes of interest easily. However, the most cumbersome part of the λ phage cloning procedure is often the preparation of high-titer lysate and subsequent isolation of DNA. To obtain high-titer phage lysate, most protocols require measurement of the exact titer of phage and host bacteria in advance (6). These procedures are not only time-consuming, but frequently they are not reproducible. Even after adequate phage lysate has been obtained, the subsequent problem generally encountered is to isolate sufficient phage DNA for cloning or mapping. The established methods for λ DNA isolation generally involve the following steps: (i) precipitate and purify the phage particles by cesium chloride gradient (6), DEAE-dextran (4), polyethylene glycol (PEG)/NaCl (1,2,6) or zinc chloride (7); (ii) deproteinate the preparation either by sodium dodecyl sulfate (SDS)/potassium acetate (7) or phenol/chloroform/isoamyl alcohol extraction (1,2); (iii) precipitate the DNA with ethanol or isopropanol. Problems associated with these methods include low yield, long procedural time and inability to digest the isolated DNA with restriction enzymes. An improved phage DNA isolation method has been reported by using zinc chloride as a phage-puri-

Table 1. Phage Amplification

1. Prepare an overnight culture of appropriate host bacteria at 37°C (e.g., LE392, TAP90) by inoculating a single colony into 5 mL of LB containing 10 mM Mg²⁺ (1% tryptone, 0.5% yeast extract, 0.5% NaCl, 10 mM Mg²⁺).
2. Infect 100 µL of bacterial culture with 10 µL of a series of diluted (1:100–1:1000) phage stocks and incubate the mixture at 37°C for 15 min. The phage stocks are made by resuspending a positive plaque into 1 mL of SM buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 10 mM MgSO₄, 0.01% gelatin) for 2 h at room temperature with gentle agitation.
3. Plate phage-bacteria mixture with molten top agarose onto a prewarmed agarose plate and incubate at 37°C overnight. One of the plates from the dilution series should lyse confluent.
4. Prepare plate lysates by flooding the confluent lysed plate with 5 mL of SM buffer. Then place on shaker to agitate gently at room temperature for 2 h and proceed to Step 5A. Note: If further amplification of phage is needed, then inoculate 300 µL of overnight host bacteria in 50 mL of prewarmed LB with 10 mM Mg²⁺ at this time and incubate at 37°C with vigorous shaking for 2 h. Following the incubation, proceed to Step 5B.
5A. Transfer the lysate to a screw-cap tube and clear the bacterial debris by centrifugation at 4000 × g for 10 min at room temperature. If a large amount of DNA is not required then, transfer the lysate to a new tube and proceed with DNA isolation.
5B. When the 50-mL bacterial culture reaches OD₆₀₀ = 0.5–0.9 (ca. 2 h), amplify phage in liquid culture by adding all the clear-plate phage lysate to the culture and shaking vigorously at 39°C for 2–3 h. The bacteria usually are lysed completely after 2–3 h of infection. If lysis is not obvious, add 1 mL of chloroform and shake for another 10 min. Lysis of the culture should be apparent. If lysis is still not apparent, save the culture by adding another 50 mL of prewarmed LB plus 10 mM Mg²⁺ to the culture and split the culture into two 250-

preparation, respectively.

mL flasks. Continue incubating for a further 2–3 h with vigorous agitation. Clear the bacterial debris as described in Step 5A.
Table 2. DNA Preparation

1. Add DNase I to a final concentration of 10 µg/mL and incubate at 37°C for 30 min.
2. Filter-sterilized 2 M ZnCl$_2$ is added to the phage lysate at a ratio of 1:50 (vol/vol) and incubated at 37°C for 5 min. Then the phage particles are pelleted by centrifugation at 4000× g for 5 min at room temperature. The phage pellet looks gray in color and settles on the wall or bottom of the tube.
3. Discard the supernatant and dissolve the pellet in 700 µL of TENS buffer (50 mM Tris-HCl, pH 8.0, 100 mM EDTA, 100 mM NaCl, 0.3% SDS) for each milliliter of phage lysate plus proteinase K at a final concentration of 100 µg/mL by vortex mixing and/or pipetting up and down. The solution should look clear. Then incubate at 65°C for 10 min.
4. Deproteinize the phage by extracting the solution with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) twice.
5. Collect the aqueous phase in a new tube, add an equal volume of isopropanol, mix gently and leave at room temperature for 5 min. The DNA usually is aggregated at this stage and can be picked up by a pipet with the tip cut off or pelletted at 4000× g for 10 min.
6. Wash the DNA pellet with 70% ethanol and resuspend in water or TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) with DNase-free RNase A at a final concentration of 50 µg/mL.

Figure 1. Digestions of λ DNA. Phages were amplified, and DNA was isolated from different clones derived from a λ-FIX genomic library as described in the above procedure. The isolated DNA was digested with EcoRI (lanes 1, 3, 5) and XhoI (lanes 2, 4, 6), then analyzed on a 0.8% agarose gel containing 0.5 µg/mL of ethidium bromide. Lane M: λ-HindIII markers.
ly obtain about 40 µg of DNA from a confluent-plate lysate (ca. 4–5 mL) and 150–300 µg of DNA from 50 mL of liquid lysate. The quality of DNA is suitable for restriction enzyme digestion, as shown in Figure 1, and other molecular biology applications such as cloning, Southern blot hybridization, sequencing and PCR (data not shown). Zinc chloride has been found to precipitate various biological macromolecules including DNA, bovine serum albumin (BSA) and phages other than λ, such as T4 and M13 (3,7). We have isolated single-stranded DNA from M13 by this method as well (data not shown).

We often encountered difficulties with amplifying phage reproducibly and obtaining enough λ DNA for cloning or mapping. By reviewing the present protocols, we have developed a streamlined procedure for phage amplification and DNA isolation. Two days after isolating phage plaques, we routinely obtain about 40 µg of DNA for cloning and mapping projects.

REFERENCES


We would like to thank H. Suh and K. Golden for trying out this method and providing us with the results and critical reading of the manuscript. We would also like to thank other members of Bodmer laboratory for helpful discussions. M.-T. Su is supported by a predoctoral fellowship from the Center for Organogenesis, University of Michigan. This work is supported by grants from the American Heart Association–Michigan and the National Institutes of Health. Address correspondence to Ming-Tsan Su, Department of Biology, 830 N. University Street, Natural Sciences Building, University of Michigan, Ann Arbor, MI 48109-1048, USA. Internet: mingtsan@biology.lsa.umich.edu

Received 19 December 1997; accepted 16 April 1998.

Ming-Tsan Su, Tyamagondlu V. Venkatesh and Rolf Bodmer
University of Michigan
Ann Arbor, MI, USA

Increased Efficiency of Liposome-Mediated Transfection by Volume Reduction and Centrifugation


Liposome-mediated transfection, or lipofection, has recently been widely and successfully applied to a variety of cell types (4,5,10). Although toxicity sometimes limits the applicability of lipofection, for those cell types that can withstand the treatment, lipofection often proves to be superior to other more classical transfection approaches such as calcium phosphate, DEAE-dextran sulfate and electroperoration (8,11–14). Even for cell types susceptible to lipofection, transfection efficiency often varies unpredictably between experiments, probably because of variations in liposome composition and target cell culture conditions.

While studying the efficacy of lipofection for transducing Cos-1 cells, we attempted to improve and regularize the efficiency of lipofection based on biophysical principles. Analogous to recent discussions regarding retroviral infection by Chuck et al. (3), we hypothesized that the relatively large size of the liposomes’ results directly limits how fast and how far they can diffuse through the liquid culture medium to reach their target cells. For liposomes ranging in size from 100 to 200 nm, the mean diffusion velocity is approximately 25–50 µm/h. Thus, during our transfections with durations of several hours, each liposome has only a finite chance of coming into contact with a Cos-1 cell. In this way, the size of the liposomes would obligately restrict the number of productive interactions between liposomes and target Cos-1 cells, thereby lowering the transfection efficiency. Such Brownian limitations have been demonstrated by several groups of investigators for retroviral infection (2,3,9), leading to the development of flow sedimentation and centrifugation techniques to improve retroviral infection. We therefore asked whether physical techniques might be used to force an increased interaction between lipo-