High Yield Method for Isolation of DNA

1. Single colony of host in 50mls LB supplemented with methio and myxyl (0.05% methio, 0.2% myxyl)

2. Incubate for 4-6 hrs at 37°C in vigorous shaking OD₆₀₀ = 0.8

3. Collect cells 4000g, 4°C 10 mins

4. Resuspend in ice-cold 10mM sodium phosphate 0.8 - 1.0

5. Pick single plaque and small amount of bacteria lawn and place in 1ml sterile 50% buffer

6. 37°C in shaking at 225rpm for at least 4 hrs

7. 1/10 vol phage to 0.5ml host bacteria 37°C 20 mins

8. Add this to 50mls LB with 0.3% glycerol 10mM sodium phosphate 37°C 280-320rpm for 14-16 hrs

9. Examine Culture for lysis. Continue growing for 1-2 hrs if no lysis dilute 1:3 to fresh LB in new flask and continue shaking 3-4 hrs. Proceed to DNA isolation

10. Add 0.5ml CitC13 and continue shaking for 30 mins.

11. Transfer to a 50ml Centrifuge tube and spin again 4000g 4°C 15 mins

12. Transfer to a fresh tube. Add 2.8g NaCl and shake 5 mins until dissolved. Add 5g PEG 8000 and mix gently for 30 mins at RT to until PEG dissolves. Place on ice for 1 hour

13. Pellet phage 4000g 15 mins. 4°C. Discard supernatant and leave tube inverted on paper towel 15 mins. Remove residual PEG from inside of tube with 2-3 tip or Kimwipe. Add 1ml 50% buffer to phage pellet and store at 0°C as stock

14. Transfer suspended phage to 1ml egg and add Dnase 1 2mg, Proteinase K 0.5g, and incubate at 37°C for 30 mins

15. Centrifuge 20000g 5 mins. Transfer Sup to fresh tube. Add SDS to 0.5%, EDTA 4H Lumin and Son proteinase K 0.5% for 30 mins

16. Extract 1x with Phenol: Chloroform (24) and 2x with Chloroform:IPA (24:1). Transfer to fresh tube each Time. Add 0.5 Vol 7.5 M NaAc at 2°C 4°C. 100% EtOH. mix gently and completely. -20°C 1 hr. 10000g at 4°C to pellet phage DNA

17. Resuspend pellet in 70% EtOH & Air-dry. Resuspend in Serial sterile TE buffer at 4°C for renaturation. Incubate 37°C 30 mins before use.
High-Yield Method for Isolation of \( \lambda \) DNA

Isolation of bacteriophage \( \lambda \) DNA is among the most common procedures used by those working with recombinant DNA methodologies. Although there are many protocols for \( \lambda \) 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 triturate 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.

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 4000x g, 4°C for 15 min to pellet the host cells.
3. Transfer 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 5 min at room temperature until PEG has dissolved. Incubate on ice for 1 hr.
4. Pellet the phage by centrifugation at 4000x 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 \( \mu \)L 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 \( \mu \)g/mL and RNase A to 50 \( \mu \)g/mL and incubate at 37°C for 30 min.
6. Centrifuge at 20000x 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 \( \mu \)g/mL. Incubate at 37°C for 30 min.
7. Extract the solution once with phenol/chloroform (1:1) and twice with chloroform/isooctyl 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 10000x g at 4°C to pellet the phage DNA.
8. Rinse the DNA pellet with 70% ethanol and air-dry. Resuspend in 500 \( \mu \)L 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 \( \mu \)g/mL 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)\(_{590}\) reaches but does not exceed, 0.3. Collect the cells by centrifugation at 4000x g, 4°C for 10 min. Resuspend in ice-cold 10 mM MgSO\(_4\) to a final OD\(_{590}\) 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 plate 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 \( \mu \)L 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

![Image of restriction digestion pattern](link-to-image)

**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 EcoRI. Lanes 2, 4, 6 and 8 were digested with PstI. M is the 1-kb DNA ladder (Life Technologies, Gaithersburg, MD, USA).
Benchmarks

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

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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-