SMALL-SCALE PREPARATIONS OF PLASMID DNA

Minipreparations of plasmid DNA can be obtained either by the alkaline lysis method presented below or by the boiling method (see page 1.29).

Harvesting and Lysis of Bacteria

HARVESTING

1. Transfer a single bacterial colony into 2 ml of LB medium containing the appropriate antibiotic in a loosely capped 15-ml tube. Incubate the culture overnight at 37C with vigorous shaking.

2. Pour 1.5 ml of the culture into a microfuge tube. Centrifuge at 12,000g for 30 seconds at 4C in a microfuge. Store the remainder of the culture at 4C.

3. Remove the medium by aspiration, leaving the bacterial pellet as dry as possible.

   The supernatant can be conveniently removed with a disposable pipette tip attached to a vacuum line (see Figure 1.3). Use a gentle vacuum and touch the tip to the surface of the liquid. Keep the tip as far away from the bacterial pellet as possible as the fluid is withdrawn from the tube. The pipette tip can then be used to vacuum the walls of the tube to remove any adherent droplets of fluid.

LYSIS BY ALKALI

This protocol is a modification of the methods of Birnboim and Doly (1979) and Ish-Horowicz and Burke (1981).

1. Resuspend the bacterial pellet (obtained in step 3 above) in 100 μl of ice-cold Solution I by vigorous vortexing.

   Solution I

   50 mM glucose
   25 mM Tris · Cl (pH 8.0)
   10 mM EDTA (pH 8.0)

   Solution I can be prepared in batches of approximately 100 ml, autoclaved for 15 minutes at 10 lb/sq. in. on liquid cycle, and stored at 4°C.

   It is essential to ensure that the bacterial pellet is completely dispersed in Solution I. This can be achieved rapidly by vortexing two microfuge tubes simultaneously with their bases touching as shown in Figure 1.2.

   The original protocol called for the use of lysozyme at this point to digest the bacterial cells. This step is unnecessary.

   Some strains of bacteria shed into the medium cell-wall components that can inhibit the action of restriction enzymes. This problem can be avoided by resuspending the bacterial cell pellet in 0.5 ml of STE (0.1 M NaCl, 10 mM Tris Cl [pH 8.0], 1 mM EDTA [pH 8.0]) and recentrifuging. After removal of the STE, resuspend the pellet in Solution I as described above.

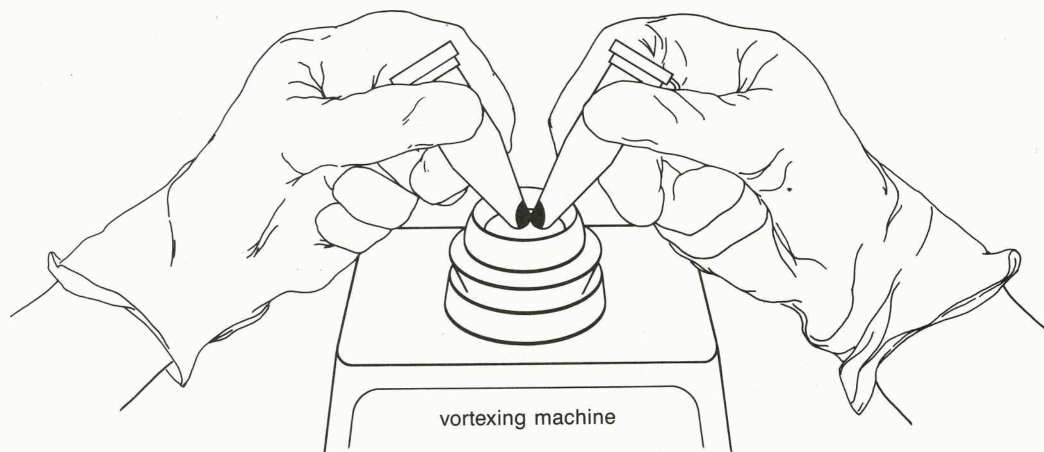

***FIGURE* 1.2**
Vortexing to disperse bacterial pellet.

2. Add 200 μl of freshly prepared Solution **II.**

> *Solution II*
>
> 0.2 N NaOH (freshly diluted from a 10 N stock)
> 1% SDS

Close the tube tightly, and mix the contents by inverting the tube rapidly five times. Make sure that the entire surface of the tube comes in contact with Solution **II.** Do not vortex. Store the tube on ice.

3. Add 150 μl of ice-cold Solution III.

> *Solution III*
>
> | | |
> |---|---|
> | 5 M potassium acetate | 60 ml |
> | glacial acetic acid | 11.5 ml |
> | H_2O | 28.5 ml |
>
> The resulting solution is 3 M with respect to potassium and 5 M with respect to acetate.

Close the tube and vortex it gently in an inverted position for 10 seconds to disperse Solution **III.** through the viscous bacterial lysate. Store the tube on ice for 3–5 minutes.

4. Centrifuge at 12,000g for 5 minutes at 4°C in a microfuge. Transfer the supernatant to a fresh tube.

5. Optional: Add an equal volume of phenol:chloroform. Mix by vortexing. After centrifuging at 12,000g for 2 minutes at 4°C in a microfuge, transfer the supernatant to a fresh tube.

Some workers find the extraction with phenol:chloroform to be unnecessary. How-

ever, for reasons that are unknown, the elimination of this step often results in DNA that is resistant to cleavage by restriction enzymes.

6. Precipitate the double-stranded DNA with 2 volumes of ethanol at room temperature. Mix by vortexing. Allow the mixture to stand for 2 minutes at room temperature.

7. Centrifuge at 12,000g for 5 minutes at 4°C in a microfuge.

8. Remove the supernatant by gentle aspiration. Stand the tube in an inverted position on a paper towel to allow all of the fluid to drain away. Remove any drops of fluid adhering to the walls of the tube.

   The supernatant can be conveniently removed with a disposable pipette tip attached to a vacuum line (Figure 1.3). Use a gentle vacuum and touch the tip to the surface

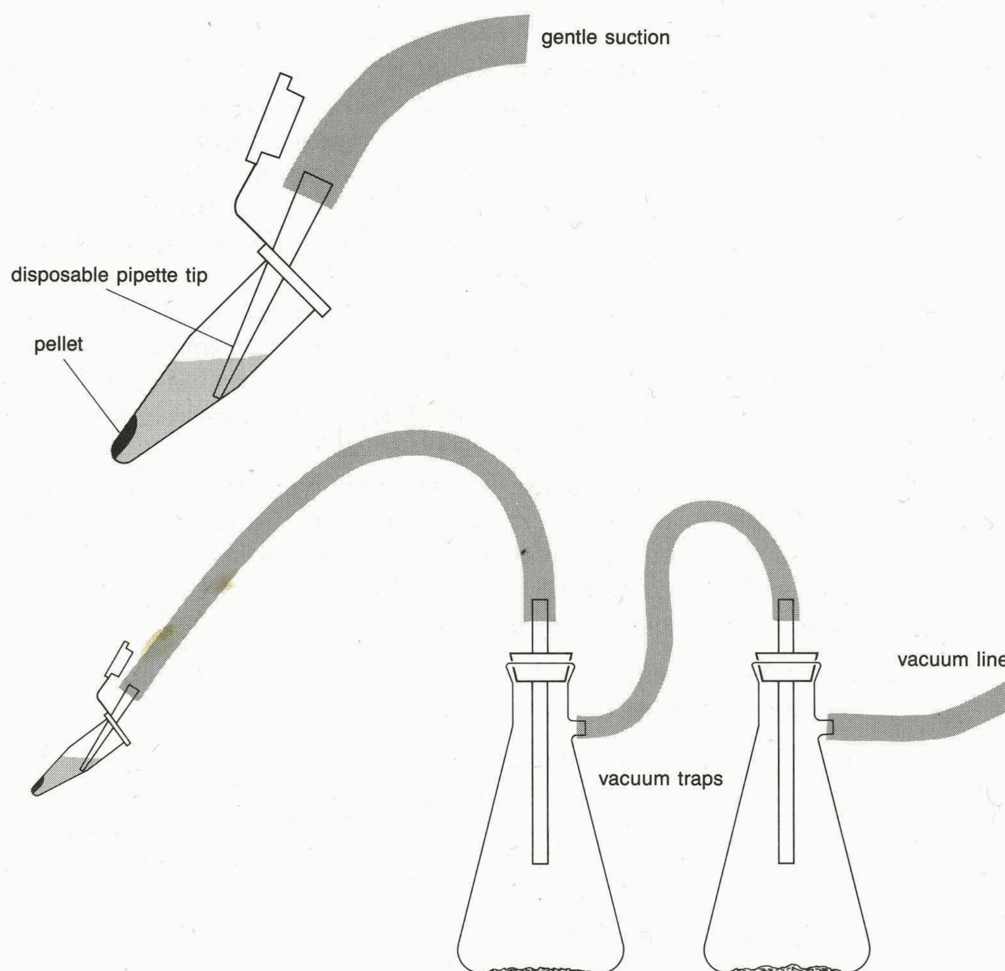

***FIGURE* 1.3**
Aspiration of supernatants. Hold the open microfuge tube at an angle, with the pellet on the upper side. Use a disposable pipette tip attached to a vacuum line to withdraw fluid from the tube. Insert the tip just beneath the meniscus on the lower side of the tube. Move the tip towards the base of the tube as the fluid is withdrawn. Use gentle suction to avoid drawing the pellet into the pipette tip. Keep the end of the tip away from the pellet. Finally, vacuum the walls of the tube to remove any adherent drops of fluid.

of the liquid. Keep the tip as far away from the pellet of nucleic acid as possible as the fluid is withdrawn from the tube. The pipette tip can then be used to vacuum the walls of the tube to remove any adherent droplets of fluid.

9. Rinse the pellet of double-stranded DNA with 1 ml of 70% ethanol at 4°C. Remove the supernatant as described in step 8, and allow the pellet of nucleic acid to dry in the air for 10 minutes.

10. Redissolve the nucleic acids in 50 μl of TE (pH 8.0) containing DNAase-free pancreatic RNAase (20 μg/ml) (see Appendix B). Vortex briefly. Store the DNA at −20°C.

Notes

i. The typical yield of high-copy-number plasmids, such as Xf3 or pUC, prepared by this method is about 3–5 μg of DNA per milliliter of original bacterial culture.

ii. To analyze the DNA by cleavage with restriction enzyme(s), remove 1 μl of the DNA solution and add it to a fresh microfuge tube that contains 8 μl of water. Add 1 μl of the appropriate 10 x restriction enzyme buffer and 1 unit of the desired restriction enzyme. Incubate the reaction for 1–2 hours at the appropriate temperature. Store the remainder of the DNA preparation at −20°C. Analyze the DNA fragments in the restriction digest by gel electrophoresis.

If the miniprep DNA is resistant to cleavage with the restriction enzyme, it is likely that insufficient care was taken to remove all of the fluid at step 3, page 1.25, or step 8 above. In this case, extract the final DNA preparation with phenol:chlorofom and reprecipitate the DNA with ethanol.

Frequently, difficulties in cleavage with restriction enzymes can be overcome by digesting in a volume of 100–200 μl and using five- to tenfold more enzyme (especially for inexpensive enzymes). After digestion, add 0.1 volume of 3 M sodium acetate (pH 5.2) and precipitate the DNA with 2 volumes of ethanol.

iii. The protocol can be modified to accommodate up to 10 ml of bacterial culture as follows:

a. Centrifuge 10 ml of bacterial culture at 4000 rpm for 10 minutes at 4°C in a Sorvall SS34 rotor (or its equivalent).

b. At step 1, resuspend the bacterial pellet in 200 μl of Solution I and transfer the suspension to a microfuge tube.

c. At step 2, add 400 μl of Solution II.

d. At step 3, add 300 μl of ice-cold Solution III.

e. At step 5, transfer 600 μl of the supernatant to a fresh tube.

f. At step 6, precipitate the DNA with 600 μl of isopropanol.

The yield of DNA is often sufficient to carry out the next step in a cloning protocol without going to the trouble or expense of a large-scale plasmid preparation.