Protocol 2

Preparation of Plasmid DNA by Alkaline Lysis with SDS: Midipreparation

PLASMID DNA MAY BE ISOLATED FROM INTERMEDIATE-SCALE (20–50 ml) bacterial cultures by treatment with alkali and SDS. The resulting DNA preparation is suitable for analysis by electrophoresis or restriction endonuclease digestion. After further purification by column chromatography, the preparations may be used to transfect mammalian cells.

MATERIALS

CAUTION: Please see Appendix 12 for appropriate handling of materials marked with <!>.

Buffers and Solutions

Please see Appendix 1 for components of stock solutions, buffers, and reagents. Dilute stock solutions to the appropriate concentrations.

Alkaline lysis solution I

For preparations of plasmid DNA that are to be subjected to further purification by chromatography (please see Protocol 9), sterile Alkaline lysis solution I may be supplemented just before use with the appropriate volume of 20 mg/ml DNase-free RNase A (pancreatic RNase) to give a final concentration of 100 µg/ml. Addition of RNase is not recommended at this stage if the DNA is to be further purified by other methods (please see Protocols 10 and 11).

Alkaline lysis solution II

Solution II should be freshly prepared and used at room temperature.

Alkaline lysis solution III

Antibiotic for plasmid selection
Ethanol
Isopropanol
Phenol:chloroform (1:1, v/v) <!>

STE

Optional, please see Step 3.

TE (pH 8.0) containing 20 µg/ml RNase A

Media

LB, YT, or Terrific Broth

Centrifuges and Rotors

Sorvall SS-34 rotor or equivalent
METHOD

Preparation of Cells

1. Inoculate 10 ml of rich medium (LB, YT, or Terrific Broth) containing the appropriate antibiotic with a single colony of transformed bacteria. Incubate the culture overnight at 37°C with vigorous shaking.

To ensure that the culture is adequately aerated:
- The volume of the culture tube should be at least four times greater than the volume of the bacterial culture.
- The tube should be loosely capped.
- The culture should be incubated with vigorous agitation.

2. Transfer the culture into a 15-ml tube and recover the bacteria by centrifugation at 2000g (4000 rpm in a Sorvall SS-34 rotor) for 10 minutes at 4°C.

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

This step can be conveniently accomplished with a disposable pipette tip or Pasteur pipette attached to a vacuum line and a side arm flask (please see Figure 1-7). 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. This minimizes the risk that the pellet will be sucked into the side arm flask. Alternatively, remove the supernatant using a pipettor or Pasteur pipette and bulb. Use the pipette tip to vacuum the walls of the tube to remove any adherent droplets of fluid.

The penalty for failing to remove all traces of medium from the bacterial pellet is a preparation of plasmid DNA that is resistant to cleavage by restriction enzymes. This is because cell-wall components in the medium inhibit the action of many restriction enzymes. This problem can be avoided by resuspending the bacterial pellet in ice-cold STE (0.25x volume of the original bacterial culture) and centrifuging again.

Lysis of Cells

4. Resuspend the bacterial pellet in 200 µl of ice-cold Alkaline lysis solution I by vigorous vortexing, and transfer the suspension to a microfuge tube.

Make sure that the bacterial pellet is completely dispersed in Alkaline lysis solution I. Vortexing two microfuge tubes simultaneously with their bases touching increases the rate and efficiency with which the bacterial pellets are resuspended.

The original protocol (Birnboim and Doly 1979) called for the use of lysozyme at this point to assist in dissolution of the bacterial cell walls. This step can be safely omitted when dealing with bacterial cultures of less than 10 ml in volume.

5. Add 400 µl of freshly prepared Alkaline lysis solution II to each bacterial suspension. Close the tube tightly, and mix the contents by inverting the tube rapidly five times. Do not vortex! Store the tube on ice.

Make sure that the entire surface of the tube comes in contact with Alkaline lysis solution II.

6. Add 300 µl of ice-cold Alkaline lysis solution III. Close the tube and disperse Alkaline lysis solution III through the viscous bacterial lysate by inverting the tube several times. Store the tube on ice for 3–5 minutes.

7. Centrifuge the bacterial lysate at maximum speed for 5 minutes at 4°C in a microfuge. Transfer 600 µl of the supernatant to a fresh tube.
8. Add an equal volume of phenol:chloroform. Mix the organic and aqueous phases by vortexing and then centrifuge the emulsion at maximum speed for 2 minutes at 4°C in a microfuge. Transfer the aqueous upper layer to a fresh tube.

Recovery of Plasmid DNA

9. Precipitate nucleic acids from the supernatant by adding 600 µl of isopropanol at room temperature. Mix the solution by vortexing and then allow the mixture to stand for 2 minutes at room temperature.

10. Collect the precipitated nucleic acids by centrifugation at maximum speed for 5 minutes at room temperature in a microfuge.

11. Remove the supernatant by gentle aspiration as described in Step 3 above. 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.

12. Add 1 ml of 70% ethanol to the pellet and recover the DNA by centrifugation at maximum speed for 2 minutes at room temperature in a microfuge.

13. Again remove all of the supernatant by gentle aspiration as described in Step 3.

Take care with this step, as the pellet sometimes does not adhere tightly to the tube.

14. Remove any beads of ethanol that form on the sides of the tube. Store the open tube at room temperature until the ethanol has evaporated and no fluid is visible in the tube (2–5 minutes).

   If the pellet of DNA is dried in a desiccator or under vacuum, it becomes difficult to dissolve under some circumstances and may denature (Svaren et al. 1987). Drying the pellet for 10–15 minutes at room temperature is usually sufficient for the ethanol to evaporate without the DNA becoming dehydrated.

15. Dissolve the nucleic acids in 100 µl of TE (pH 8.0) containing 20 µg/ml DNase-free RNase A (pancreatic RNase). Vortex the solution gently for a few seconds. Store the DNA solution at -20°C.

   For recommendations on troubleshooting, please see Table 1-5 in Protocol 3.