

## SMALL SCALE PREPARATIONS OF PLASMID DNA

Minipreparations of plasmid DNA can be obtained either by the alkaline lysis method presented below or by a commercial preparation.

---

### Harvesting and Lysis of Bacteria

A Similar protocol can be found in **DNA Science a First Course in Recombinant DNA Technology** by Micklos and Freyer

#### 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 37 degrees C with vigorous shaking.
2. Pour 1.5 ml of the culture into a microfuge tube. Centrifuge at 12,000 g for 30 seconds at 4 degrees C in a microfuge. Store the remainder of the culture at 4 degrees C.
3. Remove the medium by aspiration, leaving the bacterial pellet as dry as possible.

#### 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 three above) in 100 microliters of ice-cold solution I by vigorous vortexing.

Solution I 50 mM glucose

25 mM Tris HCl (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 degrees C.

2. Add 200 micro liters of freshly prepared Solution II.

Solution II

0.2 N NaOH (freshly diluted from a 10 N stock)

1% SDS

3. 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.
4. Add 150 microliters of ice-cold Solution III.

#### Solution III

5 M potassium acetate 60 ml

glacial acetic acid 11.5 ml

H<sub>2</sub>O 28.5 ml

The resulting solution is 3 M with respect to potassium and 5 M with respect to acetate.

5. 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.
6. Centrifuge at 12,000g for 5 minutes at 4 degrees C in a microfuge. Transfer the supernatant to a fresh tube.
7. OPTIONAL: Add an equal volume of phenol: chloroform. Mix by vortexing. After centrifuge at 12,000g for 2 minutes at 4 degrees C in a microfuge, transfer the supernatant to a fresh tube.
8. 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.
9. Centrifuge at 12,000g for 5 minutes at 4 degrees C in a microfuge.
10. 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.
11. Rinse the pellet of double-stranded DNA with 1 ml of 70 percent ethanol at 4 degrees C. Remove the supernatant as described in step 8, and allow the pellet of nucleic acid to dry in the air for 10 minutes.
12. Redissolve the nucleic acid in 50 µl of TE (pH 8.0) containing DNAase free pancreatic RNAase (20 micrograms/ml). Vortex briefly. Store the DNA at -20 degrees C.

**Sambrook, J., Fritsch, EF, and Maniatis, T. (1989). Molecular Cloning: A laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.**