

and to 100°.

1. Resuspend the bacterial pellet in 30 ml of an ice-cold solution of 10% sucrose in 50 mM Tris-Cl, pH 8.0. Transfer the suspension to a 50-ml tube. Repeat once.
2. Add 5 ml of a freshly made solution of lysozyme (20 mg/ml in 0.25 M Tris-Cl, pH 8.0).
3. Add 4 ml of 0.25 M EDTA. Mix by inverting the tube several times. Place on ice for 30 minutes at 0°.
4. Add 4 ml of 10% SDS. Mix quickly with a glass rod or as in dispersing the cells slowly through the bacterial suspension and gently mix at ice to disperse the bacterial bacterial DNA.
5. Immediately add 4 ml of 2 M NaCl (final concentration = 1 M). Again, mix gently but thoroughly. Place on ice for at least 1 hour.
6. Centrifuge to remove high-molecular-weight DNA and bacterial debris in a Beckman Tyro-40 rotor (or its equivalent) for 30 minutes at 30,000 rpm at 0°.
7. Pour off and save the supernatant. Discard the pellet, which should be firm and light.
8. Extract the supernatant twice with phenol:chloroform and once with chloroform. After each extraction, transfer the aqueous layer to a clean tube.
9. Transfer the aqueous phase to a 50-ml glass centrifuge bottle. Add 2 volumes (40 ml) of ethanol. Mix well. Let stand at -80° for 1-2 hours, or at -20° for 24 hours.
10. Remove the supernatant with centrifugation at 100g for 10 minutes at 0°.
11. Dissolve the supernatant. Wash the pellet with 10% ethanol at room temperature. Discard as much of the ethanol as possible. Dry the pellet briefly in a vacuum desiccator.
12. Dissolve the DNA in a total volume of 4 ml of TE (pH 8.0).
13. Purify the purified DNA by centrifugation to equilibrium in cesium chloride-ethidium bromide gradients (see page 68).

Ward and Tabor (1975)

PURIFICATION OF CLOSED CIRCULAR DNA BY CENTRIFUGATION TO EQUILIBRIUM IN CESIUM CHLORIDE-ETHIDIUM BROMIDE GRADIENTS

1. Measure the volume of the DNA solution. For every milliliter add exactly 1 g of solid cesium chloride. Mix gently until all of the salt is dissolved.
2. Add 0.5 ml of a solution of ethidium bromide (10 mg/ml in 0.4 M for every 10 ml of cesium chloride solution. Mix well. The final density of the solution should be 1.30 g/ml (or 1.0000), and the concentration of ethidium bromide should be approximately 0.06 µg/ml.

Note. The heavy, purple aggregates that float to the top of the solution are complexes formed between the ethidium bromide and bacterial proteins.

3. Transfer the cesium chloride solution together with the protein aggregates to a tube suitable for centrifugation in a Beckman Tyro-40 rotor. Fill the remainder of the tube with light paraffin oil.
4. Centrifuge at 45,000 rpm for 36 hours at 30°.
5. Two bands of DNA should be visible in ordinary light. The upper band consists of linear bacterial DNA and nicked circular plasmid DNA; the lower band consists of closed circular plasmid DNA.
6. Remove the cap from the tube. Collect the lower band of DNA into a glass tube through a #21 hypodermic needle inserted into the side of the tube as described on page 69.



Figure 2.3

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Judith Giles Morgan



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