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Molecular Cloning

A LABORATORY MANUAL

SECOND EDITION

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Isolation of DNA from Mammalian Cells: Protocol I

The following procedure is modified from Blin and Stafford (1976).

1. Depending on the type of sample, carry out one of the following procedures as step 1.

For cell samples

Cells growing in monolayers: Wash the monolayers twice with ice-cold Tris-buffered saline (TBS; see Appendix B). Using a policeman, scrape the cells into approximately 0.5 ml of TBS. Transfer the cell suspension to a centrifuge tube stored on ice. Wash the petri dish with 1 ml of TBS, and combine the washing with the cell suspension in the centrifuge tube. Recover the cells by centrifugation at 1500g for 10 minutes at 4°C. Resuspend the cells in 5–10 volumes of ice-cold TBS and repeat the centrifugation. Resuspend the cells in TE (pH 8.0) at a concentration of 5×10^7 cells/ml. Transfer the solution to an Erlenmeyer flask. (For 1 ml of cell suspension, use a 50-ml flask; for 2 ml, use a 100-ml flask; etc.) Add 10 ml of extraction buffer for each milliliter of cell suspension. Incubate the solution for 1 hour at 37°C, and then proceed to step 2.

Extraction buffer

10 mM Tris · Cl (pH 8.0)
0.1 M EDTA (pH 8.0)
20 µg/ml pancreatic RNAase
0.5% SDS

It is important that the cells be well-dispersed over the inner surface of the Erlenmeyer flask when the extraction buffer is added. This minimizes the formation of intractable lumps of DNA.

Blin and Stafford (1976) recommend the use of 0.5 M EDTA (pH 8.0) in the extraction buffer. However, the density of this solution almost equals that of phenol, which makes separation of the phases in step 4 difficult. In our hands, 0.1 M EDTA (pH 8.0) is equally effective in maintaining high-molecular-weight DNA and permits easier separation of the phenolic phase.

Pancreatic RNAase is not highly active in the presence of 0.5% SDS, but when added at high concentrations it works well enough to degrade most of the cellular RNA. Adding RNAase at this stage eliminates the need to treat semipurified DNA with the enzyme subsequently. To eliminate contaminating DNA, use RNAase that has been treated as described in Appendix B.

Cells growing in suspension: Recover the cells by centrifugation at 1500g for 10 minutes at 4°C. Resuspend the cells in a volume of ice-cold TBS equal to the volume of the original culture. Recover the cells by re-centrifugation, and repeat the washing procedure. Resuspend the cells in TE (pH 8.0) at a concentration of 5×10^7 cells/ml. Transfer the suspension to an Erlenmeyer flask of the appropriate size (see above). Add extraction buffer and incubate as described above.