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 1500*g* 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)	50µl 2.0 M
0.1 M EDTA (pH 8.0)	2 ml 0.5 M
20 µg/ml pancreatic RNAase	20 µl 10 mg/ml
0.5% SDS	250 µl 20%
	To 10 ml.

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 1500*g* 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.

2. Add proteinase K to a final concentration of 100 μg/ml. Using a glass rod, gently mix the enzyme into the viscous solution.

   Proteinase K is stored as a stock solution at a concentration of 20 mg/ml in H_2O (see Appendix B).

3. Place the suspension of lysed cells in a water bath for 3 hours at 50°C. Swirl the viscous solution periodically.

4. Cool the solution to room temperature, and, if necessary, pour the solution into a centrifuge tube. Add an equal volume of phenol equilibrated with 0.5 M Tris · Cl (pH 8.0) (see Appendix B) and *gently* mix the two phases by *slowly* turning the tube end over end for 10 minutes. If the two phases have not formed an emulsion at this stage, place the tube on a roller apparatus for 1 hour. Separate the two phases by centrifugation at 5000*g* for 15 minutes at room temperature.

   It is essential that the pH of the phenol be approximately 8.0 to prevent DNA from becoming trapped at the interface between the organic and aqueous phases.

5. With a wide-bore pipette (0.3-cm-diameter orifice), transfer the viscous aqueous phase to a clean centrifuge tube and repeat the extraction with phenol twice.

   When transferring the aqueous phase, it is essential to draw the DNA into the pipette very slowly to avoid disturbing the material at the interface. If the DNA solution is so viscous that it cannot easily be drawn into a wide-bore pipette, use a long pipette attached to a water-suction vacuum pump to remove the organic phase. *Make sure that the phenol is collected into traps and does not enter the water line.*
   With the vacuum line closed, slowly lower the pipette to the bottom of the organic phase. Wait until the viscous thread of aqueous material detaches from the pipette, and then carefully open the vacuum line and gently withdraw all of the organic phase. Close the vacuum line and quickly withdraw the pipette through the aqueous phase. Immediately open the vacuum line to transfer the residual phenol into the trap. Centrifuge the DNA solution at 5000*g* for 20 minutes at room temperature. Protein and clots of DNA sediment to the bottom of the tube. Pour the DNA solution into a 50-ml centrifuge tube, leaving behind the protein and clots of DNA.

6. *To isolate very-high-molecular-weight DNA (~200 kb):* After the third extraction with phenol, dialyze the pooled aqueous phases at 4°C four times against 4 liters of a solution of 50 mM Tris · Cl (pH 8.0), 10 mM EDTA (pH 8.0) until the OD_{270} of the dialysate is less than 0.05. Allow room in the dialysis bag for the volume of the sample to increase 1.5- to 2.0-fold. Continue at step 7.

   To isolate DNA whose size is 100–150 kb: After the third extraction with phenol, transfer the pooled aqueous phases to a fresh centrifuge tube and add 0.2 volume of 10 M ammonium acetate. Add 2 volumes of ethanol at room temperature and swirl the tube until the solution is thoroughly mixed. The DNA will immediately form a precipitate that can usually be removed from the ethanolic solution with a pasteur pipette whose end has been sealed and shaped into a U. Most of the contaminating oligoribonucleotides are left behind. If the DNA precipitate becomes fragmented, collect it by centrifugation at 5000*g* for 5 minutes at room temperature in

a swinging-bucket rotor. Wash the DNA precipitate twice with 70% ethanol, and collect the DNA by centrifugation as described above. Remove as much as possible of the 70% ethanol, and store the pellet in an open tube at room temperature until the last visible traces of ethanol have evaporated. *Do not allow the pellet of DNA to dry completely; otherwise, it will be very difficult to dissolve.*

Add 1 ml of TE (pH 8.0) for each $\sim 5 \times 10^6$ cells. Place the tube on a rocking platform and gently rock the solution until the DNA has completely dissolved. This usually takes 12–24 hours.

7. Measure the absorbance of the DNA at 260 nm and 280 nm. The ratio of A_{260} to A_{280} should be greater than 1.75. A lower ratio is an indication that significant amounts of protein remain in the preparation. In this case, add SDS to a concentration of 0.5% and then repeat steps 2–7.

8. Calculate the concentration of the DNA (a solution with an OD_{260} of 1 contains approximately 50 μg of DNA per milliliter), and analyze an aliquot by pulsed-field gel electrophoresis or by electrophoresis through a 0.3% agarose gel poured on a 1% agarose support (see Chapter 6, pages 6.9–6.13). The DNA should be larger than 100 kb in size and should migrate more slowly than linear dimeric molecules of intact bacteriophage λ DNA. Store the DNA at 4°C.

Notes

i. Starting with 5×10^7 cultured aneuploid mammalian cells (e.g., HeLa cells), this method should yield approximately 200 μg of high-molecular-weight DNA. The usual yield of DNA from 20 ml of normal blood is approximately 250 μg.

ii. High-molecular-weight markers can be synthesized rapidly by incubating linear bacteriophage λ DNA (20 μg/ml) for 30 minutes at 16°C in 1× bacteriophage T4 DNA ligase buffer (see Appendix B) containing 1 mM ATP and 2 Weiss units/ml bacteriophage T4 DNA ligase.

Before ligation, heat the bacteriophage λ DNA to 56°C for 5 minutes in TE (pH 7.6) to melt the cohesive termini. At the end of the ligation reaction, add EDTA to a final concentration of 0.01 M and heat the solution to 65°C for 15 minutes to inactivate the ligase.