PURIFICATION OF NUCLEIC ACIDS

Perhaps the most basic of all procedures in molecular cloning is the purification of nucleic acid. The key step, the removal of proteins, can often be carried out simply by extracting aqueous solutions of nucleic acids with phenol and/or chloroform. Such extractions are used whenever it is necessary to inactivate and remove enzymes that are used in one step of a cloning operation before proceeding to the next. However, additional measures are required when nucleic acids are purified from complex mixtures of molecules such as cell lysates. In these cases (see Chapter 9), it is usual to remove most of the protein by digesting with proteolytic enzymes such as pronase or proteinase K (see Table A.4), which are active against a broad spectrum of native proteins, before extracting with organic solvents.

Extraction with Phenol/Chloroform

The standard way to remove proteins from nucleic acid solutions is to extract once with phenol, once with a 1:1 mixture of phenol and chloroform, and once with chloroform. This procedure takes advantage of the fact that deproteinization is more efficient when two different organic solvents are used instead of one. Furthermore, although phenol denatures proteins efficiently, it does not completely inhibit RNase activity, and it is a solvent for RNA molecules that contain long tracts of poly(A) (Brawerman et al. 1972). Both of these problems can be circumvented by using a mixture of phenol and chloroform (1:1). Also, the final extraction with chloroform removes any lingering traces of phenol from the nucleic acid preparation.

Remember that "chloroform" means a 24:1 (v/v) mixture of chloroform and isoamyl alcohol. "Phenol" means phenol equilibrated with buffer and containing 0.1% hydroxyquinoline and 0.2% β-mercaptoethanol (see page 438).

1. Mix the DNA sample with an equal volume of phenol or phenol/chloroform in a polypropylene tube with a plastic cap.

2. Mix the contents of the tube until an emulsion forms (see note below).

3. Centrifuge for 3 minutes at 1600g or for 15 seconds in an Eppendorf centrifuge at room temperature. If the organic and aqueous phases are not well-separated, centrifuge again for a longer time or at a higher speed.

4. Use a pipette to transfer the upper, aqueous phase to a fresh polypropylene tube. For small volumes ($<$200 μl), use an automatic pipettor fitted with a disposable tip. Discard the interface and lower organic phase.

Note. To achieve the best recovery, the organic phase and interface may be "back-extracted" as follows. After the first aqueous phase has been transferred as described above, add an equal volume of TE (pH 7.8) to the organic phase and interface. Mix well. Separate the phases by centrifugation. Combine the second aqueous phase with the first and proceed to step 5.

5. Add an equal volume of a 1:1 mixture of phenol and chloroform. Repeat steps 2, 3, and 4.

6. Add an equal volume of chloroform and repeat steps 2, 3, and 4.

7. Recover the DNA by precipitation with ethanol as described on page 461.

Note

The organic and aqueous phases may be mixed by vortexing when isolating small DNAs (< 10 kb) or by gentle shaking when isolating DNAs of moderate size (10–30 kb).

More extensive precautions are necessary to avoid shearing large (> 30 kb) DNA molecules:

a. The organic and aqueous phases should be mixed by rotating the tube slowly on a wheel (20 rpm).

b. Large-bore pipettes should be used to transfer the DNA from one tube to another.

c. The DNA should not be precipitated with ethanol (step 7). Instead, traces of chloroform should be removed either by dialyzing the DNA solution extensively against large volumes of ice-cold TNE *or* by extraction with water-saturated ether.

Extraction of Phenol/Chloroform with Water-saturated Ether

Ether can be used to remove traces of phenol or chloroform from DNA solutions. Ether is highly volatile and extremely flammable and should be worked with and stored in an explosion-proof chemical hood.

1. Combine the DNA sample with an equal volume of water-saturated ether. Mix. Let the organic and aqueous phases separate by standing for 2–5 minutes.

2. Remove and discard the upper layer (ether is less dense than water).

3. Repeat steps 1 and 2.

4. Remove traces of ether by heating the DNA solution to 68°C for 5–10 minutes with gentle mixing; or by blowing a stream of nitrogen gas over the surface of the solution for 10–30 minutes.

5. Precipitate the DNA with ethanol or (in the case of high-molecular-weight DNA) dialyze extensively against ice-cold TE (pH 7.8) containing 0.1 M NaCl (STE).