CONCENTRATION OF NUCLEIC ACIDS

Precipitation with Ethanol or Isopropanol

The most widely used method for concentrating DNA is precipitation with ethanol. The precipitate of DNA, which is allowed to form at low temperature (−20°C or less) in the presence of moderate concentrations of monovalent cations, is recovered by centrifugation and redissolved in an appropriate buffer at the desired concentration. The technique is rapid and is quantitative even with nanogram amounts of DNA.

1. Estimate the volume of the DNA solution.

2. Adjust the concentration of monovalent cations either by dilution with TE (pH 8.0) if the DNA solution contains a high concentration of salts or by addition of one of the salt solutions shown in Table A.5.

3. Mix well. Add exactly 2 volumes of ice-cold ethanol and mix well. Chill to −20°C.

4. Store at low temperature to allow the DNA precipitate to form. Usually 30–60 minutes at −20°C is sufficient, but when the size of the DNA is small (< 1 kb) or when it is present in small amounts (< 0.1 μg/ml), the period of storage should be extended and the temperature should be lowered to −70°C.

5. Centrifuge at 0°C. For most purposes, 10 minutes in an Eppendorf centrifuge or at 12,000*g* is sufficient. However, when low concentrations of DNA or very small fragments are being processed, more extensive centrifugation (e.g., Beckman SW50.1 at 30,000 rpm for 30 minutes) may be required.

6. Discard the supernatant. Stand the tube in an inverted position on a layer of absorbent paper to allow as much of the supernatant as possible to drain away. Use capillary pipettes to remove any drops of fluid that adhere to the walls of the tube. Traces of supernatant may be removed by brief treatment (1–2 minutes) in a vacuum desiccator or lyophilizer.

TABLE A.5. SALT SOLUTIONS

	Concentrated solution	Final solution
Sodium acetate	2.5 M (pH 5.2)	0.25 M
Sodium chloride	5.0 M	0.1 M
Ammonium acetate	10.5 M	2.0 M

7. Dissolve the DNA pellet (which is often invisible) in the desired volume of buffer. Rinse the walls of the tube well with the buffer or scrape them with a sealed pipette to aid in the recovery of the DNA. The sample can be heated to 37°C for 5 minutes to assist in dissolving the pellet.

Notes

i. Isopropanol (1 volume) may be used in place of ethanol (2 volumes) to precipitate DNA. Precipitation with isopropanol has the advantage that the volume of liquid to be centrifuged is smaller. However, isopropanol is less volatile than ethanol and it is more difficult to remove the last traces; moreover, solutes such as sucrose or sodium chloride are more easily coprecipitated with DNA when isopropanol is used, especially at −70°C. In general, precipitation with ethanol is preferable unless it is necessary to keep the volume of fluid to a minimum.

ii. To remove any solutes that may be trapped in the precipitate, the DNA pellet may be washed with a solution of 70% ethanol. To make certain that no DNA is lost during washing, add 70% ethanol until the tube is $^2/_3$ full. Vortex briefly, and recentrifuge as described above. After the 70% ethanol wash, the pellet does not adhere tightly to the wall of the tube, so great care must be taken when removing the supernatant.

iii. Very short DNA molecules (<200 bp) are precipitated inefficiently by ethanol. However, adjusting the DNA solution to 0.01 M $MgCl_2$ before addition of ethanol considerably improves the efficiency with which small DNA molecules are recovered.

iv. In general, DNA precipitated from solution by ethanol can be easily redissolved in buffers of low ionic strength such as TE. Occasional difficulties arise when buffers containing $MgCl_2$ or >0.1 M NaCl are added directly to the DNA pellet. It is therefore preferable to dissolve the DNA in a small volume of low-ionic-strength buffer and to adjust the composition of the buffer later. If the sample does not easily dissolve in a small volume, add a larger volume of buffer and repeat the precipitation with ethanol.

v. To be precipitated from solution, RNA requires slightly higher concentrations of ethanol (2.5 volumes) than does DNA.

vi. Triphosphate can be removed from DNA by two sequential precipitations with ethanol from DNA solutions containing 2 M ammonium acetate.

Concentration by Extraction with Butanol

During extraction of aqueous solutions with solvents such as secondary butyl alcohol (2-butanol) or *n*-butyl alcohol (1-butanol), some of the water molecules (but not DNA or solutes) become partitioned into the organic phase. By carrying out several cycles of extraction, the volume of a DNA solution can be reduced significantly. This method of concentrating DNA is used to reduce the volume of dilute DNA solutions to the point where the DNA can be easily recovered by precipitation with ethanol.

1. Add an equal volume of 2-butanol to the DNA sample and mix well.

   Note. Addition of too much 2-butanol can result in removal of all the water and precipitation of the DNA.

2. Centrifuge at 1600*g* for 1 minute. Remove and discard the upper (2-butanol) phase.

3. Repeat steps 1 and 2 until the desired volume is achieved.

4. Extract the sample twice with water-saturated ether to remove the 2-butanol. Remove the ether by evaporation.

Note

Because 2-butanol extraction does not remove salt, the salt concentration increases in proportion to the reduction in volume of the solution. Therefore, adjust the buffer concentration by dialysis or recover the DNA by precipitation with ethanol.