Protocol

Precipitation of DNA with Ethanol

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DNA can be precipitated out of solution for the removal of salts and/or for resuspension in an alternative buffer. Either ethanol or isopropanol can be used to achieve this purpose; however, the use of ethanol is generally preferred. Cations, provided as salts, are typically included to neutralize the negative charge of the DNA phosphate backbone. This method describes ethanol precipitation of DNA in microcentrifuge tubes.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous material used in this protocol.

RECIPE: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Carrier (yeast tRNA, glycogen, or linear polyacrylamide; see Table 1) (optional; see Step 3 and Discussion)
DNA sample
Ethanol (95%, ice cold; 70%)
Isopropanol (optional; see Step 9)
$MgCl_2$ (1 M) (optional; see Step 3)
Salt solution (10 M ammonium acetate, 8 M LiCl, 5 M NaCl, or 3 M sodium acetate; see Table 2) (optional; see Step 2 and Discussion)
TE buffer, 10× <R> (pH 8.0)

Equipment

Container with ice
Vacuum aspirator equipped with traps (optional; see Step 5)

METHOD

1. Estimate the volume of the DNA solution.

From the Molecular Cloning collection, edited by Michael R. Green and Joseph Sambrook.

Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot093377

TABLE 1. Carriers used in ethanol precipitation

Carrier	Working concentration	Advantages and disadvantages
Yeast tRNA	10–20 µg/mL	Inexpensive, but cannot be used in precipitations of nucleic acids that will be used as substrates in reactions catalyzed by polynucleotide kinase or terminal transferase
Glycogen	50 µg/mL	Used when nucleic acids are precipitated with ammonium acetate and isopropanol; does not compete with nucleic acid substrates in enzyme-catalyzed reactions. However, glycogen can interfere with DNA–protein interactions (Gaillard and Strauss 1990).
Linear polyacrylamide	10–20 µg/mL	An efficient neutral carrier; used only when precipitating very small (picogram) quantities of nucleic acids (Strauss and Varshavsky 1984; Gaillard and Strauss 1990)

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 the appropriate amount of one of the salt solutions shown in Table 2 (see Discussion).

   If the volume of the final solution is 400 µL or less, perform precipitation in a single microcentrifuge tube. Larger volumes can be divided among several microcentrifuge tubes, or the DNA can be precipitated and centrifuged in tubes that will fit in a medium-speed centrifuge or ultracentrifuge.

3. Mix the solution well. Add exactly 2 volumes of ice-cold ethanol and again mix the solution well. (Optional: Add carrier DNA at the appropriate concentration; see Table 1 and Discussion.) Store the ethanolic solution on ice to allow the precipitate of DNA to form.

   Usually 15–30 min is sufficient, but when the size of the DNA is small (<100 nt) or when it is present in small amounts (<0.1 µg/mL), extend the period of storage to at least 1 h and add $MgCl_2$ to a final concentration of 0.01 M.

   DNA can be stored indefinitely in ethanolic solutions at 0°C or at −20°C.

4. Recover the DNA by centrifugation at 0°C.

   For most purposes, centrifugation at maximum speed for 10 min in a microcentrifuge is sufficient. However, as discussed above, when low concentrations of DNA (<20 ng/mL) or very small fragments are being processed, more extensive centrifugation may be required.

5. Carefully remove the supernatant with an automatic micropipettor or with a disposable pipette tip attached to a vacuum line. Take care not to disturb the pellet of nucleic acid (which may be invisible). Use the pipette tip to remove any drops of fluid that adhere to the walls of the tube.

   It is best to save the supernatant from valuable DNA samples until recovery of the precipitated DNA has been verified.

6. Fill the tube halfway with 70% ethanol and recentrifuge at maximum speed for 2 min at 4°C in a microcentrifuge.

7. Repeat Step 5.

8. Store the open tube on the bench at room temperature until the last traces of fluid have evaporated.

   It was once common practice to dry pellets of nucleic acid in a lyophilizer. This step is not only unnecessary, but also undesirable, because it causes denaturation of small (<400 nt) fragments of DNA (Svaren et al. 1987) and greatly reduces the recovery of larger fragments of DNA.

   See Discussion.

TABLE 2. Cations commonly used in ethanol precipitation of DNA

Salt	Stock solution (M)	Final concentration (M)
Ammonium acetate	10.00	2.0–2.5
Lithium chloride	8.0	0.8
Sodium chloride	5.0	0.2
Sodium acetate (pH 5.2)	3.0	0.3

BOX 1. HISTORICAL FOOTNOTE

Ethanol precipitation predates molecular cloning by ~50 yr. It was first used as a method to concentrate biologically active nucleic acid by J. Lionel Alloway, who worked at the Rockefeller Institute in the early 1930s. His project was to prepare active cell-free extracts of S-type *Streptococcus pneumoniae* that would permit bacterial transformation of R-type organisms in vitro. At that time, transformation had been achieved only with intact, heat-killed donor cells. After many frustrating failures, Alloway reported in 1932 that he could get the substance responsible for transformation into solution by heating a freeze/thaw extract of the S organisms to 60°C, removing particulate matter by centrifugation, and passing the solution through a filter made of porous porcelain (Alloway 1932). This last step was included to silence skeptics who believed that transformation was an artifact caused by an occasional S-type organism that survived the extraction procedure.

Alloway's success at eliminating the need for heat-killed donor cells was a major step on the road that eventually led to the discovery of DNA as the transforming material (Avery et al. 1944). However, not all of Alloway's cell-free preparations worked, and, even when transformation was obtained, the efficiency was very low. Alloway must have realized that these problems were caused by the dilute nature of the extract, for he began to search for different ways to lyse the pneumococci and for different methods to concentrate the transforming activity. Alloway (1933) describes how, after addition of ethanol, "a thick stringly precipitate formed, which slowly settled out after standing." Alloway's colleague Maclyn McCarty (1985) subsequently described the discovery of ethanol precipitation as follows:

> Alloway then introduced another new procedure that became an indispensable part of all work on the transforming substance from that time forward. He added pure alcohol in a volume five times that of the extract which resulted in precipitation of most of the material that had been released from the pneumococci…. The precipitated material could be redissolved in salt solution and shown to contain the active substance in transformation tests. Alcohol precipitation and resolution could be repeated at will without loss of activity.

Alloway was certainly not the first person to precipitate nucleic acids with ethanol. This technique had already been explored as a purification step by several generations of organic chemists who were puzzling over the structure of the bases in DNA. However, Alloway was the first to use ethanol precipitation to prepare material that could change the phenotype of recipient cells. Final proof that the transforming factor was DNA still lay a dozen or more years into the future. But Alloway could fairly claim to be the inventor of a technique that is now second nature to us all.

9. Dissolve the DNA pellet (which is often invisible) in the desired volume of buffer (usually TE, pH between 7.6 and 8.0). Rinse the walls of the tube well with the buffer.

See Discussion.

One volume of isopropanol may be used in place of 2 volumes of ethanol 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 is therefore more difficult to remove; moreover, solutes like sucrose or sodium chloride are more easily coprecipitated with DNA when isopropanol is used. In general, precipitation with ethanol is preferable, unless it is necessary to keep the volume of fluid to a minimum.

Historical background on this ethanol precipitation method is provided in Box 1.

DISCUSSION

Because of its exposed backbones of negatively charged phosphate residues, DNA is a highly polar molecule. In aqueous solutions, the charged residues attract a hydration shell of water molecules that suppresses binding of positively charged ions to the DNA. Ethanol disrupts the hydration shells of DNA and allows the unshielded phosphate residues to form ionic bonds with cations in the solvent. When the concentration of ethanol approaches 70% in the presence of 300 mM Na^+ ions, the repulsive forces between the polynucleotide chains are reduced to the point where the DNA precipitates. Ethanol precipitation can therefore only occur if cations are available in sufficient quantity to neutralize the charge on the exposed phosphate residues. The most commonly used cations are shown in Table 2 and are described below.

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Ammonium Acetate

Ammonium acetate is frequently used to reduce the coprecipitation of unwanted contaminants (e.g., dNTPs or oligosaccharides) with nucleic acids. For example, two sequential precipitations of DNA in the presence of 2 M ammonium acetate result in the removal of >99% of the dNTPs from preparations of DNA (Okayama and Berg 1983). Ammonium acetate is also the best choice when nucleic acids are precipitated after digestion of agarose gels with agarase. The use of NH_4^+ as a cation reduces the possibility of coprecipitation of oligosaccharide digestion products. However, ammonium acetate should not be used when the precipitated nucleic acid is to be phosphorylated, because bacteriophage T4 polynucleotide kinase is inhibited by ammonium ions.

Lithium Chloride

Lithium chloride is used when high concentrations of ethanol (>75%, v/v) are required for precipitation (e.g., when precipitating RNA). LiCl is very soluble in ethanolic solutions and is not coprecipitated with the nucleic acid. Small RNAs (tRNAs and 5S RNAs) are soluble in solutions of high ionic strength (without ethanol), whereas large RNAs are not. Because of this difference in solubility, precipitation in high concentrations of LiCl (0.8 M) can be used to purify large RNAs.

Sodium Chloride

Sodium chloride (0.2 M) should be used if the DNA sample contains SDS. The detergent remains soluble in 70% ethanol.

Sodium Acetate

Sodium acetate (0.3 M, pH 5.2) is used for most routine precipitations of DNA and RNA.

Contrary to folklore, precipitation of DNA is suppressed at low temperatures and occurs more efficiently at room temperature rather than −20°C or −80°C (Zeugin and Hartley 1985). The minimum incubation time depends on the length and concentration of the DNA. The smaller the DNA fragments and the lower their concentration, the longer is the time required for precipitation. For very small fragments and low DNA concentrations, overnight incubation at 4°C is recommended. To suppress formation of salt precipitates during extended periods of storage at low temperature, ammonium acetate (final concentration 500 mM) rather than sodium acetate should be used as the counterion (Zeugin and Hartley 1982).

At 4°C in the absence of carrier, DNA in concentrations as low as 20 ng/mL will form precipitates that can be quantitatively recovered by centrifugation in a microcentrifuge. However, when lower concentrations of DNA or very small fragments (<100 nt in length) are processed, more extensive centrifugation may be necessary to cause the pellet of nucleic acid to adhere tightly to the centrifuge tube. Centrifugation at 100,000*g* for 20–30 min allows the recovery of picogram quantities of nucleic acid. When dealing with small amounts of DNA, it is prudent to save the ethanolic supernatant from each step until all of the DNA has been recovered. This precaution is especially important after precipitates of DNA have been washed with 70% ethanol, a treatment that often loosens the precipitates from the wall of the tube.

Carriers (or *coprecipitants*) are inert substances that are used to improve the recovery of small quantities of nucleic acids during ethanol precipitation. Insoluble in ethanolic solutions, carriers form a precipitate that traps the target nucleic acids. During centrifugation, carriers generate a visible pellet that facilitates handling of the target nucleic acids. Three substances are commonly used as carriers: yeast tRNA, glycogen, and linear polyacrylamide. Their advantages and disadvantages are listed in Table 1.

Adding a carrier like glycogen to the ethanolic DNA solution can improve recovery without interfering with most downstream enzymatic reactions. The final concentration of glycogen in a precipitation mixture should be between 0.05 and 1 mg/mL (Tracy 1981; Hengen 1996).

Dissolving DNA Precipitates

Until a few years ago, DNA precipitates recovered after ethanol precipitation were dried under vacuum before being redissolved. This practice has now been abandoned because (1) desiccated pellets of DNA dissolve slowly and inefficiently; and (2) small fragments of double-stranded DNA (<400 bp) become denatured following drying, probably as a result of loss of the stabilizing shell of bound water molecules (Svaren et al. 1987).

These days, the best practice is to remove ethanol from the nucleic acid pellet and from the sides of the tube by gentle aspiration and then to store the open tube on the bench for ~15 min to allow most of the residual ethanol to evaporate. The still-damp pellet of nucleic acid can then be dissolved rapidly and completely in the appropriate buffer. If necessary, the open tube containing the redissolved DNA can be incubated for 2–3 min at 45°C in a heating block to allow any traces of ethanol to evaporate.

The precipitated DNA is not all found at the bottom of the tube after centrifugation in an angle-head rotor. In the case of microcentrifuge tubes, for example, at least 40% of the precipitated DNA is plastered on the wall of the tube. To maximize recovery of DNA, use a pipette tip to roll a bead of solvent several times over the appropriate segment of the wall. This step can easily be done by pushing the bead of fluid over the surface with a disposable pipette tip attached to an automatic micropipettor. If the sample of DNA is radioactive, check that no detectable radioactivity remains in the tube after the dissolved DNA has been removed.

In general, DNA precipitated from solution by ethanol can be redissolved easily in buffers of low ionic strength, such as TE (pH 8.0). Occasionally, 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 subsequently. If the sample does not dissolve easily in a small volume, add a larger volume of buffer and repeat the precipitation with ethanol. The second precipitation may help eliminate additional salts or other components that may be preventing dissolution of the DNA.

RECIPE

TE Buffer, 10×

100 mM Tris-Cl (desired pH)
10 mM EDTA (pH 8.0)

Sterilize solutions by autoclaving for 20 min at 15 psi (1.05 kg/cm^2) on liquid cycle. Store the buffer at room temperature.

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