Purification of plasmid DNA from cleared bacterial lysates was traditionally performed using cesium chloride (CsCl) ultracentrifugation. Today, a variety of commercially available plasmid purification kits offer easy procedures for different throughput requirements and applications.

Isopropanol Precipitation of DNA

Alcohol precipitation is commonly used for concentrating, desalting, and recovering nucleic acids. Precipitation is mediated by high concentrations of salt and the addition of either isopropanol or ethanol. Since less alcohol is required for isopropanol precipitation, this is the preferred method for precipitating DNA from large volumes. In addition, isopropanol precipitation can be performed at room temperature, which minimizes co-precipitation of salt that may interfere with downstream applications.

This section provides hints on how to perform an effective isopropanol precipitation and to help ensure maximum recovery of DNA. The range of values given reflects protocol variation depending on the scale and type of preparation.

Protocol 7. Isopropanol precipitation procedure

Protocol 7

1. Adjust the salt concentration if necessary, e.g., with sodium acetate (0.3 M, pH 5.2, final concentration) or ammonium acetate (2.0–2.5 M, final concentration).
2. Add 0.6–0.7 volumes of room-temperature isopropanol to the DNA solution and mix well.

Tip Use all solutions at room temperature to minimize co-precipitation of salt.

3. Centrifuge the sample immediately at 10,000–15,000 x *g* for 15–30 min at 4°C.

Tip Centrifugation should be carried out at 4°C to prevent overheating of the sample. (When precipitating from small volumes, centrifugation may be carried out at room temperature.)

4. Carefully decant the supernatant without disturbing the pellet.

Tip Marking the outside of the tube or uniformly orienting microcentrifuge tubes before centrifugation allows the pellet to be more easily located. Pellets from isopropanol precipitation have a glassy appearance and may be more difficult to see than the fluffy salt-containing pellets that result from ethanol precipitation.

Tip Care should be taken when removing the supernatant as pellets from isopropanol precipitation are more loosely attached to the side of the tube.

Tip Carefully tip the tube with the pellet on the upper side to avoid dislodging the pellet.

Tip For valuable samples, the supernatant should be retained until recovery of the precipitated DNA has been verified.

5. Wash the DNA pellet by adding room-temperature 70% ethanol. This removes co-precipitated salt and replaces the isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

protocol continues overleaf

Protocol 7. Continued

6. Centrifuge at 10,000–15,000 x *g* for 5–15 min at 4°C.

Tip Centrifuge the tube in the same orientation as previously to recover the DNA in a compact pellet.

7. Carefully decant the supernatant without disturbing the pellet.

Tip The QIAprecipitator™ (QIAGEN) ensures recovery of precipitated DNA without centrifugation, considerably reducing plasmid purification time and eliminating the risk of DNA pellet loss.

8. Air-dry the pellet for 5–20 min (depending on the size of the pellet).

Tip Do not overdry the pellet (e.g., by using a vacuum evaporator) as this will make DNA, especially high-molecular-weight DNA, difficult to redissolve.

9. Redissolve the DNA in a suitable buffer.

Tip Choose an appropriate volume of buffer according to the expected DNA yield and the desired final DNA concentration.

Tip Use a buffer with a pH ≥8.0 for redissolving, as DNA does not dissolve easily in acidic buffers. (If using water, check pH.)

Tip Redissolve by rinsing the walls to recover all the DNA, especially if glass tubes have been used. To avoid shearing DNA do not pipet or vortex.

Tip High-molecular-weight DNA should be redissolved very gently to avoid shearing, e.g., at room temperature overnight or at 55°C for 1–2 h with gentle agitation.

A Guide to Analytical Gels

This section is aimed at providing useful hints for effective gel analysis of nucleic acids. Firstly, the basic steps involved in pouring an agarose gel for DNA analysis are outlined. Subsequent sections look at loading and running the gel and visualization of the DNA.

Principle of gel analysis

Gels allow separation and identification of nucleic acids based on charge migration. Migration of nucleic acid molecules in an electric field is determined by size and conformation, allowing nucleic acids of different sizes to be separated. However, the relationship between the fragment size and rate of migration is non-linear, since larger fragments have greater frictional drag and are less efficient at migrating through the polymer.

Agarose gel analysis is the most commonly used method for analyzing DNA fragments between 0.1 and 25 kb. Other specialized analytical gel methods exist for analyzing extremely large or small DNA molecules. Detailed information on all types of analytical gels can be found in current molecular biology manuals (1, 2).

Pouring an agarose gel

Agarose concentration

The concentration of agarose used for the gel depends primarily on the size of the DNA fragments to be analyzed. Low agarose concentrations are used to separate large DNA fragments, while high agarose concentrations allow resolution of small DNA fragments (**Table 2**).