Purification of DNA by phenol extraction and ethanol precipitation

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Equipment and reagents

- Phenol
- TE buffer, pH 8.0 (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0)
- 24:1 (v/v) chloroform-isoamyl alcohol
- 3 M potassium acetate, pH 5.5, prepared by adding glacial acetic acid to 3 M potassium acetate until this pH is obtained
- Microcentrifuge

Method

1. Add an equal volume of phenol to the DNA containing reaction mixture and vortex gently.
2. Separate the aqueous phase which contains the DNA from the organic phase by centrifugation in the microfuge, at 2 000 rpm for 5 min or at 8 000 rpm for 1 min.
3. Remove the aqueous phase with care into a fresh microfuge tube and add an equal amount of 24:1 (v/v) chloroform-isoamyl alcohol.
4. In order to precipitate the DNA, add a 0.1 volume of 3 M sodium acetate, pH 5.5, to the aqueous phase and then 2 volumes of absolute ethanol. Incubate at –20 °C overnight or for shorter periods at –80 °C (e.g. 20–30 min).
5. Recover the precipitated DNA by centrifugation in the microfuge at 10 000 rpm for 5–15 min. Remove the ethanol with care and dry the pellet in a desiccator or 50 °C oven for 5 min. An extra wash with 70% (v/v) ethanol may be included to remove excess salt from the pellet. The dried DNA may be resuspended in sterile TE, pH 8.0, or water, and stored at 4 °C for further manipulation or at –20 °C for long-term storage.
6. This procedure denatures and removes contaminating protein from a DNA sample. A second useful method is drop dialysis, which can remove salt, SDS, and even some enzyme inhibitors. As such, it can be used with many methods involving DNA purification before or after enzymatic reactions:

(a) Gently place a drop dialysis filter, floating correct-side up, on 10–20 ml of sterile dialysis buffer (TE, pH 8.0, or water) in a Petri-dish.

(b) Gently pipette the DNA sample (10–100 μl) onto the filter.

(c) Allow to dialyse for 1–2 h before removing the DNA for further analysis.

Warning

Phenol is a hazardous organic solvent. Always use suitable laboratory gloves when handling phenol containing solutions. Specific waste procedures may be required for the disposal of phenol containing solutions.