Purification of nucleic acids

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An important step in many molecular biology and analytical chemistry experiments is to isolate, purify, or concentrate nucleic acids from aqueous solutions that contain undesired proteins, lipids, sugars, or salts.
The precipitation of nucleic acids with ethanol or isopropyl alcohol as antisolvent (*Alternative A*) is an exceptionally quantitative method that allows even picogram quantities of precious material to be recovered in high purity. The precipitate can be dissolved in any volume and buffer of choice.
For higher throughput, nucleic acids can be immobilized on glass particles or silica membranes in the presence of chaotropic agents, such that contaminants can be washed off (*Alternative B*) (Vogelstein and Gillespie, 1979). Binding is quantitative, but elution may be incomplete or volume-restricted. Fragments shorter than 50 bp or longer than 20 kbp are not efficiently recovered, making this method unsuitable for small RNAs such as siRNA, miRNA, or tRNA. Mini-, midi-, and maxi-scale formats yield up to 15 µg, 75 µg, or 250 µg.
Neither method discriminates between DNA and RNA. To enrich for one class of nucleic acids, digest with RNase A or DNase I prior to purification.

This is a bench card. Full protocol available online.

Procedures

A › ***Purification by alcohol precipitation***

- ☐ R0045 3 M Sodium acetate
- ☐ Ethanol, ice-cold
- ☐ Buffer PE (Wash buffer)
- ☐ R0056 5 mM Tris hydrochloride

(1.) Mix 1.0 vol sample with 0.1 vol 3 M sodium acetate. Mix well.

(2.) *Optional:* Add 10–20 µg/mL linear polyacrylamide to precipitate picogram quantities of nucleic acids.

(3.) Add 2.0 vol ethanol to precipitate DNA, or 2.5–3.0 vol ethanol to precipitate RNA. Mix by vortexing.

(4.) Precipitate for 15–30 min on ice.

(5.) Centrifuge at 12 000 × *g* for 15 min at 4 °C. Discard supernatant and spin briefly to remove residual solvent. The pellet will appear nearly transparent.

(6.) Wash pellet with 650 µL Buffer PE or freshly prepared 70% ethanol. Discard supernatant carefully.

(7.) Dry pellet for 10–15 min at room temperature, or for 1 min in a vacuum centrifuge.

> ***Critical:*** Avoid overdrying, which impairs dissolution and may denature small dsDNA.

(8.) Resuspend in 15–30 µL 5 mM Tris pH 8.0 or buffer of choice.

B › ***Purification over a silica matrix***

- ☐ Buffer PB (Binding buffer)
- ☐ Buffer PE (Wash buffer)
- ☐ R0056 5 mM Tris hydrochloride

(1.) Mix 1.0 vol sample with 2.0 vol Buffer PB.

(2.) Apply up to 700 µL to a silica spin column. Centrifuge at 11 000 × *g* for 30 s or use vacuum manifold.

(3.) Wash with 650 µL Buffer PE.

> ***Critical:*** Let stand for 2–5 min before spinning if DNA will be used in salt-sensitive applications such as blunt-end ligation.

(4.) Repeat wash to minimize salt carryover.

(5.) Dry membrane by centrifugation at 11 000 × *g* for 1 min.

> ***Critical:*** Residual ethanol may persist if flow-through is not discarded prior to drying.

(6.) Place spin column in a clean tube. Apply 15–30 µL 5 mM Tris pH 8.0 onto the silica membrane. Incubate 1 min. Elute at 11 000 × *g* for 1 min.

(7.) *Optional:* Repeat elution using the same or half the volume to improve yield. Pool eluates if desired.

List of references

B. Vogelstein and D. Gillespie, *Proc. Natl. Acad. Sci. U.S.A.* **76**(2), 615—619 (1979).

Recipe (available online) Troubleshooting (available online) Notes (available online)