Purification of nucleic acids

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.

Risk assessment

– Guanidine salts form TOXIC GASES with bleach or strong acids
▷ Wear gloves, safety glasses, lab coat
□ DO NOT add bleach or acidic solutions directly into sample waste

Reviewed: Jun 10, 2023

Procedures

A › *Purification by alcohol precipitation*

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

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

> ***Hint:*** Sodium acetate (0.3 M final) is standard for routine DNA or RNA precipitation. Use 0.8 M lithium chloride to selectively precipitate long RNA (not suitable for in vitro translation or reverse transcription). Use 2.0–2.5 M ammonium acetate for samples containing dNTPs or oligosaccharides (not compatible with T4 PNK). If SDS is present, use 0.2 M sodium chloride.

> ***Note:*** If the sample already contains monovalent cations, less or no additional salt may be required.

(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.

> ***Hint:*** For samples containing less than 0.2 ng/µL DNA or fragments shorter than 100 bp, incubate at least 60 min and/or add 10 mM magnesium chloride. Lowering the temperature below 0 °C increases viscosity and slows sedimentation.

(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.

> ***Hint:*** For short fragments or low concentrations, centrifuge for 30 min to improve pellet adhesion.

> ***Hint:*** Keep the supernatant until recovery has been confirmed for precious samples.

(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.

> ***Note:*** Pellet may adhere to tube walls. To maximize recovery, roll a droplet of buffer along the inner wall several times.

B › *Purification over a silica matrix*

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

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

Hint: To exclude fragments smaller than 500 bp, dilute Buffer PB to 10–15% with water before mixing.

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

Hint: Silica membranes bind up to 15–25 µg DNA. Reuse is possible for the same sample. Loading capacity varies by model.

(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.

Hint: For higher yield, warm elution buffer to 70 °C.

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

Analyses

○ Measure nucleic acid concentration and purity at 260 nm, 280 nm, and 230 nm.

Nucleic acid	A260 = 1.0	A260/A280	A260/A230
dsDNA	50 ng/µL	1.8–1.9	2.0–2.2
ssDNA	33 ng/µL	1.8–1.9	2.0–2.2
ssRNA	40 ng/µL	1.8–2.0	2.0–2.2

Note: Extinction coefficients and purity ratios vary with sequence. A260/A280 shifts with pH by 0.2–0.3 .

Quality assurance: High A230 absorbance indicates residual guanidine contamination.

Troubleshooting

Purification by alcohol precipitation

In Step 8:

– Pellet does not dissolve
  - Use low-ionic strength buffer initially; avoid $MgCl_2$ or high-salt buffers.
  - If needed, dissolve in larger volume and repeat precipitation to exchange buffer.

Purification over a silica matrix

In Step 6:

– Little or no DNA in eluate
  - Ensure elution buffer is low salt and within pH 7.0 and pH 8.5.

List of references

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

Change log

2023-06-10 Benjamin C. Buchmuller Adaptation as SOP.