Plasmid preparation by alkaline lysis

Under alkaline conditions, high molecular weight DNA is selectively denatured while covalently closed circular plasmid DNA remains double-stranded. When the solution is rapidly neutralized, denatured chromosomal DNA forms an insoluble clot, while plasmid DNA remains in solution (Birnboim and Doly, 1979).

This method yields tens of micrograms of highly pure plasmid DNA from mini-preps (a few milliliters of culture), and up to milligrams from a liter-scale culture.

The plasmids are purified over a silica matrix (*Alternative A*) or by alcohol precipitation (*Alternative B*).

Risk assessment

▷ Wear gloves, safety glasses, lab coat

Reviewed: May 30, 2023

Procedures

›› *Alkaline lysis*

- ☐ Buffer P1 (Resuspension buffer)
- ☐ Buffer P2 (Lysis buffer)
- ☐ Buffer N3 (Neutralization buffer)

(1.) Grow bacteria in 1–5 mL LB (Miller) overnight to stationary phase. Collect the cells by centrifugation.

> ***Hint:*** For culture tubes, spin at 3 000 × *g* for 5 min. For 2 mL centrifuge tubes, a brief spin at 11 000 × *g* is sufficient.

(2.) Resuspend the pellet in 150 µL Buffer P1.

> ***Hint:*** Buffers P1, P2, N3, PB, and PE refer to standard silica-column miniprep kit components (e.g., QIAGEN QIAprep). Equivalent buffers from other suppliers can be substituted. Homemade buffer recipes are available elsewhere.

(3.) Add 250 µL Buffer P2. Mix by gently inverting the tube 6–8 times. Incubate at room temperature for no more than 5 min or until the lysate appears clear.

> ***Critical:*** Do not vortex or pipette to avoid shearing chromosomal DNA.

(4.) Add 350 µL Buffer N3. Mix gently by inversion 6–8 times.

> ***Critical:*** Ensure complete neutralization to precipitate protein and chromosomal DNA. If Buffer P2 contains thymolphthalein, mix until the solution is colorless.

(5.) Centrifuge at 17 000 × *g* for 3 min to pellet precipitated material. Repeat if the lysate remains turbid.

(6.) Transfer the clear supernatant to a fresh tube for purification. Avoid any carryover of precipitate.

A › *Purification of plasmid DNA over a silica matrix*

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

(1.) Apply up to 700 µL of supernatant to a silica spin column. Centrifuge at 11 000 × *g* for 1 min, or use a vacuum manifold.

> ***Hint:*** Silica membranes typically bind 15–25 µg DNA. They may be reused to purify more of the same DNA.

(2.) *Optional:* Wash the silica matrix with 450 µL Buffer PB.

> ***Hint:*** Recommended if plasmids are isolated from strains containing high endonuclease activity such as HB101 or JM109.

(3.) Wash with 650 µL Buffer PE.

(4.) Dry the silica matrix by centrifugation at 11 000 × *g* for 2 min.

(5.) Place the spin column into a clean microcentrifuge tube.

(6.) Apply 25–55 µL 5 mM Tris pH 8.0 to the membrane. Incubate for 1 min, then elute the plasmid by centrifugation at 11 000 × *g* for 1 min.

> ***Hint:*** For higher yield or large plasmids, warm elution buffer to 70 °C.

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

B › ***Purification by alcohol precipitation***

- ☐ Isopropyl alcohol
- ☐ 5 mM Tris
- ☐ Buffer PE (Wash buffer)

(1.) Transfer up to 700 µL of supernatant to a clean microcentrifuge tube.

(2.) Add an equal volume of isopropanol to precipitate the DNA. Vortex to mix thoroughly.

(3.) Centrifuge at 11 000 × *g* for 5 min. Discard the supernatant and briefly spin to remove residual solvent. The pellet will appear nearly transparent.

(4.) Wash with 650 µL Buffer PE or freshly prepared 70% ethanol without disturbing the pellet.

(5.) Air-dry the pellet for 2–5 min, or dry in a vacuum centrifuge for 1 min.

(6.) Resuspend in 25–55 µL 5 mM Tris pH 8.0 or water.

Analyses

○ Measure DNA concentration and purity by UV absorbance 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

> ***Quality assurance:*** Extinction coefficients vary with nucleotide composition. A260/A280 ratios increase with pH and vary by 0.2–0.3 between instruments. RNA contamination elevates the ratio; protein or phenol contamination depresses it.

○ Confirm plasmid identity by restriction digestion SOP 0033 and agarose gel electrophoresis SOP 0032.

> ***Hint:*** Use one or more enzymes predicted to linearize the plasmid or release a diagnostic fragment.

Troubleshooting

Alkaline lysis

In Step 4:

– Chromosomal DNA does not form a white precipitate but remains viscous.
  - Reduce culture volume and repeat the preparation with gentler handling.

In Step 6:

– Chromosomal DNA in the eluate
  - Use cultures within 12–16 h post-inoculation. Older cultures release degraded chromosomal DNA. If not processing immediately, store the bacterial pellet at −20 °C.
  - Mix lysate gently after adding Buffer P2. Limit lysis to 5 min to avoid DNA shearing.
  - After adding Buffer N3, mix immediately and gently to allow complete neutralization.

– RNA contamination in eluate
  - Verify RNase A was added to Buffer P1 before use. Supplement fresh RNase if needed.

Purification of plasmid DNA 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.

Purification by alcohol precipitation

In Step 3:

- No visible pellet after isopropanol precipitation
  - Centrifuge for longer (10 min µN t). Mark the tube orientation before spinning to locate it on the expected side.
  - Add 0.1 vol of 3 M sodium acetate pH 5.2before adding isopropanol to improve precipitation of dilute samples.

List of references

H. Birnboim and J. Doly, *Nucleic Acids Res.* **7**(6), 1513—1523 (1979).

Change log

2016-03-15	Rahul Patharkar	Alkaline lysis with ethanol precipitation
2023-05-30	Benjamin C. Buchmuller	Adaptation as SOP