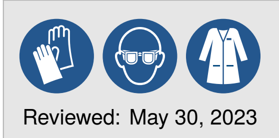
Plasmid preparation by alkaline lysis


Reviewed: May 30, 2023

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*).

This is a bench card. Full protocol available online.

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.

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

(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)
- ☐ R0056 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.

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

(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.

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

(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.

List of references

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

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

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