Purification of Plasmid DNA

**Introduction:**

The growth of colonies on antibiotic medium provides *phenotypic* evidence that cells have been transformed. To confirm this at the *genotypic* level, plasmid DNA is isolated from transformants. Restriction analysis of the purified plasmid DNA, along with the original DNAs used to make the recombinant, presents proof of its genetic identity. The small size and circular structure of plasmids make it relatively easy to separate them from the host cell’s chromosomal DNA.

A rapid method for making a small preparation of purified plasmid DNA from culture volumes as low as 1 mL is called a *miniprep*. Transformed cells from an antibiotic-resistant colony are grown to stationary phase in an overnight suspension culture. The cells are collected by centrifugation and resuspended in a buffered solution of glucose and ethylenediaminetetraacetic acid (EDTA), which binds divalent cations (such as Mg²⁺ and Ca²⁺) necessary for cell membrane stability.

The resuspended cells are then treated with a mixture of SDS and sodium hydroxide. SDS, an ionic detergent, dissolves the phospholipid and protein components of the cellular membrane. This lyses the membrane, releasing the cell contents. Sodium hydroxide denatures both plasmid and chromosomal DNAs into single strands. The chromosomal DNA separates completely into individual strands; however, the single-stranded plasmid loops remain linked together like interlocked rings.

**Figure 1: Plasmid isolation.**
Subsequent treatment with potassium acetate and acetic acid forms an insoluble precipitate of SDS/lipid/protein and neutralizes the sodium hydroxide from the previous step. At neutral pH, DNA renatures. In the miniprep, the long strands of chromosomal DNA only partially renature and become trapped in the SDS/lipid/protein precipitate. The linked, single-stranded plasmid DNA completely renatures into double-stranded molecules that remain in solution and largely escape entrapment in the precipitate.

The precipitate is pelleted by centrifugation and discarded, leaving the plasmid DNA (as well as RNA molecules) in the supernatant. Ethanol or isopropanol is added to the supernatant to precipitate the plasmid DNA out of solution. The plasmid DNA is pelleted by centrifugation, washed with ethanol, dried, and resuspended in a small volume of buffer. Subsequent treatment with RNase destroys RNA, leaving relatively clean plasmid DNA.

This protocol provides a small-scale way to purify from transformed E. coli enough plasmid DNA for restriction analysis. Cells taken from an ampicillin-resistant colony are grown to stationary phase in suspension culture. The cells from 1 mL of culture are harvested and lysed, and plasmid DNA is separated from the cellular proteins, lipids, and chromosomal DNA. This procedure should yield 2-5 μg of relatively crude plasmid DNA. In contrast, maxipreps can yield 1 mg or more of pure plasmid from a 1 liter culture.

The minipreparation is a simple and efficient procedure for isolating plasmid DNA. Become familiar with the molecular and biochemical effects of each reagent used in the protocol.

- **glucose/Tris/EDTA (GTE):** The Tris buffers the cells at pH 7.9. EDTA binds divalent cations in the lipid bilayer, thus weakening the cell envelope.
- **SDS/sodium hydroxide:** This alkaline mixture lyses the bacterial cells. The detergent SDS dissolves the lipid components of the cell membrane, as well as cellular proteins. The sodium hydroxide denatures the chromosomal and plasmid DNA into single strands. The intact circles of plasmid DNA remain intertwined.
- **potassium acetate/acetic acid:** The acetic acid returns the pH to neutral, allowing DNA strands to renature. The large, disrupted chromosomal strands cannot rehybridize perfectly, but instead collapse into a partially hybridized tangle. At the same time, the potassium acetate precipitates the SD (which is insoluble in potassium) from the cell suspension, along with proteins and lipids with which it has associated. The renaturing chromosomal DNA is trapped in the SDS/lipid/protein precipitate. Only smaller plasmid DNA and RNA molecules escape the precipitate and remain in solution.
- **isopropanol:** The alcohol rapidly precipitates nucleic acids, but only slowly precipitates proteins. Thus, a quick precipitation preferentially brings down nucleic acids.
- **ethanol:** A wash with ethanol removes some remaining salts and SDS from the preparation. Ethanol also removes the remaining isopropanol, which has a higher vapor point than does ethanol. The ethanol-isopropanol evaporates more rapidly in the drying step.
- **Tris/EDTA:** this buffers the DNA solution. EDTA protects the DNA from degradation by DNase activity. Buffering DNA is important, as low pH (<6) leads to the loss of purines (A and G) called depurination. The purines are actually cleaved from their sugars. Keep in mind that H₂O can have a pH as low as 5.
**Procedure:**

**PREPARE REAGENTS**

**0.5 M ethylenediaminetetraacetic acid (EDTA, pH 8.0)**

Makes 100 mL. Store at room temperature (indefinitely).

1. Add 18.6 g of EDTA (disodium salt, m.w. 372.24) to 80 mL of deionized or distilled water.
2. Adjust pH by slowly adding ~2.2 g of sodium hydroxide pellets (m.w. = 40.00)
3. Mix vigorously with a magnetic stirrer or by hand. EDTA will only dissolve when the pH has reached 8.0 or higher.
4. Add deionized or distilled water to make a total volume of 100 mL of solution.

**1 M Tris (pH 8.0)**

Makes 100 mL. Store at room temperature (indefinitely).

1. Dissolve 12.1 g of Tris base (m.w. 121.10) in 70 mL of deionized or distilled water.
2. Adjust the pH by slowly adding ~5.0 mL of concentrated hydrochloric acid (HCl).
3. Add deionized or distilled water to make a total volume of 100 mL of solution.
   - A yellow-colored solution indicates poor-quality Tris.
   - The pH of Tris solutions is temperature-dependent; measure pH at room temperature.

**glucose/Tris/EDTA (GTE)**

Makes 100 mL. Store at 4°C or room temperature (indefinitely).

Mix:
- 0.9 g of glucose (m.w. = 180.16) [50 mM]
- 2.5 mL of 1 M Tris (pH 8.0) [25 mM]
- 2 mL of 0.5 M EDTA [10 mM]
- 94.5 mL of deionized or distilled water.

**5 M potassium acetate (KOAc)**

Makes 200 mL. Store at room temperature (indefinitely).

1. Add 98.1 g of potassium acetate (m.w. = 98.14) to 160 mL of deionized water.
2. Add deionized or distilled water to make a total volume of 200 mL of solution.

**potassium acetate/acetic acid**

Makes 100 mL. Store at 4°C or room temperature (indefinitely).

1. Add 60 mL of 5 M potassium acetate and 11.5 glacial acetic acid to 28.5 mL of deionized or distilled water to make a total volume of 100 mL of solution.

**10% sodium dodecyl sulfate (SDS)**

Makes 100 mL. Store at room temperature (indefinitely).

1. Dissolve 10 g of electrophoresis grade SDS (m.w. = 288.37) in 80 mL of deionized water.
2. Add deionized or distilled water to make a total volume of 100 mL of solution.
   - Avoid inhaling SDS powder; wear a mask that covers both nose and mouth.
   - A precipitate may form at colder temperatures. Warm solution in a water bath, and shake gently to dissolve precipitate.
1% SDS/0.2 N NaOH
Makes 10 mL. Store at room temperature (lasts for several days).

1. Mix 1 mL of 10% SDS and 0.5 mL of 4 N NaOH into 8.5 mL of distilled water.
2. Add deionized or distilled water to make a total volume of 100 mL of solution.
   - Always use fresh SDS/NaOH solution
   - A precipitate may form at colder temperatures. Warm solution in a water bath, and shake gently to dissolve precipitate.

PREPARE E. coli CULTURE
Ideally, we should pick colonies from our own transformed plates to begin this protocol. However, the colonies must grow overnight or for at least several hours. On the day before we begin, we need to prepare an E. coli culture via overnight suspension. Inoculate the culture with a cell mass scraped from one colony selected from the +LB/amp plate from our transformation experiment. Maintain antibiotic selection with LB broth plus ampicillin. Alternatively, prepare the culture 2-3 days in advance and store at 4°C or incubate for 24-48 hours at 37°C without shaking. In either case, the cells will settle at the bottom of the culture tube. Shake the tube to resuspend cells before beginning this procedure.

PREPARE DUPLICATE MINIPREPS
1. Shake culture tube to resuspend E. coli cells.
2. Label two 1.5 mL tubes with your initials. Use a micropipettor to transfer 1000 µL of E. coli/plasmid overnight suspension into each tube.
3. Close caps, and place the tubes in a balanced configuration in the microfuge rotor. Spin for 1 minute to pellet the cells. NOTE: The cell pellet will appear as a small off-white smear on the bottom side of the tube. Although the cell pellets are readily seen, the DNA pellets in upcoming step 14 are difficult to observe. Make a habit of aligning the tube with the cap hinges facing outward in the microfuge rotor. Then, pellets should always be located at the tube bottom beneath the hinge.
4. Pour off supernatant from both tubes into a waste beaker for later disinfection. Alternatively, use a micropipettor to remove supernatant. Be careful not to disturb the cell pellets. Invert the tubes, and tap gently on the surface of a clean paper towel to drain thoroughly.
5. Add 100 µL of ice-cold GTE solution to each tube. Resuspend the pellets by pipetting the solution in and out several times. Hold the tubes up to the light to check that the suspension is homogeneous and that no visible clumps of cells remain.
6. Add 200 µL of SDS/NaOH solution to each tube. Close caps, and mix solutions by rapidly inverting tubes five times.
7. Stand tubes on ice for 5 minutes. Suspension will become relatively clear.
8. Add 150 µL of ice-cold KOAc solution to each tube. Close caps, and mix solutions by rapidly inverting tubes five times. A white precipitate will immediately appear.
9. Stand tubes on ice for 5 minutes.
10. Place tubes in a balanced configuration in the microfuge rotor, and spin them for 5 minutes to pellet the precipitate along the side of the tube.
11. Transfer 400 µL of supernatant from each tube into clean 1.5 mL tubes. If measured correctly, a small amount of supernatant remains behind, which provides a small buffer between the tip and
the precipitate. *Avoid pipetting precipitate*, and wipe off any precipitate clinging to the outside of the tip prior to expelling supernatant. Discard the old tubes containing precipitate. **NOTE:** In this step, the supernatant is saved and precipitate is discarded. This will be reversed in the upcoming steps 14 and 17. **ALSO NOTE:** Do step 12 quickly and make sure the microfuge will be available for step 13.

12. Add 400 µL of isopropanol to each tube of supernatant. Close caps, and mix vigorously by rapidly inverting tubes five times. *Stand at room temperature for only 2 minutes.* (Isopropanol preferentially precipitates nucleic acids rapidly; however, proteins remaining in solution also begin to precipitate with time.)

13. Place tubes in a *balanced* configuration in the microfuge rotor, and spin for 5 minutes to pellet the nucleic acids. Align tubes in rotor so that the cap hinges point outward. The nucleic acid residue, visible or not, will collect on the tube side under the hinge during centrifugation. **NOTE:** The pellet may appear as a tiny smear or small particles on the bottom-side of each tube.

14. Pour off supernatant from both tubes. *Be careful not to disturb nucleic acid pellets.* Alternatively, remove the supernatant with a large range micropipettor. Place tip away from the pellet. If you are concerned that the pellet has been drawn up the tip, transfer the supernatant to another 1.5 mL tube, recentrifuge, and try again. Invert tubes, and tap gently on the surface of a clean paper towel to drain thoroughly.

15. Add 200 µL of 100% ethanol to each tube and close caps. Flick tubes several times to wash pellets. Nucleic acid pellets are not soluble in ethanol and will not resuspend during washing.

**STOP: STORE DNA IN ETHANOL @ -20°C UNTIL READY TO CONTINUE**

16. Place tubes in a *balanced* configuration in the microfuge and spin for 2-3 minutes.

17. Pour off supernatant from both tubes. *Be careful not to disturb nucleic acid pellets.* Alternatively, remove the supernatant with a large range micropipettor. Place tip away from the pellet. If you are concerned that the pellet has been drawn up the tip, transfer the supernatant to another 1.5 mL tube, recentrifuge, and try again. Invert tubes, and tap gently on the surface of a clean paper towel to drain thoroughly.

18. Dry nucleic acid pellets by one of the following methods:
   a. Direct a stream of warm air from a hair dryer across the open ends of the tubes for about 3 minutes. *Be careful not to blow the pellets out of the tube!*
   b. Close caps, and pulse tubes in the microfuge to pool remaining ethanol. *Carefully* draw off drops of ethanol using a small range micropipettor. Leave cap open and place tube upright in rack, allowing pellets to air-dry for 10 minutes at room temperature.

19. At the end of the drying period, hold each tube up to the light to check that no ethanol droplets remain. If ethanol is still evaporating, an alcohol odor can be detected by sniffing the mouth of the tube. All ethanol must be evaporated before proceeding to step 20.

20. Add 15 µL of TW to each tube. Resuspend the pellets by smashing with micropipettor tip and pipetting in and out vigorously. Rinse down the side of the tube several times, concentrating on the area where the pellet should have formed during centrifugation (beneath cap hinge). Check that all DNA is dissolved and that no particles remain in the tip or on the side of the tube.

21. Pool DNA/TE solution into one tube.

**STOP: STORE DNA/TE SOLUTION @ -20°C UNTIL READY TO USE**
1. Consider the three major classes of biologically important molecules: proteins, lipids, and nucleic acids. Which steps of the miniprep procedure act on proteins? On lipids? On nucleic acids?

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2. What aspect of plasmid DNA structure allows it to renature efficiently in Step 8?

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3. What other kinds of molecules, in addition to plasmid DNA, would you expect to be present in the final miniprep sample? How could you find out?

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