Protocol

Preparation of Plasmid DNA by Alkaline Lysis with Sodium Dodecyl Sulfate: Minipreps

Michael R. Green and Joseph Sambrook

In this protocol, plasmid DNA is isolated from small-scale (1–2 mL) bacterial cultures. Yields vary between 100 and 5 µg of DNA, depending on the copy number of the plasmid. Miniprep DNA is sufficiently pure for use as a substrate or template in many in vitro enzymatic reactions. However, further purification is required if the plasmid DNA is used as the substrate in sequencing reactions.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous material used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Alkaline lysis solution I <R>, ice cold
Alkaline lysis solution II <R>

Alkaline lysis solution II should be freshly prepared and used at room temperature.

Alkaline lysis solution III <R>, ice cold
Antibiotic for plasmid selection
Arginine buffer (0.1 M, pH 12.4) (optional; see Step 5)

To make this buffer, adjust the pH of a 0.1 M solution of L-arginine to 12.4 with 5 M NaOH. For more details, see Cloninger et al. (2008) and Paul et al. (2008).

Bacterial colonies, transformed with the plasmid of interest
Ethanol (70%, 95%)
Phenol:chloroform (1:1, v/v) (optional; see Step 8)
Rich medium (LB [Luria-Bertani] liquid medium <R>, YT <R>, or Terrific Broth <R>)
STE <R>, ice cold (optional; see Step 3)
TE buffer, 10× <R> (pH 8.0)

Prepare 1× TE buffer (pH 8.0) containing 20 µg/mL RNase A.

Equipment

Incubator shaker, preset to 37°C
KimWipes

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Pasteur pipette and bulb (optional; see Step 3)
Vacuum aspirator equipped with traps (optional; see Steps 3 and 13)

METHOD

Preparation of Cells

1. Inoculate 2 mL of rich medium (LB, YT, or Terrific Broth) containing the appropriate antibiotic with a single colony of transformed bacteria. Incubate the culture overnight at 37°C with vigorous shaking.

   To Ensure That the Culture Is Adequately Aerated:

   i. The volume of the culture tube should be at least four times greater than the volume of the bacterial culture.

   ii. The tube should be loosely capped.

   iii. The culture should be incubated with vigorous agitation.

2. Pour 1.5 mL of the culture into a microcentrifuge tube. Centrifuge at maximum speed for 30 sec at 4°C in a microcentrifuge. Store the unused portion of the original culture at 4°C.

3. When centrifugation is complete, remove the medium by aspiration, leaving the bacterial pellet as dry as possible.

   This step can be conveniently accomplished with a disposable pipette tip or Pasteur pipette attached to a vacuum line and a side arm flask (see Fig. 1). Use a gentle vacuum, and touch the tip to the surface of the liquid. Keep the tip as far away from the bacterial pellet as possible as the fluid is withdrawn from the tube. This minimizes the risk that the pellet will be sucked into the side arm flask. Alternatively, remove the supernatant using a pipettor or Pasteur pipette and bulb. Use the pipette tip to vacuum the walls of the tube to remove any adherent droplets of fluid. The penalty for failing to remove all traces of medium from the bacterial pellet is a preparation of plasmid DNA that is resistant to cleavage by restriction enzymes. This is because cell-wall components in the medium inhibit the action of many restriction enzymes. This problem can be avoided by resuspending the bacterial pellet in ice-cold STE (0.25× volume of the original bacterial culture) and centrifuging again.

Lysis of Cells

4. Resuspend the bacterial pellet in 100 µL of ice-cold Alkaline lysis solution I by vigorous vortexing.

   Make sure that the bacterial pellet is completely dispersed in Alkaline lysis solution I. Vortexing two microcentrifuge tubes simultaneously with their bases touching increases the rate and efficiency with which the bacterial pellets are resuspended. The original protocol (Birnboim and Doly 1979) called for the use of

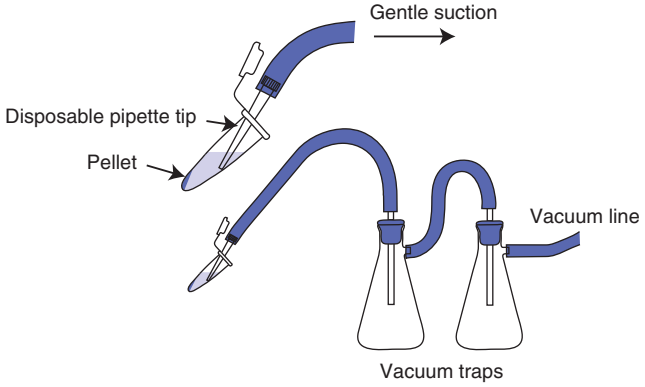

FIGURE 1. Aspiration of supernatants. Hold the open microcentrifuge tube at an angle, with the pellet on the upper side. Use a disposable pipette tip attached to a vacuum line to withdraw fluid from the tube. Insert the tip just beneath the meniscus on the lower side of the tube. Move the tip toward the base of the tube as the fluid is withdrawn. Use gentle suction to avoid drawing the pellet into the pipette tip. Keep the end of the tip away from the pellet. Finally, vacuum the walls of the tube to remove any adherent drops of fluid.

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lysozyme at this point to assist in dissolution of the bacterial cell walls. This step can be safely omitted when dealing with bacterial cultures of <10 mL in volume.

5. Add 200 µL of freshly prepared Alkaline lysis solution II to each bacterial suspension. Close the tube tightly, and mix the contents by inverting the tube rapidly five times. *Do not vortex!* Store the tube on ice.

   Make sure that the entire surface of the tube comes in contact with Alkaline lysis solution II.

   Prolonged exposure of superhelical circular DNA to alkali results in irreversible denaturation (Vinograd and Lebowitz 1966). The resulting cyclic coiled DNA, which cannot be cleaved with restriction enzymes, migrates through agarose gels at about twice the rate of superhelical, closed-circular DNA and stains poorly with SYBR dyes. Traces of this condensed form of closed-circular DNA can often be seen in plasmids prepared by alkaline lysis. The presence of a small amount of cyclic coiled DNA may be aesthetically unpleasant but is generally harmless. The easiest way to eliminate formation of cyclic coiled DNA in plasmid preparations is to follow the directions for preparing alkaline lysis buffers to the letter. Alternatively, arginine buffer may be used in place of standard Alkaline lysis buffer II in Step 5.

6. Add 150 µL of ice-cold Alkaline lysis solution III. Close the tube, and disperse Alkaline lysis solution III through the viscous bacterial lysate by inverting the tube several times. Store the tube for 3–5 min on ice.

7. Centrifuge the bacterial lysate at maximum speed for 5 min at 4°C in a microcentrifuge. Transfer the supernatant to a fresh tube.

8. (Optional) Add an equal volume of phenol:chloroform. Mix the organic and aqueous phases by vortexing, and then centrifuge the emulsion at maximum speed for 2 min at 4°C in a microcentrifuge. Transfer the aqueous upper layer to a fresh tube.

   Some investigators find the extraction with phenol:chloroform to be unnecessary. However, the elimination of this step sometimes results in DNA that is resistant to cleavage by restriction enzymes. The purpose of extracting with chloroform is to remove residual phenol from the aqueous phase. Phenol is slightly soluble in H_2O, but it can be displaced into the organic phase by chloroform.

Recovery of Plasmid DNA

9. Precipitate the nucleic acids from the supernatant by adding 2 volumes of ethanol at room temperature. Mix the solution by vortexing, and then allow the mixture to stand for 2 min at room temperature.

10. Collect the precipitated nucleic acids by centrifugation at maximum speed for 5 min at 4°C in a microcentrifuge.

    It is best to get into the habit of always arranging the microcentrifuge tubes in the same way in the microcentrifuge rotor (i.e., in order, with their plastic hinges always pointing outward). The precipitate will collect on the inside surface furthest from the center of rotation. Knowing where to look makes it easier to find visible precipitates and to dissolve "invisible" precipitates efficiently. Labeling both the sides and the tops of tubes provides clear identification of each tube, even if the ink becomes smudged.

11. Remove the supernatant by gentle aspiration as described in Step 3 above. Stand the tube in an inverted position on a paper towel to allow all of the fluid to drain away. Use a KimWipe or disposable pipette tip to remove any drops of fluid adhering to the walls of the tube.

12. Add 1 mL of 70% ethanol to the pellet, and invert the closed tube several times. Recover the DNA by centrifugation at maximum speed for 2 min at 4°C in a microcentrifuge.

13. Again remove all of the supernatant by gentle aspiration as described in Step 3.

    Take care with this step because the pellet sometimes does not adhere tightly to the tube.

14. Remove any beads of ethanol that form on the sides of the tube. Store the open tube at room temperature until the ethanol has evaporated and no fluid is visible in the tube (5–10 min).

    If the pellet of DNA is dried in a desiccator or under vacuum, it becomes difficult to dissolve under some circumstances and may denature (Svaren et al. 1987). Drying the pellet for 10–15 min at room temperature is usually sufficient for the ethanol to evaporate without the DNA becoming dehydrated.

15. Dissolve the nucleic acids in 50 µL of TE (pH 8.0) containing 20 µg/mL DNase-free RNase A (pancreatic RNase). Vortex the solution gently for a few seconds. Store the DNA solution at −20°C.

Any problems encountered during plasmid DNA preparation are evident upon analysis of the DNA following restriction enzyme digestion. See Troubleshooting.

TROUBLESHOOTING

Problem (Step 15): Very little or no DNA is visible on the gel either before or after restriction digestion.

Solution: The pellet of nucleic acid was likely inadvertently discarded after ethanol precipitation in Step 11. Be sure to remove the ethanol by gentle aspiration as soon as possible after the centrifugation step. If the centrifuge tube is left to stand for too long, the pellet of DNA will become detached from the wall.

Problem (Step 15): DNA is resistant to cleavage with restriction enzymes.

Solution: Cleavage-resistant DNA can be due to a failure to remove all traces of media, which contains components that persist through purification of the plasmid DNA and inhibit the action of restriction enzymes. Consider making one or more of the following adjustments:

- Remove all traces of medium as described in Step 3.
- Resuspend the bacterial pellet in ice-cold STE (0.25× volume of the original bacterial culture) and recentrifuge. Discard every last drop of the STE, and resuspend the bacterial pellet in Alkaline lysis solution I as described in Step 4.
- Perform a phenol:chloroform extraction as described in Step 8.
- Remove all traces of liquid as described in Step 11.

However, as a quick alternative to starting over, extract the final DNA preparation with phenol:chloroform; recover by standard ethanol precipitation and subsequent washing in 70% ethanol. Alternatively, perform the restriction digest in a larger volume (100–200 µL), using fivefold more enzyme. At the end of the digestion, recover the DNA by standard ethanol precipitation.

Problem (Step 15): DNA is converted to a smear during digestion.

Solution: The DNA is most likely tainted with a bacterial DNase (e.g., EndA) that is activated following exposure to the Mg^{2+} present in the restriction buffer. The most likely source of DNase is the stock of TE used to dissolve the plasmid DNA. Unless care is taken, TE can become contaminated by bacteria. Restriction buffers are a less likely possibility. Consider taking one or more courses of action:

- Sterilize each batch of TE by autoclaving, and dispense 1-mL aliquots into sterile microcentrifuge tubes (or sterilize TE in small aliquots). Use a fresh aliquot every day.
- Try to maintain sterile technique when using stock solutions.
- If bacterial DNase copurifies with the plasmid DNA, extract the DNA with phenol:chloroform, recover by standard ethanol precipitation, and resuspend in fresh TE.

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RECIPES

Alkaline Lysis Solution I

50 mM glucose
25 mM Tris-Cl (pH 8.0)
10 mM EDTA (pH 8.0)

Prepare Solution I from standard stocks in batches of approx. 100 mL, autoclave for 15 min at 15 psi (1.05 kg/cm^2) on liquid cycle, and store at 4°C.
For plasmid preparation.

Alkaline Lysis Solution II

0.2 N NaOH (freshly diluted from a 10 N stock)
1% (w/v) SDS

Prepare Solution II fresh and use at room temperature.
For plasmid preparation.

Alkaline Lysis Solution III

5 M potassium acetate, 60.0 mL
Glacial acetic acid, 11.5 mL
H_2O, 28.5 mL

The resulting solution is 3 M with respect to potassium and 5 M with respect to acetate. Store the solution at 4°C and transfer it to an ice bucket just before use.
For plasmid preparation.

LB (Luria-Bertani) Liquid Medium

Reagent	Amount to add
H_2O	950 mL
Tryptone	10 g
NaCl	10 g
Yeast extract	5 g

Combine the reagents and shake until the solutes have dissolved. Adjust the pH to 7.0 with 5 N NaOH (~0.2 mL). Adjust the final volume of the solution to 1 L with H_2O. Sterilize by autoclaving for 20 min at 15 psi (1.05 kg/cm^2) on liquid cycle.

STE

10 mM Tris-Cl (pH 8.0)
0.1 M NaCl
1 mM EDTA (pH 8.0)

Sterilize by autoclaving for 15 min at 15 psi (1.05 kg/cm^2) on liquid cycle. Store the sterile solution at 4°C.

TE Buffer, 10×

100 mM Tris-Cl (desired pH)
10 mM EDTA (pH 8.0)

Sterilize solutions by autoclaving for 20 min at 15 psi (1.05 kg/cm^2) on liquid cycle. Store the buffer at room temperature.

Terrific Broth

Deionized H_2O, to 900 mL
Tryptone, 12 g
Yeast extract, 24 g
Glycerol, 4 mL

Shake until the solutes have dissolved and then sterilize by autoclaving for 20 min at 15 psi (1.05 kg/cm^2) on liquid cycle. Allow the solution to cool to 60°C or less, and then add 100 mL of a sterile solution of 0.17 M KH_2PO_4, 0.72 M K_2HPO_4. (This solution is made by dissolving 2.31 g of KH_2PO_4 and 12.54 g of K_2HPO_4 in 90 mL of H_2O. After the salts have dissolved, adjust the volume of the solution to 100 mL with H_2O and sterilize by autoclaving for 20 min at 15 psi [1.05 kg/cm^2] on liquid cycle.)

YT

Tryptone, 16 g
Yeast extract, 10 g
NaCl, 5 g
Deionized H_2O, to 900 mL

To prepare 2× YT medium, shake until the solutes have dissolved. Adjust the pH to 7.0 with 5 N NaOH. Adjust the volume of the solution to 1 liter with deionized H_2O. Sterilize by autoclaving for 20 minutes at 15 psi (1.05 kg/cm^2) on liquid cycle.

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