Protocol: QIAprep Spin Miniprep Kit Using a Microcentrifuge

This protocol is designed for purification of up to 20 µg of high-copy plasmid DNA from 1–5 ml overnight cultures of *E. coli* in LB (Luria-Bertani) medium. For purification of low-copy plasmids and cosmids, large plasmids (>10 kb), and DNA prepared using other methods, refer to the recommendations on page 37.

Please read "Important Notes" on pages 19–21 before starting.

Note: All protocol steps should be carried out at room temperature.

Procedure

1. **Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.**

   Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.

2. **Add 250 µl Buffer P2 and gently invert the tube 4–6 times to mix.**

   Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.

3. **Add 350 µl Buffer N3 and invert the tube immediately but gently 4–6 times.**

   To avoid localized precipitation, mix the solution gently but thoroughly, immediately after addition of Buffer N3. The solution should become cloudy.

4. **Centrifuge for 10 min at 13,000 rpm (~17,900 x *g*) in a table-top microcentrifuge.**

   A compact white pellet will form.

5. **Apply the supernatants from step 4 to the QIAprep spin column by decanting or pipetting.**

6. **Centrifuge for 30–60 s. Discard the flow-through.**

7. **(Optional): Wash the QIAprep spin column by adding 0.5 ml Buffer PB and centrifuging for 30–60 s. Discard the flow-through.**

   This step is necessary to remove trace nuclease activity when using *endA*$^+$ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5α™ do not require this additional wash step.

8. **Wash QIAprep spin column by adding 0.75 ml Buffer PE and centrifuging for 30–60 s.**

9. **Discard the flow-through, and centrifuge for an additional 1 min to remove residual wash buffer.**

   IMPORTANT: Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.

10. **Place the QIAprep column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of each QIAprep spin column, let stand for 1 min, and centrifuge for 1 min.**

Protocol: QIAprep Spin Miniprep Kit Using 5 ml Collection Tubes

The QIAprep Spin Miniprep procedure can be performed using 5 ml centrifuge tubes (e.g., Greiner, cat. no. 115101 or 115261) as collection tubes to decrease handling. The standard protocol on pages 23–24 should be followed with the following modifications:

Step 4: Place a QIAprep spin column in a 5 ml centrifuge tube instead of a 2 ml collection tube.

Step 6: Centrifuge at 3000 x *g* for 1 min using a suitable rotor (e.g., Beckman® GS-6KR centrifuge at ~4000 rpm). (The flow-through does not need to be discarded.)

Steps 7 and 8: For washing steps, centrifugation should be performed at 3000 x *g* for 1 min. (The flow-through does not need to be discarded.)

Step 9: Transfer the QIAprep spin column to a microcentrifuge tube. Centrifuge at maximum speed for 1 min. Continue with step 10 of the protocol.