QIAprep Spin Miniprep Kit

Introduction

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"This protocol is designed for the purification of up to 20 µg of high-copy plasmid DNA from 1–5 ml overnight cultures of E. coli in LB medium. For purification of low-copy plasmids and cosmids, large plasmids (>10 kb), and DNA prepared using other methods, refer to Appendix C: Special Applications, page 45."

Materials

› Time:

› Centrifuge for 10 min

Procedure

Protocol

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.
   - If LyseBlue reagent has been added to Buffer P1, vigorously shake the buffer bottle to ensure LyseBlue particles are completely dissolved. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

2. Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times.

   - This protocol is designed for the purification of up to 20 µg of high-copy plasmid DNA from 1–5 ml overnight cultures of E. coli in LB medium. For purification of low-copy plasmids and cosmids, large plasmids (>10 kb), and DNA prepared using other methods, refer to Appendix C: Special Applications, page 45.
   - If LyseBlue has been added to Buffer P1, the cell suspension will turn blue after addition of Buffer P2. Mixing should result in a homogeneously colored suspension. If the suspension contains localized colorless regions, or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

3. Add 350 µl Buffer N3. Mix immediately and thoroughly by inverting the tube 4–6 times.

   - To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer N3. Large culture volumes (e.g., ≥5 ml) may require inverting up to 10 times. The solution should become cloudy.
   - If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue has gone and the suspension is colorless. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated.

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 800 µl of the supernatant from step 4 to the QIAprep 2.0 spin column by pipetting

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

7. **Recommended:** Wash the QIAprep 2.0 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 DH5®α, do not require this additional wash step.

8. Wash QIAprep 2.0 spin column by adding 0.75 ml Buffer PE and centrifuging for 30–60 s.

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

   CRITICAL 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 2.0 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 2.0 spin column, let stand for 1 min and centrifuge for 1 min