Plasmid Purification from Bacteria

Aim

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.

Procedure

1. Spin the cell culture in a centrifuge to pellet the cells, empty the supernatant (media) into a waste collection container.

2. Resuspend pelleted bacterial cells in 250 µl Buffer P1 (kept at 4 °C) and transfer to a microcentrifuge tube. No cell clumps should be visible after resuspension of the pellet. Important: *Ensure that RNase A has been added to Buffer P1.*

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

4. Add 350 µl Buffer N3 and invert the tube immediately and 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.

5. Centrifuge for 10 min at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge. A white pellet will form.

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

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

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

9. 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.

10. *Although they call this step optional, it does not really hurt your yield and you may think you are working with an endA- strain when in reality you are not. Again for this step, spinning for 60 seconds produces good results. Not necessary for top 10.*

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

12. *Spinning for 60 seconds produces good results.*

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

14. 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. *They are right about this.*

15. 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.

Notes

Heating the elution buffer to 55°C prior to loading on the column can slightly increase yields.

Similarly, doing the elution in two steps (first a 30 µL elution and then a 20 µL dilution) can also slightly increase yields.