Protocol: QIAprep Spin Miniprep Kit Using a Microcentrifuge

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From openwetware

Background

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. In the morning start 5 mL culture in LB media with 100 ug/mL AMP from the transformation plate
2. When grown out, freeze part of the culture
    1. Remove 0.5 mL of cell
    2. Pellet cells in the centrifuge at 14000 rpm for 2 minutes
    3. Remove supernatant and resuspend in 250 uL of LB
    4. Add 250 uL of 50% glycerol solution and mix by pipetting
    5. Place in -80C freezer in IGEM box
3. With the remaining liquid culture, resuspend pelleted bacterial cells in 250 µl Buffer P1 (kept at 4 °C) 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.
4. 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.
5. 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.
6. Centrifuge for 10 min at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge.
   A compact white pellet will form.
7. Apply the supernatants from step 4 to the QIAprep spin column by decanting or pipetting.

8. Centrifuge for 30–60 s. Discard the flow-through.
   Spinning for 60 seconds produces good results.
9. (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.
   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.
10. Wash QIAprep spin column by adding 0.75 ml Buffer PE and centrifuging for 30–60 s.
    Spinning for 60 seconds produces good results.
11. 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. *They are right about this.*
12. 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.
    If you are concerned about the concentration of the DNA, you can alternatively add 30 µL water to the center of the column, incubate at room temperature on the bench for 5 mins and then centrifuge for 1 min. This will increase the concentration of DNA in your final sample which can be useful in some cases. See notes below for why you should elute in water rather than the Buffer EB they recommend if you plan to sequence your sample. Even if you are not sequencing, it may be beneficial to elute in water. For instance, if you elute in buffer EB and you are using this DNA in a restriction digest, then the additional salts in your sample can affect the salt content of your digest. This may matter with some finicky enzymes.

The next step is the digest the plasmid and make sure the digested parts are the correct length