Protocol: Plasmid DNA Purification using the QIAprep Spin Miniprep Kit and a Microcentrifuge

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Miniprep

1. **Spin down approximately 2* 1.5 ml. of bacterial culture at 16.000 rpm at 30 sec**.
2. **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
3. **Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 8-10 times**.
   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.
4. **Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 more times**. To avoid localized precipitation, mix the solution thoroughly but gently, 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 compact white pellet will form.
6. **Apply the supernatants from step 5 to the QIAprep spin column by decanting or pipetting**.
7. **Centrifuge for 50 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**. 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.
9. **Wash QIAprep spin column by adding 0.75 ml Buffer PE and centrifuging for 50 s.**
10. **Discard the flow-through, and centrifuge at full speed 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.

11. **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.**

Buffer P1

50 mM Tris-HCl pH 8.0

10 mM EDTA

100 µg/ml RNaseA*

*keep cold

Buffer P2

200 mM NaOH

1% SDS

Buffer N3

4.2 M Gu-HCl

0.9 M potassium acetate

pH 4.8

Buffer PB

5 M Gu-HCl

30% isopropanol

Buffer PE

10 mM Tris-HCl pH 7.5

80% ethanol