User-Developed Protocol:

Isolation of Plasmid DNA from *Bacillus subtilis* using the QIAprep® Spin Miniprep Kit

This protocol has been adapted by customers and is for use with the QIAprep® Spin Miniprep Kit.

Please be sure to read the background information and the protocol notes in the *QIAprep Miniprep Handbook* carefully before beginning this procedure.

Procedure

1. **Pick a single colony from a selective plate and inoculate a culture of 10 ml LB medium containing the appropriate antibiotic. Grow the culture at 37°C with vigorous shaking (~240 rpm) for approximately 4–6 h until OD_{600} = 0.8–1.2.**

   A short growth time is recommended for two reasons: 1. a lower cell density overcomes incomplete lysis due to the thick peptidoglycan cell wall of Gram-positive bacteria, and 2. *Bacillus* spp. secrete large amounts of nucleases into the media and produce lysis-resistant spores during post-exponential growth.

2. **Harvest the cells by centrifugation at 3000 x *g* for 15 min at 4°C.**

3. **Resuspend pelleted bacterial cells in 250 µl Buffer P1 containing lysozyme at a final concentration of 1 mg/ml.**

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

4. **Incubate at 37°C for 10 min.**

5. **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.

6. **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.

7. **Centrifuge at ≥10,000 x *g* for 10 min. During centrifugation, place a QIAprep Spin Column in a 2 ml collection tube.**

   A compact white pellet will form.

8. **Apply the supernatant from step 7 to the QIAprep Spin Column by decanting or pipetting.**

9. **Centrifuge at ≥10,000 x *g* for 30–60 s. Discard the flow-through.**

10. **Wash the QIAprep Spin Column by adding 0.5 ml Buffer PB and centrifuge at ≥10,000 x *g* for 30–60 s. Discard the flow-through.**

    This step removes trace nuclease activity, and is necessary for all *B. subtilis* strains.

11. **Wash the QIAprep Spin Column by adding 0.75 ml Buffer PE and centrifuge at ≥10,000 x *g* for 30–60 s.**

12. **Discard the flow-through, and centrifuge at ≥10,000 x *g* 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.

13. **Place the QIAprep Spin 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 (pH 7.0) to the center of each QIAprep Spin Column, let stand for 1 min, and centrifuge for 1 min.**

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