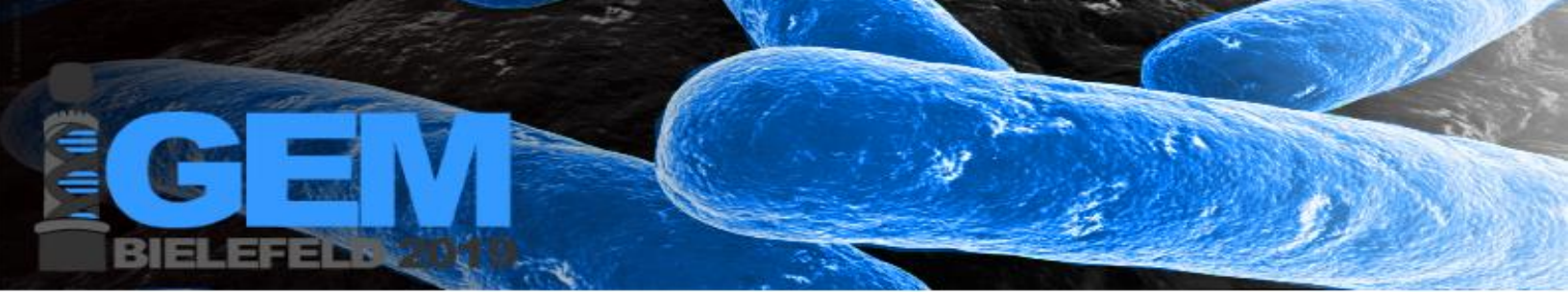

QIAprep® Spin Miniprep Kit (Plasmid Isolation)

Catalog Nos. 27104 and 27106

Centrifugation Protocol

All centrifugation steps are carried out at 13,000 rpm (*~17,900 x g*) in a conventional table-top microcentrifuge.

The following procedure should be performed at room temperature (15-30°C).

1. Pellet 1-5 ml bacterial overnight culture by centrifugation at >8000 rpm (*~6800 x g*) for 3 min at room temperature (15-25°C).

2. Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.

3. Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4-6 times until the solution becomes clear. Do not allow the lysis reaction to proceed for more than 5 min. If using LyseBlue reagent, the solution will turn blue.

4. Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4-6 times. If using LyseBlue reagent, the solution will turn colorless.

5. Centrifuge at room temperature at 13,000 rpm (*~17,900 x g*) in a table-top microcentrifuge.

6. Apply 800 µl supernatant from the last step to the QIAprep 2.0 spin column by pipetting. Centrifuge for 30-60 s and discard the flow-through.

7. **For strains with endA+ or other bacteria strains with high nuclease activity or carbohydrate content:** Wash the QIAprep 2.0 spin column by adding 0.5 ml Buffer PB. Centrifuge for 30-60 s and discard the flow-through.

8. Wash the QIAprep 2.0 spin column by adding 0.75 ml Buffer PE. Centrifuge for 30-60 s and discard the flow-through.

9. Centrifuge for 1 min to remove residual wash buffer.

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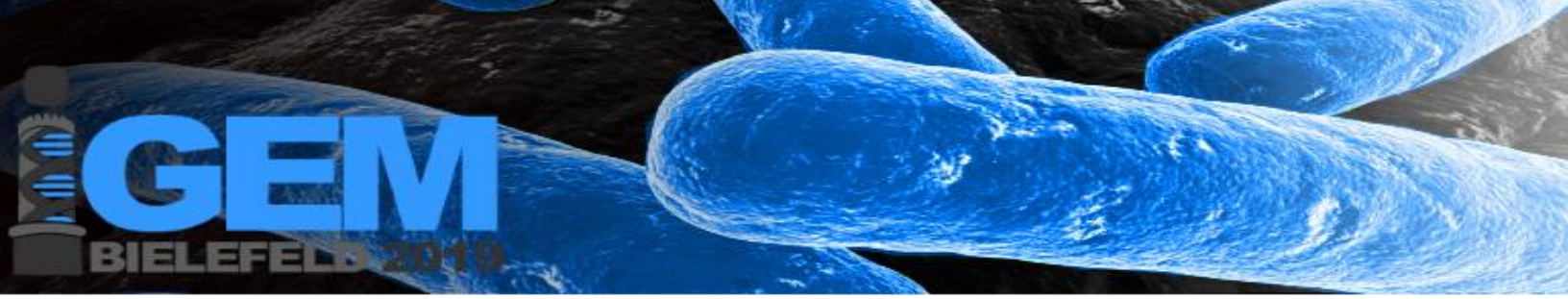

10. Place the QIAprep 2.0 column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM TrisCl, pH 8.5) or water to the center of QIAprep 2.0 spin column, let stand for 1 min, and centrifuge for 1 min.

From: iGEM Bielefeld-CeBiTec