Isolation of plasmid DNA from *Bacillus subtilis* using the QIAGEN® Plasmid Midi Kit

This procedure has been adapted by customers from the QIAGEN® Plasmid Midi Kit Protocol. It has not been thoroughly tested and optimized by QIAGEN.

The procedure has been used successfully for isolation of high- and low-copy-number plasmids from various *Bacillus subtilis* strains. Yield of plasmid DNA was typically 10–20 µg plasmid DNA from 100 ml culture.

Please be sure to read the *QIAGEN Plasmid Purification Handbook* and the detailed QIAGEN Plasmid Midi Kit Protocol carefully before beginning this procedure.

**Procedure**

1. Pick a single colony from a selective plate and inoculate a starter culture of 10 ml LB medium containing the appropriate antibiotic. Grow overnight at 37°C with vigorous shaking (240 rpm).
2. Dilute the miniculture 1:50 to 1:100 into 100 ml selective LB medium. Grow at 37°C for 3–4 hours with vigorous shaking (~240 rpm).

   The culture should reach an $A_{600}$ of 0.8–1.2 units/ml.
3. Harvest the cells by centrifugation at 3000 x $g$ for 15 min at 4°C.
4. Resuspend the bacterial pellet in 4 ml Buffer P1 containing 5 mg/ml lysozyme. Ensure that RNase A (100 µg/ml) has been added to Buffer P1.
5. Incubate at 37°C for 30 min.
6. Add 4 ml Buffer P2, mix gently but thoroughly by inverting 4–6 times, and incubate at room temperature for 5 min.

   Check Buffer P2 before use for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37°C.
7. Add 4 ml chilled Buffer P3, mix immediately but gently by inverting 4–6 times, and incubate on ice for 15 min.
8. Centrifuge at ≥20,000 x $g$ for 30 min at 4°C. Remove supernatant containing plasmid DNA promptly.
9. Centrifuge again at ≥20,000 x $g$ for 15 min at 4°C. Remove supernatant containing plasmid DNA promptly.
10. Equilibrate a QIAGEN-tip 100 by applying 4 ml Buffer QBT, and allow the column to empty by gravity flow.
11. Apply the supernatant from step 9 to the QIAGEN-tip and allow it to enter the resin by gravity flow.

12. Wash the QIAGEN-tip with 2 x 10 ml Buffer QC.

13. Elute DNA with 5 ml Buffer QF.

14. Precipitate DNA by adding 3.5 ml room-temperature isopropanol to the eluted DNA. Mix and centrifuge immediately at ≥15,000 x g for 30 min at 4°C. Carefully decant the supernatant.

15. Wash the DNA pellet with 2 ml of room-temperature 70% ethanol and centrifuge at ≥15,000 x g for 10 min. Carefully decant the supernatant without disturbing the pellet.

16. Air-dry the pellet for 5–10 min, and redissolve the DNA in a suitable volume of buffer (e.g., TE, pH 8.0, or 10 mM Tris·Cl, pH 8.5).