User-Developed Protocol:

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).**