

User-Developed Protocol:

Isolation of plasmid DNA from *Oligotropha carboxidovorans* 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 the large (128 kb), very-low-copy-number (1–2 copies per cell) plasmid pHCG3 and its derivatives from *Oligotropha carboxidovorans*. Yield of plasmid DNA was typically 3–6 µg plasmid DNA from 200 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. Dilute an overnight pre-culture into 200 ml mineral salts medium containing 0.3% nutrient broth and 0.2% pyruvate. Grow at 37°C for overnight.**
The A_{436} should be 1.0–1.2. Do not grow the culture for longer as excessive cell densities result in inefficient lysis.
- 2. Divide the culture into 6 portions and harvest the cells by centrifugation at 6000 x g for 15 min.**
- 3. Resuspend each bacterial pellet in 4 ml Buffer P1.**
Ensure that RNase A (100 µg/ml) has been added to Buffer P1.
- 4. Add 4 ml Buffer P2 to each tube, 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 by warming to 37°C.
- 5. Add 4 ml chilled Buffer P3 to each tube, mix immediately but gently by inverting 4–6 times, and incubate on ice for 15 min.**
- 6. Centrifuge at $\geq 20,000 \times g$ for 30 min at 4°C. Remove supernatants containing plasmid DNA promptly.**
- 7. Centrifuge again at $\geq 20,000 \times g$ for 15 min at 4°C. Remove supernatants containing plasmid DNA promptly.**
- 8. Equilibrate a QIAGEN-tip 100 by applying 4 ml Buffer QBT, and allow the column to empty by gravity flow.**
- 9. Pool the 6 supernatants from step 7. Apply sample to the QIAGEN-tip and allow it to enter the resin by gravity flow.**
- 10. Wash the QIAGEN-tip with 2 x 10 ml Buffer QC.**

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11. Elute DNA with 5 ml Buffer QF.

Prewarming the elution buffer to 50°C may help to increase the yield of large DNA constructs.

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

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

14. 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).

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Selected handbooks can be downloaded from www.qiagen.com/literature/handbooks/default.asp.
Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp.

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