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 ≥20,000 x *g* for 30 min at 4°C. Remove supernatants containing plasmid DNA promptly.**

7. **Centrifuge again at ≥20,000 x *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.**

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 ≥15,000 x *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 ≥15,000 x *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).**