User-Developed Protocol:

Isolation of plasmid DNA from *Citrobacter freundii* 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-copy-number plasmids from *Citrobacter freundii*. Yield of plasmid DNA was typically 3–8 µg DNA per ml culture.

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

Procedure

1. **Inoculate 40–50 µl of an overnight culture into 20 ml selective LB medium. Grow at 37°C for 12–16 hours with vigorous shaking (~300 rpm).**

   Do not grow the culture for longer since too high cell densities result in inefficient lysis and overloading of the QIAGEN-tip.

2. **Harvest the cells by centrifugation at 6000 x *g* for 15 min at 4°C.**

3. **Resuspend the 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. Mix thoroughly by gently inverting 4–6 times, and incubate at room temperature for 5 min.**

   Check Buffer P2 before use for SDS precipitation due to low storage temperature. If necessary, dissolve the SDS by warming to 37°C.

5. **Add 4 ml chilled Buffer P3, mix immediately by gently 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 supernatant containing plasmid DNA promptly.**

7. **Centrifuge again at ≥20,000 x *g* for 15 min at 4°C. Remove supernatant 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. **Apply the supernatant from step 7 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.**

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 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.**
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).**