

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.**

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

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