User-Developed Protocol:

Isolation of plasmid DNA from *Corynebacterium glutamicum* using the QIAGEN® Plasmid Mini Kit

This procedure has been adapted by customers from the QIAGEN® Plasmid Mini Purification Protocol. **It has not been thoroughly tested and optimized by QIAGEN.**

The procedure has been used successfully for isolation of different medium-copy-number plasmids carrying pHM1519 or pBL1 origins of replication from *Corynebacterium glutamicum* ATCC 13032. Yield of plasmid DNA was typically 0.4–1.5 µg per ml LB culture, although yield was dependent on the vector, the insert, and the size of the plasmid.

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

Procedure

1. **Inoculate 10 ml LB-glucose medium from a single colony or a pre-culture. Grow at 30°C for 16 hours on a roller shaker.**
   The culture should reach an A_{580} of 2–3 (A density of 1 x 10^7 *Corynebacterium* cells corresponds to an A_{580} of 0.1 for most spectrophotometers).
2. **Harvest 5 ml of the culture by centrifugation at 6000 x *g* for 5 min. Remove the supernatant completely.**
3. **Resuspend the cells in 1 ml Buffer B.**
4. **Transfer the cells to a clean microcentrifuge tube and centrifuge for 2 min. Remove the supernatant completely.**
5. **Resuspend the pellet in 0.3 ml Buffer P1 containing 15 mg/ml lysozyme.**
   Ensure that RNase A (100 µg/ml) has been added to Buffer P1.
6. **Incubate at 37°C for 2–3 hours, mixing gently occasionally.**
7. **Add 0.3 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 temperatures. If necessary, dissolve the SDS by warming to 37°C.
8. **Add 0.3 ml chilled Buffer P3, mix immediately and gently by inverting 4–6 times, and incubate on ice for 15 min.**
9. **Centrifuge at maximum speed in a microcentrifuge for 10 min. Remove supernatant promptly.**
10. **Equilibrate a QIAGEN-tip 20 by applying 1 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 4 x 1 ml Buffer QC.**
13. **Elute DNA with 0.8 ml Buffer QF.**
14. **Precipitate DNA by adding 0.56 ml room-temperature isopropanol to the eluted DNA. Mix and centrifuge immediately at ≥10,000 x *g* for 30 min in a microcentrifuge. Carefully decant the supernatant.**
15. **Wash the DNA pellet with 1 ml of room-temperature 70% ethanol and centrifuge at ≥10,000 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).**

Medium and buffer composition

LB-glucose medium (1 liter)

10 g tryptone, 5 g yeast extract, 10 g NaCl, and 2 g glucose.

Buffer B: 50 mM glucose

10 mM CDTA, 25 mM Tris·Cl, pH 8.0