User-Developed Protocol:

Isolation of plasmid DNA from *Lactobacillus* spp. 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 cryptic plasmids (pLC2-based) from mesophilic *Lactobacillus* strains such as *L. sake* and *L. curvatus*. Yield of plasmid DNA was typically 10–20 µg plasmid DNA from 100 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 miniculture 1:1000 into 100 ml MRS medium containing the appropriate antibiotic. Grow anaerobically in closed flasks without shaking at 30°C overnight.**

   Lactobacilli do not need strict anaerobic growth conditions. The A_{600} of the culture should be ~3, which corresponds to ~10^9 cfu per ml.

2. **Harvest the cells by centrifugation at 3000 x *g* for 15 min at 4°C.**

   Do not use a higher *g* force as this may result in a very compact pellet.

3. **Wash the bacterial pellet with 20 ml STE buffer. Repeat the centrifugation.**

   Lactobacilli produce huge amounts of acid during growth, and at the moment of harvesting the pH is usually <5. The STE-wash step is included in order to remove and neutralize these acids.

4. **Resuspend the cells in 4 ml STE buffer containing 10 mg/ml lysozyme and incubate at 37°C for 1–2 hours.**

   Some *Lactobacillus* strains, e.g., LTH677, are difficult to lyse, and mutanolysin (20–100 U/ml) should be added to STE buffer. Mutanolysin can be obtained from suppliers such as Sigma (product no. M9901).

5. **Add 4 ml Buffer P2, 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 the SDS by warming to 37°C.

6. **Add 4 ml chilled Buffer P3, mix immediately but gently by inverting 4–6 times, and incubate on ice for 15 min.**

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

8. **Centrifuge again at ≥20,000 x *g* for 15 min at 4°C. Remove supernatant containing plasmid DNA promptly.**
9. **Equilibrate a QIAGEN-tip 100 by applying 4 ml Buffer QBT, and allow the column to empty by gravity flow.**
10. **Apply the supernatant from step 8 to the QIAGEN-tip and allow it to enter the resin by gravity flow.**
11. **Wash the QIAGEN-tip with 2 x 10 ml Buffer QC.**
12. **Elute DNA with 5 ml Buffer QF.**
13. **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.**
14. **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.**
15. **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

MRS medium (per liter)

10 g casein peptone, tryptic digest, 10 g meat extract, 5 g yeast extract, 20 g glucose, 1 g Tween® 80, 2 g K_2HPO_4, 5 g sodium acetate, 2 g diammonium citrate, 0.2 g $MgSO_4$ x 7 H_2O, 0.05 g $MnSO_4$ x H_2O. Adjusted to pH 6.2–6.5.

de Man et al *(1960) J. Appl. Bacteriol.* ***23****, 130–135.*

STE buffer

6.7% saccharose; 50 mM Tris·Cl, pH 8.0; 1 mM EDTA.

Anderson D.G., and McKay, L. L. (1983) Appl. Environ. Microbiol. ***46****, 549–552.*

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