User-Developed Protocol:

Isolation of plasmid DNA from yeast 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.**

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. **Harvest 2 grams of yeast cells (wet weight) by centrifugation and rinse once in water.**
2. **Resuspend the cells in 4 ml of freshly prepared Buffer SCE.**
   Composition of Buffer SCE: 1M sorbitol, 0.1 M NaAc, 60 mM EDTA, pH 7.0.
3. **Add 5–8 mg of zymolase enzyme and 30 µl of ß-mercaptoethanol, and incubate for 1 hour at 37°C.**
   Zymolase is available from suppliers such as ICN (cat. no. 320921, Zymolyase 20T, 1 g).
4. **Spin down spheroplasts at 5000 rpm for 5 minutes.**
5. **Resuspend the spheroplasts in 4 ml of P1 and incubate at 37°C for 15 min.**
6. **Add 2 ml of lysis buffer (3% SDS, 60 mM EDTA). Mix gently but thoroughly, and incubate for 20 min at 65°C.**
   After addition of the lysis buffer, the volume should be 6 ml.
7. **Add 3 ml of 3 M potassium acetate, pH 5.5, mix immediately to avoid localized SDS precipitation. Incubate on ice for 15 min.**
8. **Centrifuge at 4°C for 30 minutes at 30,000 x *g*. Remove the 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 eluted DNA. Mix and centrifuge immediately at ≥15,000 x *g* for 30 min at 4°C. Carefully decant the supernatant.**

14. **Wash DNA pellet with 2 ml room-temperature 70% ethanol, and centrifuge for 10 min at ≥15,000 x *g*. 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).**