User-Developed Protocol:

Isolation of plasmid DNA from yeast using the QIAprep® Spin Miniprep Kit

This procedure has been adapted from the QIAprep® Spin Miniprep Kit Protocol by Michael Jones, Chugai Institute for Molecular Medicine, Ibaraki, Japan. **It has not been thoroughly tested and optimized by QIAGEN.**

The procedure has been used successfully for isolation of plasmid DNA (6.6 kb low-copy plasmid pGAD 10, Clontech, containing inserts of average 1.6kb) from *Saccaromyces cerevisiae* strain PJ69-4A. The plasmid DNA obtained has been used successfully for PCR and transformation of *E. coli*.

Please be sure to read the *QIAprep Miniprep Handbook* and the detailed QIAprep Spin Miniprep Kit Protocol carefully before beginning this procedure, paying special attention to the important notes and the detailed QIAprep Spin Miniprep Kit Protocol using a microcentrifuge.

Procedure

1. **Inoculate a single colony into 2–5 ml of the appropriate selective media and grow the culture for 16–24 h at 30°C.**
   SD medium: 6,7g/liter Yeast Nitrogen Base (Difco 0919-15-3) supplemented with amino acids. To select for pGAD 10 do not add leucine.
2. **Harvest the cells by centrifugation for 5 min at 5000 x *g* and resuspend cells in 250 µl Buffer P1 containing 0.1 mg/ml RNase A. Transfer the cell suspension to a 1.5 ml microcentrifuge tube.**
3. **Add 50–100 µl of acid-washed glass beads (Sigma G-8772) and vortex for 5 min. Let stand to allow the beads to settle. Transfer supernatant to a fresh 1.5 ml microcentrifuge tube.**
4. **Add 250 µl lysis buffer P2 to the tube and invert gently 4–6 times to mix. Incubate at room temperature for 5 min.**
5. **Add 350 µl neutralization buffer N3 to the tube and invert immediately but gently 4–6 times.**
6. **Centrifuge the lysate for 10 min at maximum speed in a tabletop microcentrifuge (13,000 rpm or ≥10,000 x *g*). Meanwhile, place a QIAprep Spin Column in a 2 ml collection tube.**
7. **Transfer the cleared lysate from step 6 to QIAprep Spin Column by decanting or pipetting.**
8. **Centrifuge for 30–60 s (13,000 rpm or ≥10,000 x *g*). Discard flow-through.**

9. **Wash QIAprep Spin Column by adding 0.75 ml of Buffer PE and centrifuging 30–60 s (13,000 rpm or ≥10,000 x *g*).**

10. **Discard flow-through and centrifuge for an additional 1 min to remove residual wash buffer (13,000 rpm or ≥10,000 x *g*).**

    IMPORTANT: Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.

11. **Place QIAprep Spin Column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 25 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of each QIAprep Spin Column, let stand for 1 min, and centrifuge for 1 min.**

    Typical yield is up to 1 µg. For subsequent PCR, use 0.1-1.0 µl of the eluate. For subsequent transformation into *E. coli,* 2–3 µl of eluate yields about 30 colonies.