

QIAprep Spin Miniprep Kit Protocol (for yeast)

using a microcentrifuge

Procedure

1. Grow yeast strain containing desired plasmid overnight in 10 mL of rich media (YEPD).
2. Pellet cells in 15 mL Falcon tube, resuspend in water, and transfer to 1.5 mL eppendorf tube. Pellet cells and remove water.
3. Resuspend pelleted cells in 200 μ L Buffer P1.
4. Add 100 μ L of lyticase solution[†], mix thoroughly and incubate at 37 °C for 30 minutes. (It is safe to leave cells incubating overnight.)
5. Add 300 μ L Buffer P2 and gently invert 4-6 times to mix. Incubate at 22 °C for 10 minutes.
6. Add 420 μ L Buffer N3 and invert immediately but gently 4-6 times.
7. Centrifuge for 10 minutes at 10,000g on tabletop microcentrifuge.
8. Apply supernatants from step 4 to the QIAprep column by decanting or pipetting and centrifuge for 30-60 s. Discard the flowthrough.
9. Wash the QIAprep spin column by adding 0.5 mL Buffer PB and centrifuging for 30-60 s. Discard the flowthrough.
10. Wash QIAprep spin column by adding 0.75 mL Buffer PE and centrifuging for 30-60 s. Discard the flowthrough, and centrifuge for an additional 1 min to remove residual wash buffer.
11. Place the QIAprep column in a clean 1.5 mL microcentrifuge tube. To elute DNA, add 50 μ L elution buffer (prewarmed to 65 °C for large plasmids) to the center of each QIAprep column, let stand for more than 1 min, and centrifuge for 1 min.

[†] 1.2 M sorbitol, 0.1 M NaPO₄ buffer, pH 7.5, 5 mg/ml *Arthrobacter luteus* lyticase