Protocol: DNA Purification from Yeast Using the Gentra Puregene Yeast/Bact. Kit

This protocol is for purification of genomic DNA from fresh or frozen samples of 1 ml overnight yeast cultures (approximately 1–2 x 10^8 cells) using the Gentra Puregene Yeast/Bact. Kit.

Things to do before starting

- Preheat water baths to 37°C for use in steps 6 and 20 and 65°C for use in step 21 of the procedure.
- Frozen yeast samples should be thawed and equilibrated to room temperature (15–25°C) before beginning the procedure.

Procedure

1. **Prepare an overnight culture containing 1–2 x 10^8 cells.**
2. **Transfer 1 ml of the cell suspension to a 1.5 ml microcentrifuge tube on ice.**
3. **Centrifuge for 5 s at 13,000–16,000 x *g* to pellet cells.**
4. **Carefully discard the supernatant by pipetting or pouring.**
5. **Add 300 µl Cell Suspension Solution, and pipet up and down.**
6. **Add 1.5 µl Lytic Enzyme Solution, and mix by inverting 25 times. Incubate for 30 min at 37°C.**
7. **Centrifuge for 1 min at 13,000–16,000 x *g* to pellet cells.**
8. **Carefully discard the supernatant by pipetting or pouring.**
9. **Add 300 µl Cell Lysis Solution, and pipet up and down to lyse the cells.**
10. **Add 100 µl Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.**
11. **Centrifuge for 3 min at 13,000–16,000 x *g*.**

    The precipitated proteins should form a tight pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation.
12. **Pipet 300 µl isopropanol into a clean 1.5 ml microcentrifuge tube and add the supernatant from the previous step by pouring carefully.**

    Be sure the protein pellet is not dislodged during pouring.
13. **Mix by inverting gently 50 times.**
14. **Centrifuge for 1 min at 13,000–16,000 x *g*.**

    The DNA may be visible as a small white pellet.
15. **Carefully discard the supernatant, and drain the tube by inverting on a clean piece of absorbent paper, taking care that the pellet remains in the tube.**

16. **Add 300 µl of 70% ethanol and invert several times to wash the DNA pellet.**
17. **Centrifuge for 1 min at 13,000–16,000 x *g*.**
18. **Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube. Allow to air dry for 5 min.**

    The pellet might be loose and easily dislodged. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.
19. **Add 100 µl DNA Hydration Solution and vortex for 5 s at medium speed to mix.**
20. **Add 1.5 µl RNase A Solution, and mix by vortexing by 1 s. Pulse spin to collect liquid, and incubate at 37°C for 15–60 min.**
21. **Incubate at 65°C for 1 h to dissolve the DNA.**
22. **Incubate at room temperature (15–25°C) overnight with gentle shaking. Ensure tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.**