

Preparation of genomic DNA for PCR

1. Centrifuge 5 mL of a saturated overnight culture of yeast cells at 5000 x *g* for 5 min at RT.
2. Decant supernatant and resuspend cell pellet in 1 mL of sterile water.
3. Transfer volume to screw-cap eppi tube and centrifuge at 5,000 x *g* for 5 min at RT.
4. Aspirate supernatant.
5. Resuspend cell pellet in 400 μ L of Rescue Buffer.
 - Rescue Buffer:
6. Add 200 μ L of sterile glass beads and 200 μ L of phenol-chloroform-isoamyl alcohol.
7. Vortex 15 min at RT.
8. Centrifuge 5 min at 5,000 x *g* for 5 min.
9. Transfer 300 μ L of aqueous phase (top) to new eppi tube.
10. Add 300 μ L of chloroform and vortex briefly.
11. Centrifuge 5 min at 5,000 x *g* for 5 min.
12. Transfer 200 μ L of aqueous (top) phase to new eppi tube.
13. Add 20 μ L of 3 M NaOAc pH 5.2; vortex briefly.
 - 3 M NaOAc pH 5.2:
14. Add 200 μ L of 100% isopropanol (or 400 μ L of 95 – 100% ethanol); vortex briefly.
15. Incubate on ice (or at -20°C) for \geq 20 min.
16. Centrifuge 10 min at 14,000 x *g* at 4°C.
17. Aspirate supernatant.
18. Add 500 μ L of 75 – 80% ethanol.
19. Vortex briefly.
20. Centrifuge 10 min at 14,000 x *g* at RT.
21. Aspirate supernatant.
22. Dry pellet completely by speed-vac for 10 – 15 min (or leave tubes open at RT overnight).
23. Add 500 μ L of TE; allow DNA to resuspend completely (e.g., incubate at 42°C for 30 min).
24. Vortex thoroughly.
25. Use 5 μ L as a template for PCR.