

Protocol: Easy Yeast DNA Extraction

DNA is isolated from yeast using the crude procedure below. This protocol involves lysing the cells in the presence of lithium acetate (LiOAc) and sodium dodecyl sulfate (SDS). The lysate is treated with RNase A to degrade RNA, and DNA is precipitated in ethanol.

Yeast DNA Lysis Buffer:

| Stock | Volume | Final Concentration |
|------------------|---------------|----------------------------|
| 1M LiOAc | 2 ml | 200mM |
| 10% SDS | 1 ml | 1% |
| H ₂ O | 8 ml | |

Protocol:

1. Move 500 μ l of a 24-hour old culture of yeast to a microcentrifuge tube.
2. Centrifuge at 6,000 x g for 1 minute. Decant supernatant.
3. Add 100 μ l of Yeast DNA Lysis Buffer and resuspend the pellet thoroughly.
4. Incubate on a 70°C heat block for 15 minutes.
5. Place the lysate on ice for 5 minutes to cool.
6. Add 1 μ l RNase A and incubate at 37°C for 15 minutes.
7. Add 300 μ l 100% EtOH to the lysate and vortex.
8. Centrifuge the lysate at 20,000 x g for 3 minutes.
9. Decant supernatant, and wick away residual liquid using a Kimwipe. Allow any residual ethanol to evaporate.
10. Add 100 μ l TE to the pellet and resuspend thoroughly. Allow the sample to rest for 30 minutes at room temperature.
11. Centrifuge at 20,000 x g for 1 minute. Remove the clear liquid portion of the sample (containing DNA) to a new microcentrifuge tube and label appropriately.