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GENOMIC DNA PREPARATION FROM *SACCHAROMYCES CEREVISIAE* MINI-PROTOCOL

1. Grow a 10ml overnight culture to saturation ($OD_{600} \sim 2-3$).
2. Spin down the cells in a centrifuge ($\sim 2,000$ rpm in Sorvall) and wash with an equal volume of cold, distilled, sterile water.
3. Resuspend the cell pellet in 200 μ l lysis buffer, then add ~ 300 μ l glass beads and 200 μ l 1:1 phenol:chloroform.
4. Vortex for 1-2min.
5. Add 200 μ l TE buffer and mix 6-10 times by inversion. WARNING: vortexing from this step on can shear the DNA.
6. Spin samples for 5min at 13,000rpm in a microfuge
7. Transfer the aqueous (top) phase to a fresh eppendorf tube.
8. Add 1 volume (400 μ l) chloroform and mix by inversion.
9. Centrifuge as in step 6
10. Transfer the aqueous (top) phase to a fresh eppendorf tube.
11. Add 1ml 100% EtOH and mix by inversion.
12. Spin in a microfuge for 2min at 13,000rpm.
13. Remove the supernatant and wash the pellet with 1ml 70% EtOH.
14. Remove the supernatant completely and dry the pellet at room temperature.
When dry the pellet will be whitish and should not smell like ethanol.
15. Resuspend the pellet in 400 μ l TE buffer containing a final concentration of 2 μ g/ml RNaseA.
16. Incubate 10min at 37°C.
17. Repeat steps 9-12.
18. Resuspend the pellet in 50 μ l TE.

Lysis buffer:

2% (v/v) Triton X-100
1% (v/v) SDS
100mM NaCl
10mM Tris base, pH 8.0
1mM EDTA, pH 8.0

TE buffer:

10mM Tris base, pH 8.0
1mM EDTA, pH 8.0

YPD:

1% yeast extract
2% bacto peptone
2% glucose
autoclave 20min/liter

Acid-washed glass beads

425-600 μ m, from Sigma, G 8772
autoclave before use