TEN-MINUTE GENOMIC DNA PREPARATION FROM YEAST

Reagents:

-YPD

-Sterile water

-Lysis buffer: 2% Triton X-100, 1% SDS, 100 mM NaCl, 10mM Tris-HCl (pH 8.0), 1mM Na_2EDTA.

-Phenol:Chloroform:isoamyl alcohol (25:24:1).

-Glass beads: 0.45-0.5 mm beads (Sigma) soaked in nitric acid and washed in distilled water. Beads should be dried before use.

-TE (pH 8.0): 10 mM Tris-HCl, 1 mM Na_2EDTA

-100% EtOH

-Rnase A (10 mg/ml)

-7.5 M ammonium acetate

Protocol:

1. Grow 10-ml yeast culture to saturation in YPD at 30°C.
2. Collect the cells by centrifugation for two minutes in a clinical centrifuge. Remove the supernatant and resuspend the cells in 0.5 ml of distilled water. Transfer the cells to a 1.5-ml microfuge tube and collect them by centrifugation for 5 seconds in a microfuge,
3. Decant the supernatant and briefly vortex the tube to resuspend the pellet in the residual liquid.
4. Add 0.2 ml of lysis buffer and 0.2 ml phenol:chloroform:isoamyl alcohol. Then add 0.3 g of glass beads.

5. Vortex for 3-4 minutes. Then add 0.2 ml of TE (pH 8.0)
6. Centrifuge for 5 minutes in a microfuge. Transfer the aqueous layer to a fresh tube. Add 1 ml of 100% ethanol. Mix by inversion.
7. Centrifuge for 2 minutes in a microfuge. Discard the supernatant. Resuspend the pellet in 0.4 ml of TE plus 3 μl of a 10 mg/ml solution of RNase A. Incubate for 5 minutes at 37°C. Add 5.4 μl of 7.5 M ammonium acetate plus 1 ml of 100% ethanol. Mix by inversion.
8. Centrifuge for 2 minutes in a microfuge. Discard the supernatant. Air-dry the pellet and resuspend in 50 μl of TE.
9. Use 10μl for each sample to be analyzed by Southern blotting. This is ~2-4 μg of DNA.