玻璃珠法快速制备酵母基因组 DNA*

Additional Materials

TE buffer
1 mg/ml DNase-free RNase A
4 M ammonium acetate solution
100% ethanol
18 × 150–mm glass culture tubes *or* 17 × 100–mm disposable polypropylene tubes, sterile
Tabletop centrifuge

Prepare yeast cells

1. Grow a 10-ml culture of yeast in YPD overnight to stationary phase in either 18 × 150–mm sterile glass culture tubes or in 17 × 100–mm sterile, polypropylene tubes.

2. Spin culture 5 min in a tabletop centrifuge at 1200 × *g* (3000 rpm), room temperature.
Aspirate or pour off supernatant, and resuspend cells in 0.5 ml water.

3. Transfer the resuspended cells to a microcentrifuge tube and spin 5 sec at room temperature. Pour off supernatant and disrupt pellet by vortexing briefly.
This wash step in water removes any remaining medium.

Break open the cells

4. Resuspend cells in 200 µl breaking buffer. Add 0.3 g glass beads (～200 µl volume) and 200 µl phenol/chloroform/isoamyl alcohol and vortex at highest speed for 3 min.
The amount of vortexing required can vary depending upon the vortex used. Determine by microscopic examination the minimum vortexing required for a particular machine to break 80% to 90% of the cells.
If using a multi-tube vortex rather than a single-tube vortex, the time of vortexing may need to be increased up to 4 or 5 min to get more efficient breakage of cells. However, longer vortexing can result in shearing of DNA molecules.

5. Add 200 µl TE buffer and vortex briefly.

6. Microcentrifuge 5 min at high speed, room temperature, and transfer aqueous layer to a clean microcentrifuge tube. Add 1 ml of 100% ethanol and mix by inversion.

7. Microcentrifuge 3 min at high speed, room temperature. Remove supernatant and resuspend pellet in 0.4 ml TE buffer.

Degrade RNA contaminants and recover DNA

8. Add 30 µl of 1 mg/ml DNase-free RNase A, mix, and incubate 5 min at 37°C.

9. Add 10 µl of 4 M ammonium acetate and 1 ml of 100% ethanol. Mix by inversion.

10. Microcentrifuge 3 min at high speed, room temperature. Discard supernatant and dry pellet. Resuspend DNA in 100 µl TE buffer.
Yields of ～20 g of chromosomal DNA should be obtained. This DNA is ready to use for

restriction digestion, in vitro PCR amplification, or Southern blotanalysis. For Southern blots, best results are obtained when 5 l DNA (～1 g) is digested in a total volume of 20 l. To amplify by PCR, 2 l of DNA should be used in a50-l reaction.

REAGENTS AND SOLUTIONS

Breaking buffer

2% (v/v) Triton X-100
1% (v/v) sodium dodecyl sulfate (SDS)
100 mM NaCl
10 mM Tris~Cl, pH 8.0
1 mM EDTA, pH 8.0
Store ≤1 year at room temperature

*:出自 *Current Protocols in Molecular Biology* [B], *Wiley Online Library*,2007