Protocols

Yeast genomic DNA extraction with LiOAc-SDS

PROTOCOL FOR:

Extraction of genomic DNA from yeasts for PCR-based applications

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We have developed a quick and low-cost genomic DNA extraction protocol from yeast cells for PCR-based applications. This method does not require any enzymes, hazardous chemicals, or extreme temperatures, and is especially powerful for simultaneous analysis of a large number of samples. DNA can be efficiently extracted from different yeast species (*Kluyveromyces lactis*, *Hansenula polymorpha*, *Schizosaccharomyces pombe*, *Candida albicans*, *Pichia pastoris*, and *Saccharomyces cerevisiae*). The protocol involves lysis of yeast colonies or cells from liquid culture in a lithium acetate (LiOAc)–SDS solution and subsequent precipitation of DNA with ethanol. Approximately 100 nanograms of total genomic DNA can be extracted from 1×10^7 cells. DNA extracted by this method is suitable for a variety of PCR-based applications (including colony PCR, real-time qPCR, and DNA sequencing) for amplification of DNA fragments of ≤3500 bp.

Procedure

1. Pick one yeast colony from the plate or spin down 100–200 µL liquid yeast culture (OD_{600} = 0.4). Suspend cells in 100 µL 200 mM LiOAc, 1% SDS solution.
2. Incubate for 5 min at 70°C.
3. Add 300 µL 96–100% ethanol, then vortex.
4. Spin down DNA and cell debris at 15,000× *g* for 3 min.
5. Wash pellet with 70% ethanol.
6. Dissolve pellet in 100 µL H_2O or TE and spin down cell debris for 15 s at 15,000× *g*.
7. Use 1 µL supernatant for PCR.

Reagents

0.2 M lithium acetate (LiOAc) 1%
SDS solution
Ethanol, 96–100% and 70%.

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