Yeast Genomic DNA Miniprep Using A FastPrep Cell Lyser

Xiyan Li*

Department of Genetics, Stanford University, Stanford, USA
*For correspondence: lixiyan@stanford.edu

[Abstract] This method is a convenient way to purify high-quality genomic DNA from yeast cells. It is suitable for PCR and other assays that require genomic DNA of higher quality.

Materials and Reagents

1. 5 M Ammonium acetate (pH 7.0)
2. Chloroform
3. Isopropanol
4. 70% Ethanol
5. Lysis buffer (see Recipes)

Equipment

1. Adapted for Fastprep machine
2. Screw-tube
3. Glass beads
4. Microfuge

Procedure

1. Grow 5 ml yeast cells overnight at 30 °C.
2. Spin, wash once with 1 ml H_2O.
3. Resuspend in 500 µl lysis buffer.
4. Transfer to a screw-tube with acid washed glass beads.
5. Fastprep at 6.0 speed for 2 min.
6. Recover liquid phase with blue tip into another tube.
7. Add 385 µl 5 M ammonium acetate pH 7.0.
8. Incubate 5 min at 65 °C, then 5 min on ice.
9. Add 500 µl chloroform, vortex, spin 2 min in microfuge.
10. Take supernatant and precipitate with 1 ml isopropanol.

11. Incubate 5 min at room temprature, then spin 5 min.
12. Wash pellet with 70% ethanol, dry and dissolve in 50 µl H_2O.
    Note: For Southern, digest 5 µl DNA; For PCR, use 0.5-1 µl DNA. For E coli transformation, use 1-5 µl DNA.

Recipes

1. Lysis buffer
   100 mM Tris (pH 8.0)
   50 mM EDTA
   1% SDS
   For 50 ml: 5 ml 1 M Tris, 5 ml 0.5 M EDTA, 5 ml 10% SDS