A rapid and simple method for the preparation of yeast mitochondrial DNA

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A rapid method for the extraction of yeast mitochondrial DNA (mtDNA) is described. The method is based on: preparation of spheroplasts, lysis of the cellular membrane, isonicotic purification of mitochondria, lysis of the mitochondrial membranes, and mtDNA isolation without using CsCl gradient centrifugation. In comparison with previous methods (1, 2), the method described here consists of rapid and simple-to-perform steps, and does not require neither phenol treatment nor the use of carcinogenic dyes (ethidium bromide and bis-benzimide). The resulting mtDNA is sufficiently pure for RFLP analyses, and the yield is high even from wild strains.

The extraction is made using 250 ml of an overnight grown culture in YEPG medium. Cells are pelleted by low-speed centrifugation (at 3,800 ×g for 5 min). Approximately 3 g (wet weight) of cells are generally obtained. Pellet is resuspended in 1 ml per g of cells of a solution 1M sorbitol, 0.1M EDTA pH 7.5 and 0.5 mg/ml zymolyase 20T (freshly made), and incubated at 37°C for 30–60 min. Spheroplasts are pelleted at 3,800 ×g for 5 min, and resuspended in 10 ml per g of cells of a solution 0.5 mM Tris-HCl pH 7.5, 1 mM EDTA, 0.25M sorbitol, and incubated at 4°C for 30 min. Nuclei and cell debris are pelleted at 2,000 ×g for 20 min. Supernatant is transferred to a new tube and centrifuged at 15,000 ×g for 20 min. Mitochondria are resuspended in 5 ml of 20% sucrose, 10 mM Tris-HCl, 0.1 mM EDTA pH 7.5, and after centrifugation at 15,000 ×g for 20 min, are resuspended in 2 ml of 60% sucrose, 10 mM Tris-HCl 0.1 mM EDTA pH 7.5. The mitochondrial suspension is transferred to an Ultra-Clear tube and then overlaid with 4 ml of 50% sucrose, 10 mM Tris-HCl pH 7.5 solution followed by 4 ml of 44% sucrose, 10 mM Tris-HCl pH 7.5 solution. After ultracentrifugation at 40,000 rpm for 90 min with a Beckman SW41 rotor, mitochondria form a tight band at the interface of the 44% and 55% sucrose layers. Mitochondria are collected with a syringe needle, and diluted in 4 ml of 10 mM Tris-Cl, 1 mM EDTA, 50 mM NaCl pH 7.5, and then centrifugated at 15,000 ×g for 20 min. The pellet is resuspended in 2 ml of the last solution added with SDS up to 0.5% and 50 μg/ml of proteinase K, and incubated at 37°C for 3 h. 0.5 ml of 5M potassium acetate is added and the mixture is incubated at −20°C for 15 min and centrifugated at 17,000 ×g at 4°C for 10 min. The supernatant is transferred to a Corex tube and the mtDNA is precipitated by adding 1 vol. of isopropanol, and after incubation at room temperature for 10 min is centrifugated at 17,000 ×g at room temperature for 10 min. The DNA is washed with 70% ethanol and vacuum dried and dissolved in 200 or 300 μl TE. Between 80 and 120 μg of mtDNA are routinely obtained. The ratio of the absorption at 260 nm over that at 280 nm of this mtDNA is 1.8–1.9. It is free enough of proteins and RNA to serve as a substrate for restriction endonucleases and other modifying enzymes (Fig. 1).

REFERENCES

Figure 1. Agarose gel electrophoresis of EcoRV-digested mtDNA prepared from wild wine strains of Saccharomyces cerevisiae (lanes 2–5). Lane 1, a mixture of lambda DNA digested with HindIII, and with HindIII-EcoRI.