A rapid miniprep method for the preparation of yeast mitochondrial DNA

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Two protocols have been recently proposed to speed up the preparation of *Saccharomyces cerevisiae* mitochondrial DNA (mtDNA). The first (1) is a simplified procedure derived from pre-existing methods using a CsCl gradient, while the other (2) is based on the isopycnic purification of the mitochondrial fraction from lysed spheroplasts. Both the above procedures require an ultracentrifugation step. We present here a rapid, simple and inexpensive miniprep method which requires no more than usual bench equipment and which gives reliable results for the routine analysis of mtDNA from both wild and laboratory strains of *S. cerevisiae*.

For each strain, the extraction of mtDNA was performed from an overnight 20 ml culture in YPD (1% yeast extract, 2% peptone, 2% dextrose) at 28°C under shaking. The cells were harvested by centrifugation at 500×g for 5 min and the pellet, which corresponds to 0.3—0.4 g (wet weight) was washed twice in sterile distilled water and then once in 1.2 M sorbitol, 50 mM EDTA, 2% mercaptoethanol. The pellet was then resuspended in 5 ml of Sol A (0.5 M sorbitol, 10 mM EDTA, 50 mM Tris, pH 7.5) containing 2% mercaptoethanol and 0.2—1 mg/ml of zymolyase 20 T, and then incubated at 37°C for 45 min under gentle agitation. During this step the majority of the formed spheroplasts were osmotically lysed. The suspension can be sonicated using an ultrasonic cell disruptor (Bioruptor UCD 130, Tosho Denki Co Ltd) at 19.3—19.5 KHz for 1 to 5 min (until the sample becomes visually more clear). This step, which breaks open the unlysed protoplasts, was not absolutely necessary but did improve the final result. The cellular lysate was then transferred into microfuge tubes and centrifuged at 1000×g for 10 min. The supernatant containing the mitochondria was centrifuged at 15000×g for 15 min and the crude mitochondrial pellet was carefully washed three or four times with Sol A to eliminate genomic DNA contamination (this step is crucial). The mitochondria were resuspended in 0.2—0.4 ml of Sol B (100 mM NaCl, 10 mM EDTA, 1% Sarkosyl, 50 mM Tris, pH 7.8) and allowed to lyse for 30 min at room temperature. The nucleic acids were then purified from the mitochondrial lysate by extraction with phenol-chloroform and ethanolic precipitation. RNAs could be further degraded by RNase A. Good yields were obtained (10 to 20 μg DNA per 0.3—0.4 g of wet weight) and the mt DNA was pure enough (absorption ratio at 260/280 nm = 1.8—1.9) for RFLP analysis (fig. 1).

This protocol is efficient for many yeast strains and should take no more than 4—6 hours. It is cheap and easy to perform (since it avoids ultracentrifugation steps and the use of carcinogenic dyes) and can be adapted for obtaining much larger amounts of mtDNA. It is suitable for studying the polymorphism of a large number of wild strains or for strain identification.

REFERENCES


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Figure 1. Agarose gel electrophoresis of EcoRV digested mtDNA of *Saccharomyces cerevisiae* strains. Lanes 2—3: industrial enological strains EG 8 and 522 Davis; lanes 4—5 wild strains; lanes 7 and 8: undigested and digested mtDNA of the laboratory strain S 288 C; lane 7, L:L dsRNA; lanes 1 and 6: HindIII restriction fragments of Lambda DNA and HaeIII restriction fragments of PhiX DNA.

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