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A rapid, efficient method for isolating DNA from yeast

(Recombinant DNA; genome; plasmid; restriction analysis; chaotropic; guanidine; proteinase K; zymolyase; *Saccharomyces cerevisiae*)

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SUMMARY

A method is described for the purification of chromosomal and plasmid DNA from the yeast *Saccharomyces cerevisiae*. This method is rapid, gives 75% of theoretical yield, and produces DNA that can be cut with restriction endonucleases. Yeast cells are treated with zymolyase, and the resulting spheroplasts are lysed in the presence of the chaotropic agent guanidine hydrochloride. After a brief ethanol precipitation, protein is removed by treatment with proteinase K followed by phenol-chloroform extraction. After ethanol precipitation, the DNA is sufficiently pure for restriction analysis or for the transformation of *Escherichia coli*.

INTRODUCTION

To isolate density-labeled DNA, our laboratories required an efficient purification method to produce yeast chromosomal DNA of high M_r. Unlike most yeast DNA purification schemes, the procedure described here utilizes the chaotrophic agent GuHCl during spheroplast lysis to disrupt protein structure and inhibit nuclease activity. Spheroplast lysis in the presence of GuHCl was adapted from a procedure by Kielland-Brant et al. (1979). Our procedure is a technically simple, rapid method for isolating plasmid as well as chromosomal DNA.

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Abbreviations: *ARS*, autonomously replicating sequence; DABA, diaminobenzoic acid; EtdBr, ethidium bromide; GuHCl, guanidine hydrochloride; RF, replicative form; SCE, 1 M sorbitol, 0.1 M sodium citrate, 0.06 M EDTA, pH 7.0; 10 × TE, 100 mM Tris, 10 mM EDTA, pH 8.0; [ ], designates plasmid-carrier state.

MATERIALS AND METHODS

(a) Enzymes and chemicals

Reagents were purchased from the following suppliers: Zymolyase 60000 from Kirin Brewery Co., Tokyo (Japan); restriction enzymes from New England Biolabs; [^{3}H]uracil and [^{32}P]dCTP from New England Nuclear; ribonuclease A from Sigma; and proteinase K from Boehringer Mannheim.

(b) Strains, plasmids and media

S. cerevisiae strain DBY1033 is *MATα ura3*-52 *SUC2*$^+$, and it comes from M.I.T. Strain LH330-SD, provided by Dr. Leland Hartwell, is a haploid strain that is disomic for chromosome VII; it has the following genotype: *MATα trp1 leu2 ura3 can1 hom3 fcy1 snp3 ade2* $\frac{lys5\ +\ +\ ade6\ +}{+\ aro2\ cyh2\ +\ ade3}$.

E. coli strain HB101(LM1035) (also called DB1142) was derived from strain HB101 in a screen for improved transformation efficiency. In our hands the efficiency of transformation is approximately five times higher with this strain than it is with HB101. The strain is *leu*$^-$ *pro*$^-$ *thr*$^-$ r_B^- m_B^- *recA*$^-$ obtained from Connie Cepko at M.I.T. (Cepko et al., 1984).

All of the plasmids used in this study can be maintained in yeast or as episomes in *E. coli*. Plasmid YIp5 is an integrating yeast plasmid (Botstein et al., 1979) that was used as reference DNA. Plasmid YCp19 contains a yeast centromere as well as an *ARS* (Stinchcomb et al., 1982). Plasmid YEp24 contains a portion of the endogenous yeast plasmid, 2μ DNA (Botstein et al., 1979).

The media used in these experiments have been described. When [^{3}H]uracil was used to quantitate DNA yield, the cells were grown in synthetic medium (Zakian et al., 1979) at pH 3.5. For transformation, the cells were grown in SD medium (Wickerham et al., 1946) supplemented with 2% glucose. For experiments involving gel electrophoresis, the cells were grown in YM-1 medium (Hartwell, 1967).

(c) DNA isolation

Logarithmically growing haploid yeast cells (5×10^8 cells) were harvested by centrifugation, resuspended in water, and centrifuged again. The pellet was resuspended in 150 μl SCE buffer and transferred to a 1.5-ml microfuge tube. Zymolyase solution (10 μl; 3 mg/ml Zymolyase 60000, 10% 2-mercaptoethanol in SCE) was added, and the resuspended cells were incubated at 37°C until spheroplasting was complete (usually 20 to 30 min). The spheroplasted cells were then spun very briefly in a microfuge (2 s, or as long as minimally necessary to sediment the cells), and the resulting pellet was drained well. The pellet was slowly resuspended in 150 μl of a GuHCl solution [4.5 M GuHCl (99% grade), 0.1 M EDTA, 0.15 M NaCl, 0.05% sarkosyl, pH 8.0] and then incubated at 65°C for 10 min with occasional swirling. After cooling to room temperature, 150 μl of cold ethanol were added, and the mixture was centrifuged for 5 min in a microfuge. The pellet was then drained well, and 0.3 ml of 10 × TE were added slowly. If removal of RNA was desired, 3 μl of 5 mg/ml RNase A were added, and the solution was incubated at 37°C for 1 h. Whether or not the RNase step was included, 3 μl of 20 mg/ml proteinase K were then added, and the solution was incubated at 65°C for 30 min. It was then extracted twice with 0.5 ml of 25 : 24 : 1 phenol–chloroform–isoamyl alcohol. The aqueous phase was made 0.3 M in Na · acetate, and the DNA was precipitated with the addition of 0.6 ml ethanol. After cooling at −70°C for 15 min, the DNA was sedimented in a microfuge, rinsed with cold 70% ethanol, allowed to dry, and resuspended in the desired buffer.

NOTE 1: Spheroplasting was monitored under the microscope by adding 10% sarkosyl to the edge of the coverslip under which a small quantity of cells was placed. Spheroplasts were recognized by their sudden darkening when exposed to the sarkosyl. Logarithmically growing cells usually reach 100% spheroplasting; stationary-phase cells do not.

NOTE 2: Special handling was required to resuspend the first ethanol pellet thoroughly. Our best results came from stirring the pellet with an empty Pipetteman tip for 2 to 3 min, until it liquified. 10 × TE was then added slowly, initially while stirring with a Pipetteman tip.

(d) Quantitation of DNA yield

To quantitate the recovery of DNA, samples labeled with [^{3}H]uracil were assayed for alkali-stable, acid-precipitable radioactivity using Liquifluor (New England Nuclear) in toluene. To confirm the results obtained by this method, we also monitored the yield of DNA using a DABA assay (described below) and by comparing the intensity of EtdBr fluorescence with that produced by a known amount of DNA subjected to electrophoresis in the same agarose gel.

DABA was used to quantitate DNA yield as follows. The assay was performed by placing 1 to 20 μl of a DNA preparation (0.1 to 10 μg of DNA) in duplicate into 10 × 75 mm glass tubes. The tubes

were placed in a 65°C drying oven for 3 h. DABA solution [400 mg/ml w/v; recrystallized (Hopper and Hall, 1975) DABA (Aldrich) in water] was prepared, and 50 μl were added to each tube. The tubes were covered with aluminum foil and incubated at 65°C for 30 min. After the samples cooled to room temperature, 1.6 ml of 1.0 N HCl was added to each tube. Fluorescence was measured using an American Instrument Company fluoro-colorimeter with a Wratten 405 filter as the primary filter, and Wratten 3 and 65A filters as secondary filters. The concentration of DNA in each preparation was determined by comparison of fluorescence to a standard curve generated using DNA of known quantity in the range of 0.1 to 10 μg.

RESULTS AND DISCUSSION

(a) DNA recovery

The procedure presented in MATERIALS AND METHODS, section **c**, is the most efficient protocol we found for isolating yeast DNA. To quantitate recovery, cells were prelabeled with [^{3}H]uracil, and samples from each step in the procedure were subjected to alkali hydrolysis and acid precipitation; total radioactivity in each fraction was assessed by scintillation counting. The recovery of DNA was 75% of the starting material, or approx. 6 μg of DNA from 5×10^8 cells based on the estimate of haploid DNA content of *S. cerevisiae* made by Lauer et al. (1977). This recovery was confirmed by estimation of DNA yield by DABA assays and by EtdBr staining of agarose gels.

This rate of recovery compares favorably with that obtained from other simple methods for isolating yeast DNA. The TNE method (Winston et al., 1982), for example, produces only a 5–10% yield, which is ten-fold less than the method described here. In contrast, the alkali-lysis procedure of Devenish and Newlon (1982) gives almost 100% yield. However, it is more cumbersome than the present method, and it is not useful for isolating genomic DNA; furthermore, additional purification is required for successful transformation.

In the course of developing the GuHCl procedure, several modifications were found to lower the yield of DNA. In particular, omitting the proteinase K step decreased the yield by almost half (46% yield vs. 75% yield); reducing the volume of the phenol extractions also decreased the yield of DNA significantly. In contrast, neither increasing the duration of

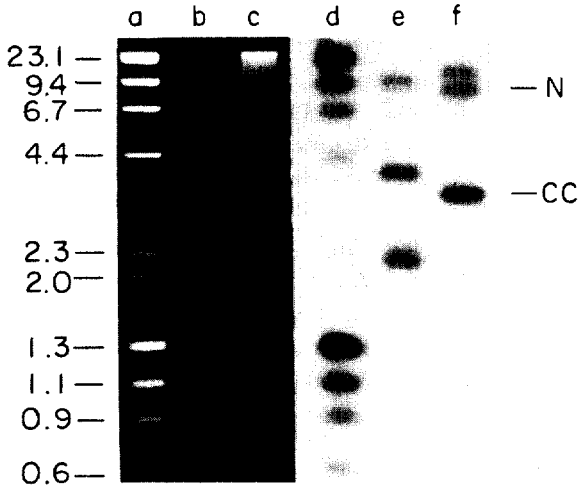

Fig. 1. Electrophoresis of isolated yeast DNA. DNA was cut with restriction enzymes and subjected to electrophoresis in an 0.8% agarose gel. Lanes a–c show DNA stained with EtdBr: (a) Size standards; they are a mixture of phage λ DNA digested with *Hin*dIII and phage φX174 RF DNA digested with *Hae*III. (Sizes on the left margin are in kb.) (b) Yeast DNA digested with *Eco*RI. (c) Yeast DNA digested with *Bam*HI. Lanes d–f are the autoradiograms corresponding to lanes a–c. The probe consists of a mixture of nick-translated phage λ and phage φX174 DNAs, to allow visualization of the size standards, and plasmid YEp24, which contains the *URA3* gene of yeast as well as 2μ DNA sequences. Hybridization of this filter with the isolated *URA3* gene reveals that the top band in lanes e and f corresponds to the chromosomal *URA3* gene (data not shown). In lane e, the two additional bands are the expected *Eco*RI restriction fragments of 2μ DNA. In lane f, the lower two bands correspond to nicked (N) and covalently closed (CC) 2μ DNA molecules. (The DNA in lane f was digested with *Bam*HI, which does not cleave 2μ DNA.) *Methods*: DNA was prepared from a 5-ml culture (1×10^7 cells/ml) of strain LH330-SD grown in YM-1 medium. Nucleic acids were resuspended in 60 μl of 1 × TE buffer. Aliquots of 5 μl were removed and digested with 0.5 units of *Eco*RI or *Bam*HI; complete digestion was routinely obtained using 4 to 6 units of restriction enzyme per μg of yeast DNA. The samples were electrophoresed in a 0.8% agarose gel at 1 V/cm for 16 h as described by Brewer et al. (1980). M_r standards were phage λ DNA digested with *Hin*dIII, and phage φX174 RF DNA digested with *Hae*III. The DNA was transfered to nitrocellulose paper by the method of Southern (1975). Hybridization was done at 65°C with shaking for 18 h. DNA probes were nick-translated as described by Palmiter et al. (1982). The blots were washed as described by Nasmyth (1982), and exposure to X-ray film was described by Fangman et al. (1983).

the proteinase K treatment from 30 min to 60 min nor omitting the optional RNase step had any significant effect on the yield of DNA. Efficient recovery of DNA has also been obtained upon scaling up this procedure for one-liter cultures of exponentially growing yeast cells (approx. 1×10^{10} haploid cells).

(b) Sensitivity to restriction enzymes

DNA isolated by this procedure can be efficiently cut with restriction endonucleases. Fig. 1 shows DNA that has been treated with *Eco*RI and *Bam*HI. The bands are sharp, which indicates that little DNA degradation has taken place. In addition, lane f shows that only minimal degradation of the DNA takes place even when it is incubated in the presence of magnesium. Southern analysis of the migration of the yeast plasmid 2μ DNA shows that only about one-third of the circular molecules become nicked after incubation in restriction enzyme buffer for 1 h; no linear molecules are produced at all. Thus, DNA prepared by this method is probably sufficiently free of contaminating enzyme activities for most types of restriction analysis.

(c) Genetic transformation

DNA isolated from plasmid-bearing yeast cells can also be used to transform *E. coli*. As has been observed with other procedures (Devenish and Newlon, 1982), DNA isolated from yeast appears to contain an inhibitor of *E. coli* transformation. However, when 1/100 of a DNA preparation from a strain containing plasmid YCp19 was mixed with CsCl-purified YIp5 DNA and used to transform *E. coli*, it was found that overall transformation efficiency was reduced only three-fold (from 4.0×10^6 to 1.3×10^6 transformants per μg of YIp5; Table I). Thus, it is possible with appropriate dilution to recover even single-copy plasmids from yeast cells using this protocol (Table I). When total DNA was isolated from 2.5×10^8 yeast cells containing the centromere plasmid YCp19, 1/100 of the preparation yielded an average of 16 *E. coli* transformants; this is an efficiency of transformation of 8.5×10^5 transformants per μg of plasmid DNA if one assumes that each yeast cell contains one plasmid. The number of transformants is correspondingly higher for DNA from cells bearing multicopy plasmids such as the 2μ DNA vector YEp24. When 1/100 of a DNA preparation from 4×10^8 cells containing YEp24 was used to transform *E. coli*, an average of 136 transformants was produced; this recovery represents an efficiency of 6.7×10^5 tranformants per μg of plasmid DNA if one assumes that each cell contains an average of 10 copies of YEp24

(d) Conclusions

The described method of isolating DNA is fast and efficient, and the DNA it produces is useful for both restriction analysis and transformation of *E. coli*. It yields total cellular DNA with little evidence of degradation, and it has been useful for

TABLE I

Transformation of *E. coli* by plasmids isolated from yeast

Source of yeast and/or plasmid DNA[a]	YIp5 DNA added (μg)	Average number of transformants[b]	Transformants per μg of DNA[c]
None	6×10^{-5}	237	4.0×10^6
DBY1033[YCp19]	6×10^{-5}	93	1.3×10^6 [d]
DBY1033[YCp19]	–	16	0.9×10^6
DBY1033[YEp24]	–	136	0.7×10^6
DBY1033	–	0	0

[a] See MATERIALS AND METHODS, section **b**, for description of plasmids and yeast strains.

[b] Calcium chloride-mediated transformation of *E. coli* strain HB101(LM1035) was carried out according to Mandel and Higa (1970).

[c] Transformation frequencies were calculated based on standard yields of DNA. See RESULTS AND DISCUSSION, section c.

[d] Transformants per μg of YIp5, minus estimated contribution from YCp19.

isolating both circular and linear species of nucleic acids, such as the linear DNA plasmid described by Dunn et al. (1984). It has also been used for isolating DNA from other cells with tough walls, such as *Chlamydomonas* (S. Dutcher, personal communication) and *Neurospora crassa* (D. Stadler, personal communication). It is hoped that this procedure will prove useful for other laboratories investigating the molecular biology of eukaryotic cells.

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REFERENCES

Botstein, D., Falco, S.C., Stewart, S.E., Brennan, M., Scherer, S., Stinchcomb, D.T., Struhl, K. and Davis, R.W.: Sterile host yeast (*SHY*): a eukaryotic system of biological containment for recombinant DNA experiments. Gene 8 (1979) 17–24.

Brewer, B.J., Zakian, V.A. and Fangman, W.L.: Replication and meiotic transmission of yeast ribosomal RNA genes. Proc. Natl. Acad. Sci. USA 77 (1980) 6739–6743.

Cepko, C.L., Roberts, B.E. and Mulligan, R.C.: Construction and applications of a highly transmissible murine retrovirus shuttle vector. Cell 37 (1984) 1053–1062.

Devenish, R.J. and Newlon, C.S.: Isolation and characterization of yeast ring chromosome III by a method applicable to other circular DNAs. Gene 18 (1982) 277–288.

Dunn, B., Szauter, P., Pardue, M.L. and Szostak, J.W.: Transfer of yeast telomeres to linear plasmids by recombination. Cell 39 (1984) 191–201.

Fangman, W.L., Hice, R.H. and Chlebowicz-Sledziewska, E.: *ARS* replication during the yeast S phase. Cell 32 (1983) 831–838.

Hartwell, L.H.: Macromolecule synthesis in temperature-sensitive mutants of yeast. J. Bacteriol. 93 (1967) 1662–1670.

Hopper, A.K. and Hall, B.D.: Mating type and sporulation in yeast, I. Mutations which alter mating type control over sporulation. Genetics 80 (1975) 41–59.

Kielland-Brandt, M.C., Nilsson-Tillgren, T., Holmberg, S., Petersen, J.G.L. and Svenningsen, B.A.: Transformation of yeast without the use of foreign DNA. Carlsberg Res. Commun. 44 (1979) 77–87.

Lauer, G.D., Roberts, T.M. and Klotz, L.C.: Determination of the nuclear DNA content of *Saccharomyces cerevisiae* and implications for the organization of DNA in yeast chromosomes. J. Mol. Biol. 144 (1977) 507–576.

Nasmyth, K.A.: The regulation of yeast mating-type chromatin structure by *SIR*: an action at a distance affecting both transcription and transposition. Cell 30 (1982) 567–578.

Palmiter, R.D., Chen, H.Y. and Brinster, R.L.: Differential regulation of metallothionein-thymidine kinase fusion genes in transgenic mice and their offspring. Cell 29 (1982) 701–710.

Southern, E.M.: Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98 (1975) 503–517.

Stinchcomb, D.T., Mann, C. and Davis, R.W.: Centromeric DNA from *Saccharomyces cerevisiae*. J. Mol. Biol. 158 (1982) 157–179.

Wickerham, L.J.: A critical evaluation of the nitrogen assimilation tests commonly used in the classification of yeasts. J. Bacteriol. 52 (1946) 293–301.

Winston, F., Chumley, F. and Fink, G.R.: Eviction and transplacement of mutant genes in yeast. Methods Enzymol. 101 (1983) 211–228.

Zakian, V.A., Brewer, B.J. and Fangman, W.L.: Replication of each copy of the yeast 2 micron DNA plasmid occurs during the S phase. Cell 17 (1979) 923–934.

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