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Research Article

Factors affecting chemical-based purification of DNA from *Saccharomyces cerevisiae*

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ABSTRACT

Extraction of high molecular weight chromosomal DNA from yeast cells is a procedure that is performed frequently for experiments involving polymerase chain reaction (PCR), Southern blotting and other DNA analysis techniques. We have investigated several parameters affecting DNA yield and quality, using a simple chemical-based purification procedure that was modelled on alkaline lysis methods developed for bacterial cells. The three major steps of the procedure, cell lysis, protein removal and DNA precipitation, were optimized by testing the impacts of several chemicals, including sodium dodecyl sulphate (SDS), sodium hydroxide, Tris buffer, sodium acetate and potassium acetate. Other parameters, such as the effect of elevated temperatures on cell lysis, were also investigated. A rapid, optimized protocol was derived for the purification of DNA from small cell cultures that can be readily digested with restriction enzymes and used as a template for PCR. Average yield was calculated to be approximately 1.7 μg DNA/10^8 cells, which is similar to the theoretical maximum amount obtainable from haploid yeast cells. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: **DNA purification; cell lysis; PCR; Southern blot; alkaline lysis**

Introduction

Purification of chromosomal and/or plasmid DNA from small yeast cell cultures is a common procedure performed to obtain DNA for amplification by PCR, for digestion with restriction enzymes for Southern blots, and for subsequent transformation of DNAs into other cells. Several different approaches have been described for lysing yeast cells to release DNA, including the use of cell wall-degrading enzymes, glass bead vortexing, repeated freeze–thawing and grinding in liquid nitrogen (Akada *et al*., 2000; Borman *et al*., 2010; Burke *et al*., 2000; Fujimura and Sakuma, 1993; Harju *et al*., 2004; Hoffman and Winston, 1987; Kabir *et al*., 2003; Lee, 1992; Mann and Jeffery, 1989; Polaina and Adam, 1991). These lysis procedures are typically used in conjunction with other chemicals, such as detergents (e.g., SDS) or sodium hydroxide (NaOH) to aid in lysis of the cells. After lysis, proteins are typically removed by extraction, using organic solvents such as phenol, chloroform and/or ether. The resulting nucleic acid suspensions containing DNA and RNA are then concentrated and purified by precipitation with either ethanol or isopropanol, followed by degradation of contaminating RNA molecules with RNase enzymes. For some rapid PCR-based applications, it is sufficient to simply lyse the cells and centrifuge away the cellular debris prior to use (e.g. Akada *et al*., 2000), but such 'dirty' preparations are not pure enough for many downstream procedures, such as digestion with restriction enzymes.

Several simple and rapid chemical-based protocols have been developed for purification of plasmid and/or chromosomal DNAs from small bacterial cell cultures (Sambrook and Russell, 2001; Feliciello and Chinali, 1993; Zhou *et al*., 1997). These methods avoid many of the negatives associated with the yeast-based protocols cited above, e.g. the

shearing of high molecular weight chromosomal DNA that occurs with glass beads or the use of harsh organic chemicals such as phenol and chloroform. One of the most common methods used for isolation of DNA from *E. coli* cells is an alkaline lysis prep. This protocol is used primarily for purification of plasmids, but it can also release chromosomal DNA from cells when lysis is allowed to progress for longer times (Sambrook and Russell, 2001; Feliciello and Chinali, 1993; Zhou *et al.*, 1997). The method involves only three major steps: lysis of the cells with SDS + NaOH, precipitation and removal of proteins and cell debris with potassium acetate (KOAc) and precipitation and concentration of nucleic acids with isopropanol. It would clearly be advantageous if such a simple protocol could be applied to fungal cells for preparation of high molecular weight chromosomal and plasmid DNAs.

Polaina and Adam (1991) described a relatively simple procedure for purification of *S. cerevisiae* plasmid and chromosomal DNA that involves alkaline lysis of cells with SDS and NaOH, neutralization of pH with sodium acetate, phenol/ether extraction and isopropanol precipitation of the nucleic acids. In the current study the earlier steps of this procedure were combined with the simpler downstream steps of well-established bacterial alkaline lysis methods to create a new protocol, which was then modified after testing several variables and multiple chemicals. The result is an optimized method that produces high yields of DNA from yeast cells and also releases plasmid DNA which can be used to efficiently transform competent *E. coli* cells.

Materials and methods

Chemicals and enzymes

Agarose, disodium ethylenediaminetetraacetic acid (EDTA), potassium acetate (KOAc) and Trizma (Tris) base were obtained from Sigma-Aldrich Chemical Co. Sodium hydroxide was purchased from EM Science. Sodium acetate and sodium dodecyl sulphate (SDS) were purchased from Mallinckrodt Baker. Ethanol was purchased from Pharmco-Aaper and isopropanol from Fisher Scientific. *Eco*RI, *Xho*I, RNase I_f and 1 kb ladder DNA molecular weight standards were obtained from New England Biolabs. RNase A was purchased from Invitrogen.

Yeast growth media

All amino acids, bases and ampicillin were purchased from Sigma-Aldrich. Bacto peptone, bacto yeast extract and bacto agar were obtained from Becton-Dickinson Microbiological Systems. Anhydrous D-glucose (dextrose) was purchased from Mallinckrodt. Yeast cells were grown on YPDA (rich) medium (1% bacto yeast extract, 2% bacto peptone, 2% dextrose, 2% bacto agar and 0.002% adenine) for general, non-selective growth. For plasmid selection, cells were grown on synthetic media with drop-out mix (2% glucose, 2% bacto agar, plus all essential amino acids and bases, exempting those used for plasmid selection) as described (Sherman, 2002).

DNA purification from yeast cells

Haploid *Saccharomyces cerevisiae* strain BY4742 (*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) (Brachmann *et al.*, 1998) was used for all DNA purification experiments. Cells were shaken overnight (18–24 h) at 30°C in YPDA broth, so that final titres were approximately 2×10^8 cells/ml. Procedures involving precipitation of cells, proteins or DNA utilized an Eppendorf 5415D microcentrifuge spinning at 16,100 × *g*. Heating of cell extracts at 65–75°C was accomplished using a Thermolyne dri-bath with an attached heating block designed for 1.5 ml microfuge tubes. DNA pellets were dried in an SC110 speed vac concentrator (Savant). Yields of total double-stranded DNA (dsDNA) were determined using a Hoefer DynaQuant 200 fluorometer and the DNA-binding fluorophore Hoechst 33258.

SET (SDS–EDTA–Tris) cell lysis solutions prepared in 50 ml screwcap plastic tubes remained stable at room temperature for at least 1 year. EDTA was present in all SDS lysis solutions (with or without NaOH) at a final concentration of 10 mM. Stock solutions of NaOAc and KOAc (3 M) were stored at 4°C, 70% and 100% ethanol solutions were maintained at −20°C, and isopropanol was stored at room temperature prior to use. For all optimization experiments performed for Figures 1 and 2, four or five independent cell cultures were tested and averages and standard deviations (SDs) calculated.

Shuttle plasmids pRS425 (2μ *LEU2* Amp^r) and pRS426 (2μ *URA3* Amp^r) (Christianson *et al.*, 1992) were transformed into BY4742 cells, using a rapid lithium acetate-based protocol (Soni *et al.*,

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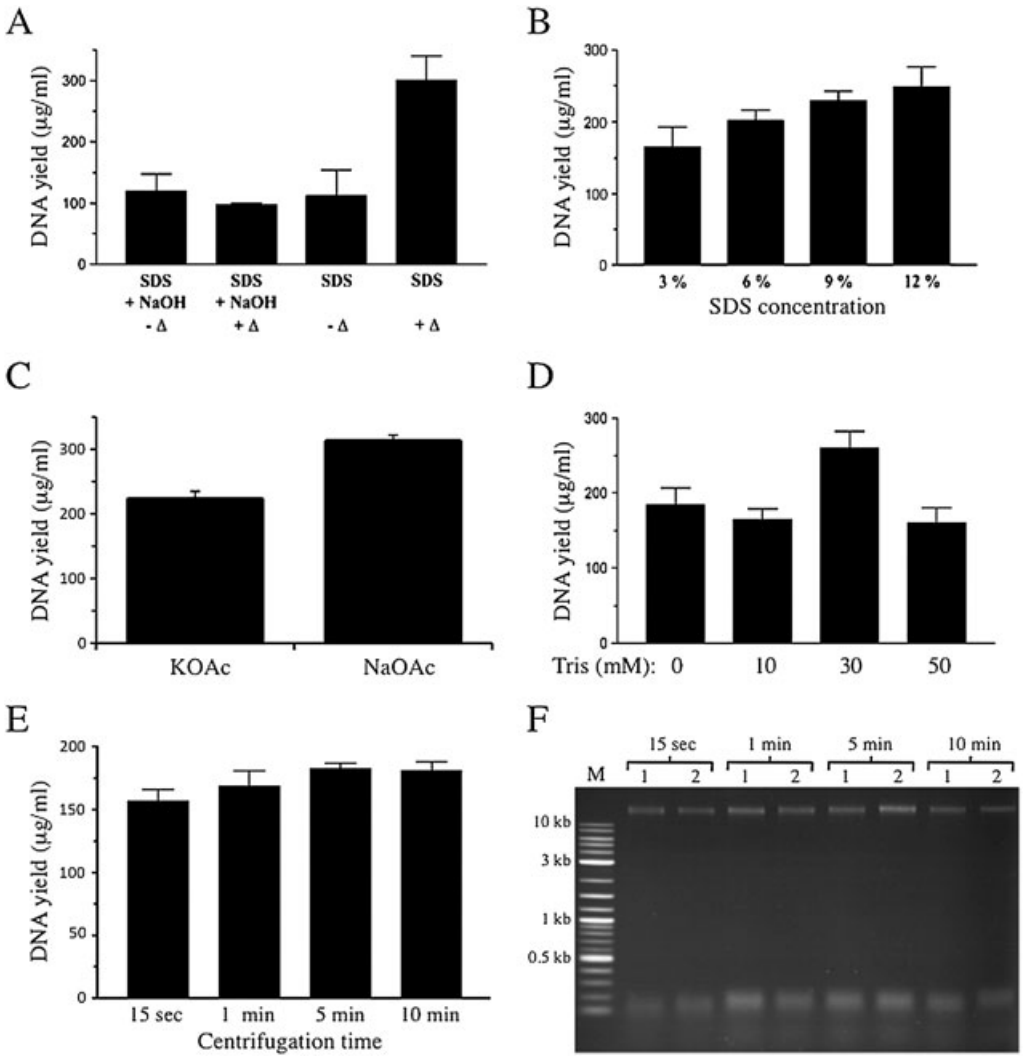

Figure 1. Investigation of factors affecting cell lysis, protein precipitation and nucleic acid precipitation. (A) Impact of inclusion of 3% SDS, with or without NaOH and with or without heating at 65°C for 15 min; −Δ, no heating; +Δ, with heating. DNA yields are shown as final concentrations determined by fluorometry. Four or five cultures were tested and the results averaged. Error bars indicate SDs. (B–D) Effects of variation of SDS concentration during lysis, use of different 3 M salts for protein precipitation and addition of varying amounts of Tris, pH 8.0, to the cell resuspension solution. (E) Impact of changing centrifugation times after addition of isopropanol. (F) Assessment of the quality of DNAs prepared with different isopropanol precipitation times, using 0.7% agarose gel electrophoresis. 1 and 2, individual 3 ml DNA isolates

1993), propagated on selective synthetic glucose plates and then shaken overnight at 30°C in YPDA broth for purification of chromosomal and plasmid DNA. Aliquots (2 µl) of each DNA prep were subsequently transformed into 80 µl competent 5α *E. coli* cells (New England Biolabs) and colonies selected on LB plates containing 100 µg/ml ampicillin. The procedure was performed as recommended by the supplier, except that the final 1 ml suspension of cells in SOC broth was centrifuged for 30 s, the supernatant removed and the cell pellet resuspended in 100 µl SOC broth. All of the resulting solution was spread onto plates; 20–100 colonies/transformation were produced for each plasmid using this procedure.

PCR reactions were performed using Taq DNA polymerase (New England Biolabs) along with primers specific for the *MRE11* gene, generating a

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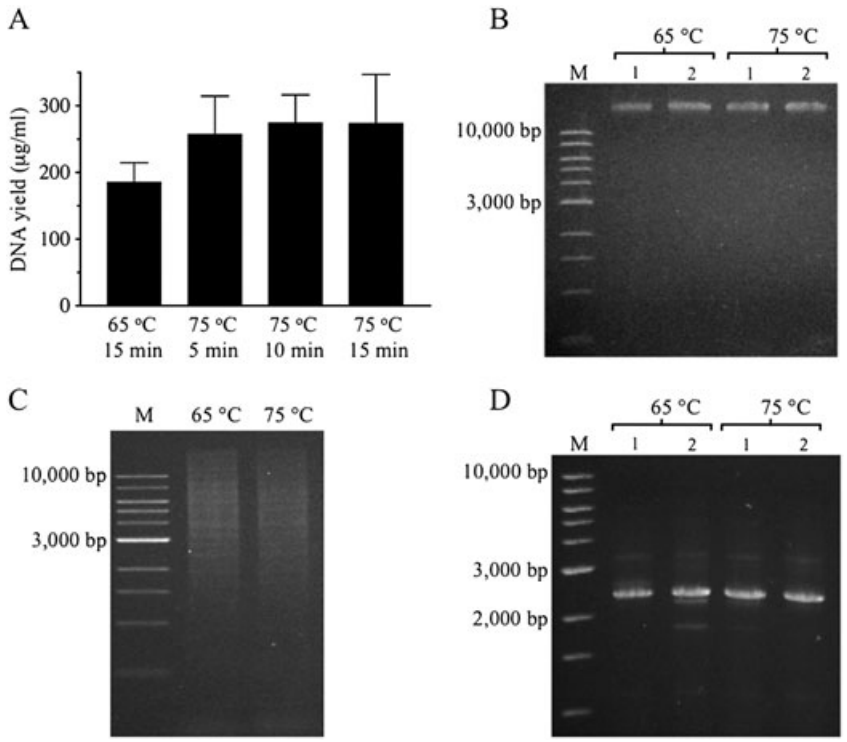

Figure 2. Analysis of the quality of DNAs prepared by lysis at 65°C and 75°C. (A) Average DNA yields produced by lysis of cells at 75°C for 5–15 min compared to the standard lysis at 65°C. (B–D) Use of agarose gel electrophoresis to assess uncut DNAs, DNAs digested to completion with *Eco*RI and DNA fragments produced by PCR amplification of the *MRE11* gene. DNAs digested in (C) were from one of four independent purifications and are representative of several digests done with either *Eco*RI or *Xho*I. 1 and 2, individual 3 ml DNA preparations

2341 bp product. The primers used were 5′-Bamre (TTAGGATCCCAACCAAACTGACTTAAGGTT) and 3′-Bamre (TTAGGATCCATACCTTGTTGTT-CGCGAAGGCA). For each reaction, 30 cycles (94°C, 0.5 min; 50°C, 0.5 min; 72°C, 2 min) were performed using an Applied Biosystems 2720 thermal cycler. Restriction digests were performed using a universal enzyme buffer (1 × KGB) (McClelland *et al.*, 1988). Ten ml 5 × KGB was prepared as described, except that 1.1 g potassium glutamate was used instead of 926 mg, which results in less non-specific cleavage by some restriction enzymes after dilution to 1× concentrations (unpublished results). Digestions with *Eco*RI or *Xho*I were performed for ≥ 3 h at 37°C using approximately 20 U enzyme/µg DNA.

The final optimized protocol arising from this study is as follows. Shake a 4–5 ml YPDA broth culture of yeast cells overnight at 30°C. Transfer 1.5 ml to a microfuge tube, spin at full speed in a microcentrifuge for 15–30 s, pour off the supernatant, add 1.5 ml more cells, re-spin and pour off the supernatant again. Add 300 µl 6% SET (SDS + EDTA + Tris; 3% SDS can also be used) and vortex or scrape the tube across the bottom of a test tube rack several times to resuspend cells. Incubate at 65°C for 15 min and transfer to ice. Add 150 µl cold 3 M NaOAc, invert several times to mix and leave on ice for 5 min. Spin 10 min and transfer supernatant to a new tube. Add 500 µl isopropanol, invert vigorously to mix and spin for 1 min. Remove the supernatant and rinse the pellet with 200 µl cold 70% ethanol (do not vortex). Remove liquid and dry in a speedvac for 5–10 min (tubes may also simply be inverted to dry for 30 min). Resuspend the DNA pellet in 50 µl TE + 1 µl 1 mg/ml RNase A or RNase I_f. Incubate at 37°C for 10–15 min.

We note that lysis at 75°C for 5 min produces yields that are similar to lysis at 65°C for 15 min and the resulting DNAs are of similar quality (see Results). In addition, 3 M KOAc, which is employed in many bacterial DNA isolation protocols and should be prepared with glacial acetic acid as

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described by Sambrook and Russell (2001), can also be used in place of NaOAc with only a slightly reduced yield. 6% SET solution (10 ml) was prepared by combining 3 ml 20% SDS, 0.2 ml 0.5 M EDTA, pH 8.0, 0.3 ml 1 M Tris, pH 8.0, and 6.5 ml double-deionized water. If small RNAs and residual protein must be removed from the final preparations, we have used the isopropanol/KOAc clean-up method of Feliciello and Chinali (1993) with success (not shown).

Results

The goal of this study was to develop a simplified chemical-based procedure for the purification of DNA from small volumes of *S. cerevisiae* cells. A previously published method (Polaina and Adam, 1991) demonstrated that yeast cells could be lysed efficiently by incubation at room temperature for 15 min in 3% SDS + 0.2 N NaOH. In that study, the lysates were diluted with Tris/EDTA (TE), neutralized with 0.3 N sodium acetate, and then proteins were extracted with phenol and ether. Isopropanol was added to the resulting solutions and they were centrifuged to precipitate nucleic acids. Although several DNA isolation protocols employ phenol/chloroform/ether extraction to remove proteins, methods developed for bacterial DNA isolation have demonstrated that organic chemical extractions are unnecessary, since proteins can be removed by precipitation with KOAc. DNA molecules in the resulting supernatant can then be quickly concentrated and purified by adding isopropanol, mixing and centrifuging to sediment the nucleic acids.

We developed an initial procedure for purifying DNA from 3 ml cultures of yeast cells that combined useful aspects of both yeast and bacterial methods and had three major steps: cell lysis with SDS/NaOH, protein removal with KOAc, and DNA precipitation with isopropanol. Haploid BY4742 cells were shaken overnight at 30°C in YPDA broth and 1.5 ml was transferred to a microfuge tube. After centrifugation for 15–30 s, the supernatant was removed and 1.5 ml more cells were added (3 ml cells total) and the tube spun again. After removal of the final supernatant, cell pellets were resuspended in 300 µl 3% SDS + 0.2 N NaOH + 10 mM EDTA. The Mg^{2+}-chelator EDTA was included in all lysis solutions to protect DNA from potential nuclease degradation. The lysis mixture was incubated at room temperature for 15 min, placed on ice and 150 µl cold 3 M KOAc added. After 5 min on ice, proteins were sedimented by centrifugation for 10 min, the supernatant containing nucleic acids was transferred to a new tube and 500 µl isopropanol was added. After mixing by inverting, DNA and RNA were pelleted by centrifugation for 10 min. The pellet was rinsed with 200 µl cold 70% ethanol, without vortexing or centrifuging, and dried for 10 min in a speed-vac concentrator. After resuspension in 50 µl TE, pH 8.0, 1 µl 1 mg/ml RNase A or RNase I_f was added, followed by incubation at 37°C for 10 min. Yields were assessed by DNA fluorometry, using the dsDNA-specific fluorophore Hoechst 33258.

Use of this protocol produced good yields of dsDNA, with final concentrations of approximately 100 µg/ml (Figure 1A, column 1). Since some published procedures have incubated cells at elevated temperatures to enhance lysis (e.g., Burke *et al.*, 2000; Fujimura and Sakuma, 1993; Lee, 1992), DNA was isolated as before, except that lysis with SDS + NaOH was performed at 65°C for 15 min. Heating did not improve yields under these conditions (Figure 1A, column 2). Use of 3% SDS alone without heating also did not improve product yield but, surprisingly, heating at 65°C for 15 min without the strong base NaOH increased DNA concentrations by over two-fold to 290 µg/ml in this experiment (Figure 1A, columns 3 and 4). Based on these results, the basic protocol was adjusted so that lysis was performed at 65°C with 3% SDS + 10 mM EDTA, but without NaOH. We note that all of the experiments shown in Figure 1A were performed on the same day with the same stock solutions. Four or five trials were performed for each variable tested and averages and SDs are shown.

Using the adjusted protocol in conjunction with 6%, 9% or 12% SDS revealed that higher levels of the detergent increased DNA yields modestly (Figure 1B). However, SDs overlapped one another and the results therefore represented a trend and were not statistically significant. After addition of KOAc and centrifugation of the solutions containing 9% or 12% SDS, the supernatants were noticeably more turbid, suggesting that these concentrations of the detergent were too high. These results indicated that 3–6% SDS was optimum for the procedure. Replacement of 3 M KOAc with 3 M NaOAc for protein precipitation reproducibly generated a modest increase in the final yield of DNA and this

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increase was statistically significant (Figure 1C). Since some DNA isolation protocols lyse cells in the presence of Tris buffer, addition of Tris, pH 8.0, at several concentrations was also tested. Interestingly, addition of 30 mM Tris to the lysis buffer, so that it contained 3% SDS + 10 mM EDTA + 30 mM Tris, pH 8.0, consistently improved yields (Figure 1D). This increase was observed when either KOAc or NaOAc was used for protein precipitation and also when either 3% or 6% SDS was used for lysis (not shown).

Protocols for precipitation of DNA with isopropanol or ethanol recommend centrifugation at ~15 000 × *g* for times varying from 10 s to 10 min (Burke *et al.*, 2000; Sambrook and Russell, 2001). We tested 16,100 × *g* centrifugation times of 15 s, 1, 5 and 10 min and observed good yields of total DNA, with final concentrations falling within a narrow range of 150–180 μg/ml in this experiment (Figure 1E). DNA from the preparations (4 μl each sample) migrated as large bands (~50,000 bp) on 0.7% agarose gels, without exhibiting the smear of lower molecular weight fragments typically seen with glass bead-based lysis procedures (Holt and Winston, 1987; Lewis *et al.*, 1998) (Figure 1F). Levels of both DNAs and small RNAs (the diffuse bands at the bottom of each lane) were reduced in samples spun for 15 s relative to the other centrifugation times, but DNA yields were quite similar between 1 and 10 min.

Having established that lysis of cells with SDS + EDTA + Tris (SET) at elevated temperatures, precipitation of proteins with NaOAc and centrifugation of DNA for ≥ 1 min produced the highest yields, we investigated whether incubation at a higher temperature, 75°C, could improve lysis and DNA recovery. As shown in Figure 2A, lysis at 75°C for 5, 10 or 15 min consistently increased yields compared to 65°C, but variability among samples also increased, leading to large error bars and overlapping SDs. The results therefore suggest that the increases were not significant. We also tested incubation at 65°C for shorter or longer times, but once again no statistically significant increases in DNA recovery were observed (not shown).

The quality of DNA prepared by this method, using either 65°C or 75°C for cell lysis, was assessed in three ways. DNA isolated by both methods migrated as high molecular weight bands in gels, was efficiently digested with restriction enzymes and was successfully employed as a template for PCR amplification of a 2341 bp fragment containing the *MRE11* gene (Figure 2B–D). Our laboratory has also routinely digested DNAs from cells lysed at 65°C with *Xho*I for use with Southern blots to analyse yeast telomere fragment sizes with good reproducibility (not shown). The final optimized protocol arising from all of these experiments is presented in Materials and methods.

The final concentrations of DNA obtained using this method varied in the approximate range 150–300 μg/ml. Since DNA preps were resuspended in 50 μl TE, each 3 ml cell culture produced approximately 7.5–15 μg total DNA. Based on a conservative estimate of 200 μg/ml final DNA concentration after resuspension in TE and 2×10^8 cells/ml in each overnight culture, we calculate that the protocol produces a typical yield of 1.67 μg DNA/10^8 cells.

Haploid *Saccharomyces cerevisiae* cells contain 1.21×10^7 bp chromosomal DNA (Goffeau *et al.*, 1996). Multiplying 1.21×10^7 bp by 650 g/(mol of bp) and by 1 mol/6.02×10^{23} molecules indicates that there is 1.31×10^{-14} g chromosomal DNA bp per haploid genome (per cell). Yeast cells also contain dsDNA within their mitochondria that represents 10–20% of total extractable DNA (Taylor *et al.*, 2005). If chromosomal DNA is 1.31×10^{-14} g/cell and mitochondrial DNA is 15% of each cell's total DNA, then there is 1.31×10^{-14}/0.85, or 1.54×10^{-14} g total dsDNA/cell. This number corresponds to 1.54 μg extractable DNA/10^8 cells. The calculated yield of 1.67 μg/10^8 cells described above is therefore close to the theoretical maximum yield that can be achieved using haploid cells.

To determine whether circular plasmids were copurified along with the chromosomal DNA, total DNA was isolated from 30 ml YPDA broth cultures of BY4742 cells containing either pRS425 or pRS426 (Christianson *et al.*, 1992), using a scaled-up version of the protocol. This DNA produced large numbers of colonies on LB plates containing ampicillin upon transformation into competent *E. coli* 5α cells (see Materials and methods). These results confirmed that both chromosomal and plasmid DNAs are efficiently purified by the new protocol.

Discussion

In this study we tested several variables affecting chemical-based DNA purification and developed

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a simple method for the purification of chromosomal and plasmid DNAs from small yeast cell cultures. The method avoids use of liquid nitrogen, freeze-thawing, organic extractions, glass beads or cell wall-digesting enzymes that have been employed in many previous approaches. The procedure is rapid and consists of only three major steps – cell lysis with SDS plus heat, protein removal by NaOAc precipitation, and DNA concentration and purification by precipitation with isopropanol. The resulting chromosomal DNA is of high molecular weight and is readily digested with restriction enzymes and used for PCR amplification reactions.

Approximately 7.5–15 μg DNA was obtained from each 3 ml cell culture in the current study. Final titres of overnight yeast cultures grown in YPDA broth on different days varied in the range 1×10^8–3×10^8 cells/ml (unpublished observations). This variation is normal and is dependent on several factors, such as the starting inoculum of cells, the batch of YPDA broth used and hours of growth at 30°C before the cells were harvested. We suggest that this fluctuation in final titres is likely to be the major factor causing variation in DNA yields.

The protocol of Polaina and Adam (1991), which served as a starting point for this work, reported yields of 0.5 μg DNA/10^8 cells. As described in Results, using a conservative estimate of 200 μg/ml DNA after resuspension in TE and 2×10^8 cells/ml in each overnight culture, the new protocol produces a typical yield of 1.67 μg DNA/10^8 cells. This number compares favourably with the 0.5 μg DNA/10^8 cells of the earlier method and is also higher than the 1.0 μg/10^8 cells yield reported recently for a different type of chemical-based method (Looke *et al*., 2011). The latter protocol was developed primarily to create rapid extracts for PCR from colonies and did not include a protein extraction or precipitation step, so the purity of the DNA produced by that method and the current one are also likely to be different. Including both chromosomal and mitochondrial DNAs as the major sources of dsDNA inside cells, there is 1.54 μg extractable dsDNA within 10^8 haploid *S. cerevisiae* cells (derived in Results). This indicates that the yield of 1.67 μg/10^8 cells obtained with the new method is similar to the theoretical maximum yield that can be achieved from haploid cells.

We observed that plasmid DNAs were co-purified along with the chromosomal DNA and that they could be efficiently transformed into competent *E. coli* cells. We did not optimize the protocol for plasmid purification and it is possible that adjustments to the procedure might further increase yields of these molecules.

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