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Full Length Research Paper

Simple and rapid protocol for isolation of chromosomal and plasmid DNA from *Saccharomyces cerevisiae* suitable for polymerase chain reaction analysis

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Majority of the shuttle vectors developed for the metabolic engineering of yeast have low copy number, hence the need to be rescued and optimized in *Escherichia coli* prior to genetic manipulation studies. Methods that are currently available are complicated, contain lot of detergents, time consuming and often yield poor quality plasmid DNA. The present method describes a simple and rapid protocol for isolation of plasmids from yeast using buffer without any detergents. The plasmids isolated by this protocol can be transformed efficiently in *E. coli*. Further, we also demonstrate that *Saccharomyces cerevisiae* DNA preparations are best suited for PCR amplification.

Key words: *Saccharomyces cerevisiae,* plasmid, chromosomal DNA, plasmid rescue, PCR, transformation.

INTRODUCTION

Saccharomyces cerevisiae is the widely used eukaryotic model system in molecular biology similar to *Escherichia coli* in prokaryotes. Yeast-*Escherichia coli* shuttle vectors play a significant role in understanding the regulation and expression of heterologous genes. These vectors can be moved back and forth between the hosts and will have selection markers and replication machinery that allows its propagation in both the hosts. Although several successful shuttle vectors have been developed, the copy number of these vectors is usually very low in yeast and hence needs to be rescued in *E. coli* for genetic manipulations (Burke et al., 2000; Sambrook and Russel, 2001; Gietz and Schiestl, 1995; Guthrie and Fink, 1991). Development of alternative, simple and rapid method for isolation of intact high molecular weight DNA as well as plasmid DNA for use in various molecular biology analyses is essential, as the available methods are elaborate, time consuming and require relatively expensive reagents. Lower eukaryotes possess turgid cell wall compared to the bacterial system, and require the preparation of spheroplasts to get quality DNA (Burke et al., 2000; Sambrook and Russel, 2001; Guthrie and Fink, 1991; Hanna and Xiao, 2006). Further, alternative methods involve the use of high concentration of detergents and harsh treatments (Burke et al., 2000; Sambrook and Russel, 2001) that often results in poor quality DNA. Most of the methods described earlier use high salt buffers containing SDS, Triton-X100 followed by vigorous vortexing with phenol: chloroform reagent (Burke et al., 2000; Sambrook and Russel, 2001; Gietz and Schiestl, 1995; Guthrie and Fink, 1991). Plasmid DNA isolation methods invariably use zymolyase to release spheroplasts followed by various lengthy steps for isolation and purification of DNA (Burke et al., 2000; Hoffman and Winston, 1987; Sambrook and Russel, 2001). Plasmids yields are low in yeast, and the usual practice is to rescue them in *E. coli,* and amplify

them to be used in genetic manipulation studies (Becker and Guarente, 1991; Elble, 1992; Gietz et al., 1992; Gietz and Schiestl, 1995). In this paper, we describe a simple and easy protocol for isola-tion of genomic and plasmid DNA, which takes less time and could be directly used for *E.coli* transformation during plasmid rescue and for PCR amplification.

MATERIALS AND METHODS

Yeast strains, plasmids and transformation

S. cerevisiae strains, *BWG7a, DBY746*, *NY1489, J69-4A* and *W303-a* used in the analysis were grown on YPD media. The shuttle vector, *pADGAL4.2-1*, containing *YNK1* (*pADGAL4.2-1::YNK1*) and *CPR3* (*pADGAL4.2-1::CPR3*) were transformed into yeast strains using the standard protocol and selected on SD media supplemented with amino acids except leucine (Burke et al., 2000; Elble, 1992). Plasmid isolated from yeast was transformed in to *E. coli* XL1 blue MRF strain using calcium chloride-heat shock method and selected on LB ampicillin (Sambrook and Russel, 2001).

Isolation of yeast genomic and plasmid DNA

Yeast strains were grown on YPD medium and yeast strains harboring plasmid vectors were grown on SD media lacking leucine (Burke et al., 2000) at 30°C till the OD_{600nm} reached ~0.4 to 0.5. 1.5 ml of the culture was centrifuged at 4000 rpm for 2 min and washed twice with sterile water. The cells were suspended in $1/10^{th}$ volume of 50 mM Tris-HCl pH 7.4 containing 10 mM EDTA, 0.3 M sucrose and 10 mM β-mercaptoethanol, and disrupted with glass beads by vortexing for about six to eight times for 30 s at 4°C. Glass beads and cells debris with nuclei were pelleted by centrifugation at 2000 rpm for 10 min at 4°C and the supernatant was stored for plasmid isolation. The pellet was suspended in 200 µl in 50mM Tris-HCl pH 7.4 buffer containing 10mM EDTA and 10 mM β-mercaptoethanol for genomic DNA isolation. Crude genomic and plasmid DNA preparations were extracted once each with phenol:chloroform: isoamyl alcohol (25:24:1,V/V) and chloroform: isoamyl alcohol (24:1,V/V). The aqueous phase was transferred to separate tubes, $1/10^{th}$ volume of 0.3 M sodium acetate, pH 5.0 and two volume of 100% ethanol was added, mixed gently and kept for precipitation at –70°C for one hour. Centrifuged at 12000 rpm for 15 min at 4°C and washed with 70% aqueous ethanol before air-drying. Dried pellet was dissolved in 100 µl of sterile distilled water and treated with RNAse (1 µg/µl) for 30 min at 37°C. Extracted once with phenol:chloroform:isoamyl alcohol (25:24:1,V/V) followed by chloroform: isoamyl alcohol (24:1, V/V). DNA was precipitated by the addition of $1/10^{th}$ volume of 0.3 M sodium acetate pH 5.0 and two volume of 100% ethanol. Washed with 70% aqueous ethanol and air-dried. The dried pellet was dissolved in 20 µl of TE (10 mM Tris-HCl pH 8.0 containing 1 mM EDTA) and used for PCR amplification and transformation.

PCR analysis

PCR amplification of the genomic DNA isolated from yeast strains was carried out using *RAS1* specific primers (5'CGG AAT TCG ACA TGC AGG GAA ATA AAT C3' and 5'CCG CTC GAG TGT CAT ATC AAG AGA GCA GG3', amplicon of ~950bp). 30 ng of genomic DNA was amplified with 50 pmol of primers and with initial denaturation at 94°C for 2 min. PCR amplification condition included, denaturation at 94°C for 1 min, annealing at 52°C for 30 s, and extension at 72°C for 30 s for 25 cycles in 50 µl reaction volume. Final extension was carried out at 72°C for 10 min. Gene specific primers for *YNK1* in *pADGAL4.2-1::YNK1* (forward: 5'CCC GAA TTC CCT GCA AAA ATG TCT ATG3' and reverse: 5'CCG CTC GAG TTA TTC ATT CAT AAA TCC ACT TAG C3', amplicon of ~450bp) and *CPR3* in *pADGAL4.2-1::CPR3* (forward: 5'CCG CTC GAG GCC TTA GCT TCA TAT AAG C3' and reverse: 5'CGG AAT TCA CCA TGT TTA AAC GTT CC3', amplicon of ~550 bp) were used for amplification with conditions described earlier. 10 µl of the amplified products were analyzed on the 0.8% agarose gel using TBE and stained with ethidium bromide for visualization (Sambrook and Russel, 2001).

RESULTS AND DISCUSSION

S. cerevisiae strains, BWG7a, DBY746, J69-4A, NY1489 and W303a, commonly used host strains in our lab, were grown on YPD medium and yeast transformants on SD-leucine selection media. The steps involved in the isolation of yeast genomic and plasmid DNA are summarized in Figure 1. Mid log phase cells (0.5 OD at 600 nm, ~10^7) were suspended in 50 mM Tris-HCl pH 7.4 containing 10 mM EDTA, 0.3 M sucrose and 10 mM β-mercaptoethanol. We used sucrose in the buffer to maintain the osmoticum during disruption. Vortexing the cells with the glass beads (0.5 mm size, Biospec Products Inc., USA, Cat No.11079105) for about 30 s each at 4°C for six to eight times was found sufficient for disruption. Low temperature and EDTA in the buffer was used to minimize the nuclease activity during vortexing and extraction at various levels.

Genomic DNA samples isolated from five different yeast strains showed compact crisp bands on agarose gel electrophoresis (Figure 2A). Most of the methods used to isolate yeast chromosomal DNA and plasmid DNA employs detergents and high salt buffers. Incomplete removal of these compounds was found to hamper the use of these DNA in transformation and pose problems for restriction digestion and PCR amplification (Burke et al., 2000; Gietz and Schiestl, 1995; Guthrie and Fink, 1991; Sambrook and Russel, 2001). With our protocol, it is possible to get enough and high quality genomic and plasmid DNA from small concentrations of cells, that can be used as template for PCR amplification (Figure 2A, B, 3A and B). In Figure 3A, lanes 1 to 5 represent the transformed yeast strains with shuttle vectors. We can hardly detect the plasmids on the gel suggesting that they are present in very low copy number. However, the same plasmids rescued from yeast cells in *E. coli* showed high level replication (Figure 3A, lanes 6 to 10). Further, we used primers specific for RAS1 (typical yeast chromosomal gene of ~950 bp) and plasmid vector containing YNK1 (~450 bp) and *CPR3* (~550 bp) for PCR amplification. The results in clearly demonstrate the amplification of the specified genes with no contamination (Figures 2B and 3B). Further, the plasmid isolated from yeast strain could be transformed into *E. coli* with very high efficiency (~10^8 colonies per µg DNA, Figure 3C). Results of PCR amplification and transformation of the shuttle vectors used in our study were

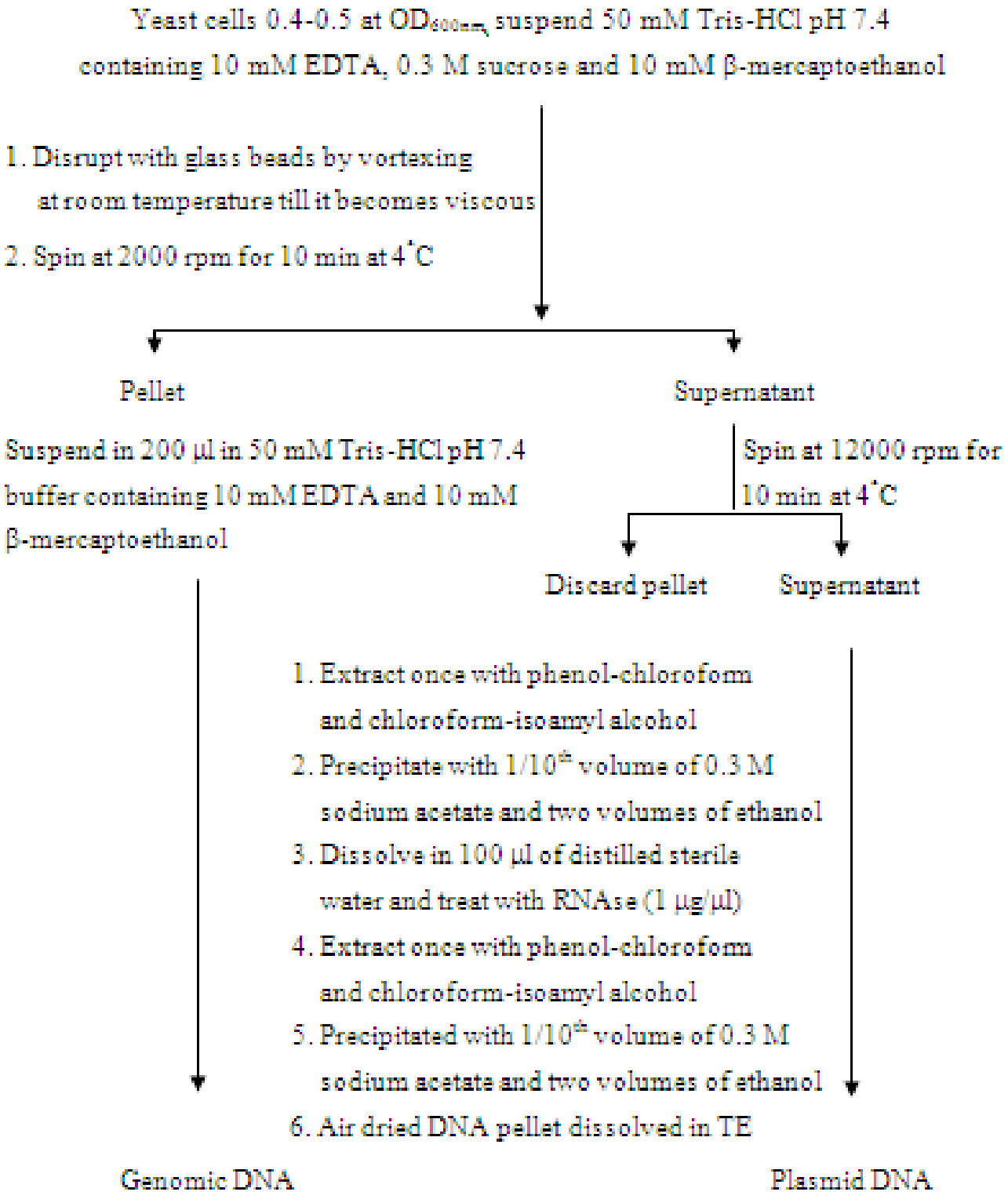

Figure 1. Flow chart depicting the steps for isolation of genomic DNA and Plasmid DNA from *S. cerevisiae.*

found to be highly reproducible. These results clearly demonstrate that irrespective of the vector types and host strains used in this study, our protocol works better than the current methods under use. Therefore, we opine that this protocol is very simple, rapid, user friendly and provides good quality genomic as well as plasmid DNA to be used in genetic manipulation studies using *E. coli*/ yeast shuttle vectors.

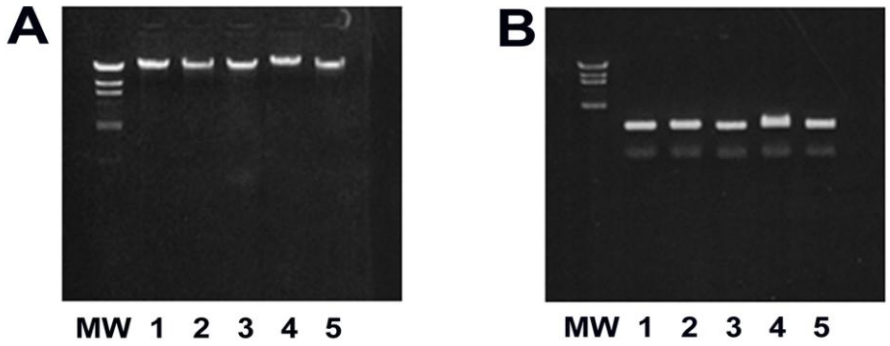

Figure 2. (A) Genomic DNA from 5 yeast strains. Lane1: *BWG7a*, Lane 2: *DBY746*, Lane 3: *J69-4A*, Lane 4: *NY1489*, Lane 5: *W303-a*. (B) PCR amplification from the genomic DNA using *RAS1* specific primers (5'CGG AAT TCG ACA TGC AGG GAA ATA AAT C3' and 5'CCG CTC GAG TGT CAT ATC AAG AGA GCA GG3', amplicon size ~950 bp). 30 ng of genomic DNA is used for amplification with 50 pmol of primers and with initial denaturation at 94°C for 2 min, followed by PCR cycle-denaturation at 94°C for 1 min, annealing at 52°C for 30 s, and extension at 72°C for 30 s for 25 cycles in 50 µl reaction volume. Final extension was carried out at 72°C 10 min. 10 ul of the amplified product was separated on the 0.8% agarose gel.

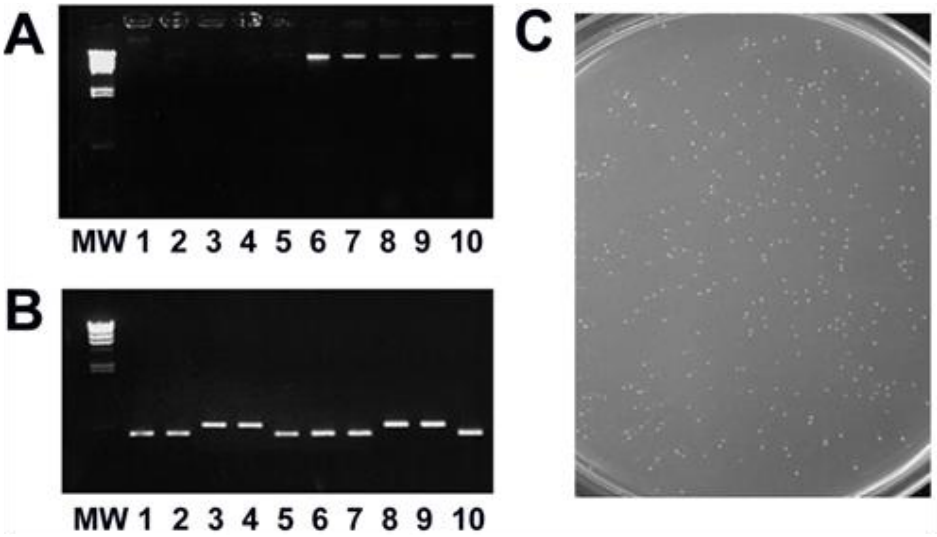

Figure 3. (A) Plasmid DNA from yeast transformants. Lane1: *BWG7a – pADGAL4.2-1::YNK 1*, Lane 2: *DBY746- pADGAL4.2-1::YNK1*, Lane 3: *J69-4A-pADGAL4.2-1::CPR3*, Lane4: *NY1489-pADGAL4.2-1:: CPR3*, Lane 5: *W303a-pADGAL4.2-1:: YNK1*. Lanes 6 to 10: Plasmids isolated from *E. coli* after rescued from the yeast strains as in the lanes 1 to 5. B)PCR amplification using *YNK1* and *CPR3* specific primers. Lanes 1, 2, 5 to 7, 10 with *YNK1* forward and reverse primers (*YNK1* forward: 5'CCC GAA TTC CCT GCA AAA ATG TCT ATG3' and reverse: 5'CCG CTC GAG TTA TTC ATT CAT AAA TCC ACT TAG C3', ~450 bp), and Lane 3, 4, 8, 9 with *CPR3* forward and reverse primers (*CPR3* forward: 5'CCG CTC GAG GCC TTA GCT TCA TAT AAG C3' and reverse: 5'CGG AAT TCA CCA TGT TTA AAC GTT CC3', ~550 bp). Lanes 1 to 10 are as in Figure 3A. C) *E. coli* XL1-blue MRF strain transformed with plasmid, *pADGAL4.2-1::YNK1*, isolated by the above protocol from yeast.

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