Biotechnology Letters (2006) 28: 55–59
DOI 10.1007/s10529-005-4688-z

Extremely rapid extraction of DNA from bacteria and yeasts

Hai-Rong Cheng[1,2] & Ning Jiang[1,*]
[1]*Center for Microbial Biotechnology, Institute of Microbiology, Chinese Academy of Sciences, 100080, Beijing, China*
[2]*Graduate School, Chinese Academy of Sciences, 100080, Beijing, China*
**Author for correspondence (Fax: +86-10-62553081; E-mail: jiangn@sun.im.ac.cn)*

Received 8 June 2005; Revisions requested 16 June 2005 and August 2005; Revisions received 22 August 2005 and 19 October 2005; Accepted 28 October 2005

Key words: bacteria, DNA extraction, yeast

Abstract

A very simple and rapid method for extracting genomic DNA from Gram-negative bacteria, Gram-positive bacteria and yeasts is presented. In this method, bacteria or yeasts are lysed directly by phenol and the supernatant is extracted with chloroform to remove traces of phenol. The supernatant contains DNA that is suitable for molecular analyses, such as PCR, restriction enzyme digestion and genomic library construction. This method is reproducible and simple for the routine DNA extraction from bacteria and yeasts.

Introduction

The rapid availability of genomic DNA (gDNA) from microorganisms is necessary for cloning genes and selecting recombinant constructs, and for taxonomy (Niemi *et al.* 2001) and diagnostics (Müller *et al.* 1998). Previous methods for gDNA extraction from bacteria or yeasts take several hours to complete. These methods include using SDS/CTAB/proteinase K (Wilson 1990), SDS lysis (Syn *et al.* 2000), lysozyme/SDS (Flamm *et al.* 1984), lysozyme/SDS/proteinase K (Neumann *et al.* 1992), bead-vortexing/SDS lysis (Sambrook *et al.* 2001), and mechanical lysis using high-speed cell disruption (Müller *et al.* 1998). Although these methods are suitable for DNA extraction from bacteria or yeasts, they still have the drawbacks of including laborious manipulations, such as four to six changes of microcentrifuge tubes, incubation, precipitation, elution or washing and drying steps or even special equipment. Thus the release of DNA is often poor due to the multiple manipulations. All these methods use detergents such as SDS to lyse the cell wall and this often remains in the DNA solution and inhibits further manipulations.

Here we describe a relatively simple method for the isolation of gDNA from Gram-negative or Gram-positive bacteria and yeasts.

Materials and methods

Strains and culture conditions

Escherichia coli DH5α, *Escherichia coli* HB101, *Klebsiella pneumoniae* subsp. *pneumoniae* ATCC 49790 were cultivated in Lubia-Bertani broth (LB) at 37 °C and 250 rpm. *Corynebacterium glutamicum* ATCC 13032, *Bacillus subtilis* ATCC 6633, *Agrobacterium tumefaciens* EHA 105 and *Pseudomonas fluorescens* ATCC 13525 were cultivated in LB medium at 30 °C and 250 rpm. *Thermoanaerobacter tengcongenis* MB4T was cultivated at 75 °C and pH 7.5 in the medium described previously (Xue *et al.* 2001). *Gluconobacter oxydans* CGMCC 1.110 and *Acetobacter suboxydans* sp. were cultivated in YDC medium broth (10 g yeast

extract, 50 g dextrose, 10 g $CaCO_3$ in 1 l distilled water, pH 7.0) at 30 °C and 250 rpm. *Pichia anomala* sp. and *Saccharomyces cerevisiae* ATCC 18824 were cultivated in YEPD broth (5 g yeast extract, 10 g peptone and 10 g dextrose in 1 l distilled water, pH 7.0) at 28 °C and 250 rpm.

Extraction of DNA from Gram-positive, Gram-negative bacteria and yeasts

The DNA extraction method presented in this paper is an improved method of the standard phenol/chloroform method (Neumann *et al.* 1992). We eliminated the lysis step that uses SDS/lysozyme or proteinase K, and lysed cells directly by phenol. To extract the DNA from Gram-negative or Gram-positive bacteria, 1 ml cell suspension was centrifuged at 8000*g* for 2 min, except for *K. pneumoniae* where 13 000*g* for 10 min was used to pellet cells. After removing the supernatant, the cells were washed with 400 μl STE Buffer (100 mM NaCl, 10 mM Tris/HCl, 1 mM EDTA, pH 8.0) twice. Then the cells were centrifuged at 8000*g* for 2 min, except for *K. pneumoniae* (13 000*g* for 10 min). The pellets were resuspended in 200 μl TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0). For yeasts, 50 mg of 425–600 μm size-fractionated glass beads (Sigma) were added to the cell suspension. Then 100 μl Tris-saturated phenol (pH 8.0) was added to these tubes, followed by a vortex-mixing step of 60 s for bacteria, 120–200 s for yeasts, to lyse cells. The samples were subsequently centrifuged at 13 000*g* for 5 min at 4 °C to separate the aqueous phase from the organic phase. 160 μl upper aqueous phase was transferred to a clean 1.5 ml tube. 40 μl TE buffer was added to make 200 μl and mixed with 100 μl chloroform and centrifuged for 5 min at 13 000*g* at 4 °C. Lysate was purified by chloroform extraction until a white interface was no longer present; this procedure might have to be repeated two to three times. 160 μl upper aqueous phase was transferred to a clean 1.5 ml tube. 40 μl TE and 5 μl RNase (at 10 mg/ml) were added and incubated at 37 °C for 10 min to digest RNA. Then 100 μl chloroform was added to the tube, mixed well and centrifuged for 5 min at 13 000*g* at 4 °C. 150 μl upper aqueous phase was transferred to a clean 1.5 ml tube. The aqueous phase contained purified DNA and was directly used for the subsequent experiments or stored at −20 °C. The purity and yield of the DNA were assessed spectrophotometrically by calculating the A_{260}/A_{280} ratios and the A_{260} values to determine protein impurities and DNA concentrations.

PCR amplification of purified DNA

The D-arabitol dehydrogenase gene (*dal*D, 1.4 kb; *ArDH*, 0.8 kb) from *K. pneumoniae* and *G. oxydans*, partial 18S rRNA gene (1.7 kb) from *P. anomala* and partial 16S rRNA gene (0.6 kb) from *B. subtilis* were amplified from their gDNA prepared using the recommended method. The primers selected to amplify the *dal*D gene were P_{dalD1} (5′-GGAGAGCAGAACATGAAC-AATCAA−3′) and P_{dalD2} (5′-AGCCACCTCTTAGTTAATCAG CGT−3′), primers selected to amplify the *ArDH* gene were P_{ardh1} (5′-ATGTACATGGAAAAACT TCGTCTC−3′) and P_{ardh2} (5′-TTACCAGACG GTGAAACCAGCATC−3′), primers selected to amplify the partial 18S rRNA gene were P_{18S1} (5′-ATCCTGCCAGTAGTCATATGCTTGTCTC−3′) and P_{18S2} (5′-GAGGCCTCACTAAGCCATTC AATCGGTA−3′), primers selected to amplify the partial 16S rRNA gene from *B. subtilis* were P_{16S1} (5′-CCGAAGTTATCATACATGGACTGC−3′) and P_{16S2} (5′-ATACAGTACATCTGTGTCCC-AGTA−3′). The 25 μl reaction mixture consisted of 20 ng genomic DNA , 15 mM Tris/HCl (pH 8.5), 100 mM KCl, 0.1% (v/v) Triton X−100, 3 mM $MgCl_2$, 0.25 mM each dNTP, 2 U *Taq* DNA polymerase and 0.5 μm each primer. The PCR was performed in a thermal cycler (Eppendorf, Germany) using cycling conditions that consisted of an initial denaturation at 94 °C for 5 min and then 33 cycles with denaturation at 94 °C for 40 s, annealing at 55 °C for 45 s, and extension at 72 °C for 45 s for 16S rRNA gene and *ArDH* gene, 1.5 min for 18S rRNA and *dal*D gene. A final extension was performed at 72 °C for 6 min. A blank that contained all the components of the reaction mixture without the DNA sample was used as a control. The PCR products were analyzed by 1% agarose gel electrophoresis.

Restriction analysis

To test whether the gDNA prepared using this method could be digested with restriction enzyme,

1–2 μg gDNA from *E. coli* TG1, *E. coli* HB101, *K. pneumoniae*, *B. subtilis*, *A. tumefaciens*, *T. tengcongenis*, *G. oxydans*, *A. suboxydans*, *P. anomala*, *C. glutamicum* was incubated with 5 U *Eco*RI in a final volume of 20 μl for 5 h at 37 °C and applied to 1% agarose gel electrophoresis.

Analysis of construction of genomic library

20 μg gDNA from *A. suboxydans* sp. was partially digested by 0.5 U *Sau*3AI, for 4 min at 30 °C, in a final 80 μl volume. Then the *Sau*3AI-digested mixture was placed into an 80 °C water-bath for 20 min for heat inactivation of *Sau*3AI. 5 μl of the digestion mixture was then ligated with 100 ng of *Bam*HI-digested and dephosphorylated vector pBluescript II SK(-) in a 20 μl volume at 16 °C for 18 h. The ligation mixture was transformed into competent *E. coli* strain TG1. The transformation conditions was: 30 min on ice, 42 °C for 2 min, 2 min on ice, 300 μl SOC broth (20 g tryptone, 5 g yeast extract, 0.5 g NaCl, 0.2 g KCl, 2 g $MgCl_6 \cdot H_2O$, 5 g $MgSO_4 \cdot 7H_2O$, 4 g dextrose in 1 l distilled water, pH 7.0) was added and incubated at 37 °C for 1 h. The cells were plated on solid minimal medium (5 g $Na_2HPO_4 \cdot 12H_2O$, 0.9 g KH_2PO_4, 0.16 g NaCl, 2 g NH_4Cl, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.01 g $CaCl_2$, 4 g D-arabitol, 15 g agar in 1 l distilled water, pH 7.0) with 100 μg ampicillin/ml and incubated at 37 °C for 72 h. Transformants were further tested for growth in liquid minimal medium with 50 μg ampicillin/ml at 37 °C and 250 rpm for 72 h.

Results and discussions

In the recommended DNA extraction protocol, Gram-negative or Gram-positive bacteria as well as yeasts were lysed by phenol without any other detergents such as SDS or guanidium thiocyanate or Triton X−100. Since phenol is a strong oxidizing reagent, it could directly disrupt the cell wall or nucleus envelope and genomic DNA was released from cells. The extracted DNA was then directly purified using chloroform and the purified supernatant could be used directly for further analyses, omitting a precipitation step with ethanol or isopropanol. This method gave reproducible yields of high-quality DNA (Table 1).

We also tested the availability of commercial DNA extraction kit (DingGuo Biotech. Co., Ltd., Beijing) to prepare gDNA from *E. coli* TG1. The mean DNA yield was 2413 μg/g dry cells, with A_{260}/A_{280} ratio of 1.68. Table 1 showed that the recommended method was in particular useful to extract gDNA from Gram-negative bacteria. We also tested four other Gram-negative bacteria including *Xanthomonas campestris* which produced extracellular material, xanthan gum (Becker *et al.* 1998), *Acetobacter aceti*, *Acetobacter pastoris*, and *E. coli* C600 using this method. The yields obtained were above 3000 μg DNA/g dry cells, A_{260}/A_{280} ratios were between 1.57 to 1.74 (data not shown). However, the gDNA yields from Gram-positive bacteria and yeasts were not so high due to the incomplete lysis of cell wall using phenol as the sole lysis reagent. But the gDNA yields obtained from two yeasts were higher than those obtained from two Gram-positive bacteria; the reason might be the use of acid-washed glass beads while extracting gDNA from yeasts. However, this method was still suitable for preparation of gDNA from Gram-positive bacteria and yeasts due to its extreme rapidness and cost-effectiveness, and the DNA yield was high enough for

Table 1. Yields and quality of DNA obtained by using the recommended method.

Microorganism	Mean DNA Yield[a] (μg/g dry cells)	A_{260}/A_{280}
Escherichia coli TG1	4327	1.77
Escherichia coli HB101	3786	1.64
Gluconobacter oxydans	3818	1.64
Acetobacter suboxydans	3759	1.71
Pseudomonas fluorescens	3942	1.67
Klebsiella pneumoniae	4244	1.71
Thermoanaerobacter tengcongenis	2116	1.65
Agrobacterium tumefaciens	3614	1.67
Bacillus subtilis	316	1.68
Corynebacterium glutamicum	344	1.72
Pichia anomala	526	1.66
Saccharomyces cerevisiae	511	1.65

Values given are the average of three replications.
[a]DNA yields were calculated after treatment with RNase. One A_{260} unit of double-stranded DNA corresponds to 50 μg/ml.

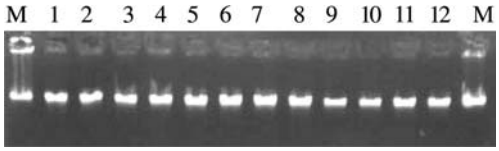

Fig. 1. The pattern of DNA electrophoresis on a 1% (w/v) agarose gel. M: lambda DNA. Lane1–12: the genomic DNA extracted from *Escherichia coli* TG1, *Escherichia coli* HB101, *Gluconobacter oxydans*, *Acetobacter suboxydans*, *Klebsiella pneumoniae*, *Pseudomonas fluorescens*, *Thermoanaerobacter tengcongenis*, *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Corynebacterium glutamicum*, *Saccharomyces cerevisiae*, *Pichia anomala*, respectively.

performing PCR. Figure 1 shows that DNA extracted using the recommended method made a clear single band which indicated that it was not digested or broken down.

We used the gDNA prepared by using this method as template to amplify partial 16S rRNA gene (0.6 kb) from *B. subtilis*, partial 18S rRNA gene (1.7 kb) from *P. anomala* and a D-arabitol dehydrogenase gene (1.4 kb and 0.8 kb) from *K. pneumoniae* and *G. oxydans*. Figure 2 shows that the corresponding genes could be amplified from the gDNA; the bands correspond to the anticipated size of 0.6, 0.8, 1.4 and 1.7 kb, respectively. The results indicated that PCR inhibitors did not exist or their concentrations were too low to inhibit the PCR reaction. These PCR products were ligated into TA cloning vector pMD18

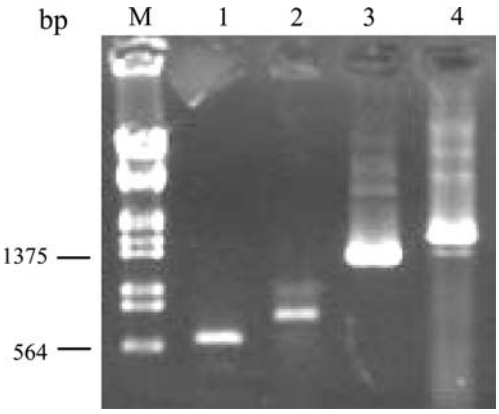

Fig. 2. The pattern of PCR products electrophoresis on a 1% (w/v) agarose gel. M: lambda DNA digested with *Eco*RI and *Hin*dIII. Lane 1–4: PCR products from *Bacillus subtilis*, *Gluconobacter oxydans*, *Klebsiella pneumoniae*, and *Pichia anomala* with different primers (see Materials and Methods).

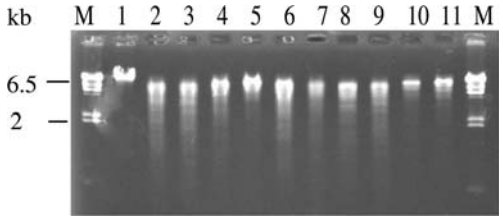

Fig. 3. The pattern of restriction enzyme digestion electrophoresis on a 1% (w/v) agarose gel. M: *Hin*dIII-digested lambda DNA; Lane 1: undigested genomic DNA from *Escherichia coli* TG1. Lane2–11: *Eco*RI-digested genomic DNA from *Escherichia coli* TG1, *Escherichia coli* HB101, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Agrobacterium tumefaciens*, *The-rmoanaerobacter tengcongenis*, *Gluconobacter oxydans*, *Acetobacter suboxydans*, *Pichia anomala*, *Corynebacterium glutamicum*, respectively.

(Takara Biotech. Co., Ltd., Japan) and the results of partial sequencing showed that they were almost identical (>98%) to sequences deposited in GenBank (data not shown).

The restriction digestion patterns of gDNA clearly showed that the gDNA obtained could be digested by *Eco*RI (Figure 3). The size of most digested DNA fragments ranged from 2 to 6.5 kb, while the size of control DNA (lane 1 in Figure 3) corresponded to that of lambda DNA (48 kb) (Figure 1). These restriction digestion results show that no restriction process was inhibited by any components in the DNA preparation.

A genomic library of *A. suboxydans* was constructed. Ninety-five constructs could grow on solid minimal medium containing 100 μg ampicillin/ml. These constructs were transferred to liquid minimal medium containing 100 μg ampicillin/ml. Seven positive clones could grow in liquid minimal medium and the inserted gene was completely sequenced. It was a short-chain dehydrogenase gene with 774 base pairs (data not shown). The result also showed that no ligation process was inhibited by any components in the DNA preparation.

This DNA extraction method has several advantages. First, the number of extraction steps was minimized to a simple phenol/chloroform extraction, and a precipitation was not necessary. So the process of DNA extraction by this method was achieved within 50 min for up to 12 samples, while other methods needed at least

2–4 h (except for DNA extraction using DNA extraction kit which could complete the process within 1 h in the case of several samples). Second, the method yielded equally high molecular weight DNA compared with standard phenol/chloroform protocol. Third, this method was cost-effective, since it only used phenol, chloroform, STE buffer, TE solution and RNase. SDS, lysozyme and proteinase K were not necessary. Fourth, the applicable range of the method was very broad: it was applicable to Gram-negative or Gram-positive bacteria, even those that could produce extracellular materials, and yeasts. Previously described methods for DNA extraction from such bacteria producing extracellular materials needed to lyse cells by freezing and grinding in liquid N_2 and were treated with SDS and purified by phenol/chloroform extraction, and precipitated in 2-propanol (Lee *et al*. 2003).

In conclusion, we have presented here a DNA extraction method that is easy to use, rapid, cost-effective and applicable for many microorganisms, especially for Gram-negative bacteria.

Acknowledgements

We are very grateful to Dr. Bernhard Seiboth (Institute of Chemical Engineering, Vienna University of Technology, Austria) for valuable discussion and critically reading the manuscript. We also thank Ms. Yu Bai for the preparation of the manuscript. The research was supported by the National Basic Research Program of China (No. 2004 CB 719002) and the National Knowledge Innovation Project from the Chinese Academy of Sciences.

References

Becker A, Katzen F, Puhler A, Ielpi L (1998) Xanthan gum biosynthesis and application: a biochemical/genetic perspective. *Appl. Microbiol. Biotechnol.* **50**: 145–152.

Flamm RK, Hinrichs DJ, Thomashow MF (1984) Introduction of pAMβ1 into *Listeria monocytogenes* by conjugation and homology between native *L. monocytogenes* plasmids. *Infect. Immun.* **44**: 157–161.

Lee YK, Kim HW, Liu CL, Lee HK (2003) A simple method for DNA extraction from marine bacteria that produce extracellular materials. *J. Microbiol. Methods* **52**: 245–250.

Müller FM, Werner KE, Kasai M, Francesconi A, Chanock SJ, Walsh TJ (1998) Rapid extraction of genomic DNA from medically important yeasts and filamentous fungi by high-speed cell disruption. *J. Clin. Microbiol.* **36**: 1625–1629.

Neumann B, Pospiech A, Schairrer HU (1992) Rapid isolation of genomic DNA from Gram-negative bacteria. *Trends Genet.* **8**: 332–333.

Niemi RM, Heiskanen I, Wallenius K, Lindstrom K (2001) Extraction and purification of DNA in rhizosphere soil samples for PCR-DGGE analysis of bacterial consortia. *J. Microbiol. Methods* **45**: 155–165.

Sambrook J, Russell DW (2001) Rapid isolation of yeast DNA. In: Sambrook J & Russell DW, eds. *Molecular Cloning, a Laboratory Manual*, New York: Cold Spring Harbor Laboratory Press, pp. 631–632.

Syn CK, Swarup S (2000) A scalable protocol for the isolation of large-sized genomic DNA within an hour from several bacteria. *Anal. Biochem.* **278**: 86–90.

Wilson K (1990) Preparation of genomic DNA from bacteria. In: Ausubel FM & Brent R eds. *Current Protocols in Molecular Biology*, New York: Greene Publ. Assoc. and Wiley Interscience, pp. 241–245.

Xue Y, Xu Y, Liu Y, Ma Y, Zhou P (2001) *Thermoanaerobacter tengcongensis* sp. nov., a novel anaerobic, saccharolytic, thermophilic bacterium isolated from a hot spring in Tengcong, China. *Int. J. Syst. Evol. Microbiol.* **51**: 1335–1341.