Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

A simplified universal genomic DNA extraction protocol suitable for PCR

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Genet. Mol. Res. 10 (1): 519-525 (2011)
Received September 8, 2010
Accepted October 27, 2010
Published March 29, 2011
DOI 10.4238/vol10-1gmr1055

ABSTRACT. Conventional genomic DNA extraction protocols need expensive and hazardous reagents for decontamination of phenolic compounds from the extracts and are only suited for certain types of tissue. We developed a simple, time-saving and cost-efficient method for genomic DNA extraction from various types of organisms, using relatively innocuous reagents. The protocol employs a single purification step to remove contaminating compounds, using a silica column and a non-hazardous buffer, and a chaotropic-detergent lysing solution that hydrolyzes RNA and allows the selective precipitation of DNA from cell lysates. We used this system to extract genomic DNA from different tissues of various organisms, including algae (*Dunaliella salina*), human peripheral blood, mouse liver, *Escherichia coli*, and Chinese hamster ovary cells. Mean DNA yields were 20-30 μg/cm^3 from fresh tissues (comparable to yields given by commercial extraction kits), and the 260/280 nm absorbance ratio was 1.8-2.0, demonstrating a good degree of purity. The extracted DNA was successfully used in PCR, restriction enzyme digestion and for recombinant selection studies.

Key words: DNA extraction; PCR; Protocol

INTRODUCTION

To develop a rapid and cost-efficient genomic DNA extraction protocol for different materials is very important in molecular biology, and the quality and quantity of template DNA influence the success of subsequent experiments, such as polymerase chain reaction (PCR), enzyme digestion and recombinant selection. Many DNA extraction protocols are known, including CTAB and salt extraction method (Doyle and Doyle, 1987) and its modification (Huang et al., 2000). Commercial genomic DNA extraction kits have also been developed. However, conventional DNA extraction protocols (Jobes et al., 1995; Cheng et al., 2003) require large quantities of tissue (in grams) to be ground (with a mortar and pestle), and the kits are generally either expensive or not readily available, especially for researchers in developing and under-developed countries worldwide (Kotchoni and Gachomo, 2009). In addition, the use of reported methods is limited to certain organisms, and they are not for universal organism DNA extraction (Hoarau et al., 2007; Ahmed et al., 2009; Margam et al., 2010). Developing a simple time- and cost-efficient protocol for the extraction of different DNAs is not only essential for molecular studies, but also highly desirable when a large number of samples are to be processed.

In the present study, a simple genomic DNA extraction protocol for different organisms is described, which is time- and cost-efficient, free of PCR-inhibiting contaminants, and not reliant on toxic reagents such as phenol/chloroform. The extracted DNA could be used in the following experiments, such as PCR, enzyme digestion, etc.

MATERIAL AND METHODS

BALB/c mice, Chinese hamster ovary (CHO) cells, *Escherichia coli* JM109, human peripheral blood, and *Dunaliella salina* were used throughout to extract genomic DNA.

Reagents and chemicals

Extraction buffer

1. Add 24 g GuSCN (guanidinium thiocyanate) and 20 mL 0.1 M Tris-HCl, pH 6.4, to a 50-mL Falcon tube.
2. Heat to 60°C to dissolve the GuSCN.
3. Add 4.4 mL 0.2 M EDTA, pH 8.0.
4. Add 0.5 mL Triton X-100. Mix by inverting.

Binding buffer

1. Combine 24 g GuSCN and 20 mL 0.1 M Tris-HCl, pH 6.4, in a 50-mL Falcon tube.
2. Heat to 60°C to dissolve the GuSCN.
3. Add 4.4 mL 0.2 M EDTA, pH 8.0. Mix by inverting.

Washing buffer: 70% EtOH supplemented with 10 mM NaCl TE buffer: 10 mM Tris-HCl buffer, pH 8.0, 1 mM EDTA, pH 8.0, PCR reagents: Taq DNA polymerase, dNTPs (TaKaRa).

DNA extraction protocol

1. Lysis/homogenization: Add 0.6 mL extraction buffer to 20-80 mg tissue (animal or plant) or 10^5-10^8 cells (cultured cells, white cells in whole blood).
   A. Tissues: Grind the tissue into a powder under liquid nitrogen or on an ice bath. Transfer up to 50 mg tissue to a 1.5- or 2.0-mL microcentrifuge tube. Add extraction buffer and store for 5-10 min at room temperature.
   B. Cells: Cells grown in monolayer should be lysed directly in a culture dish. Pour off media, then add extraction buffer. Cell pellets or suspensions: add 1 mL extraction buffer to 10^7 cells (suspended in 0.2 mL PBS) and lyse the cells by repeated pipetting. Decrease the extraction buffer by one half if using less than 5×10^6 cells. White blood cells: add 1 mL extraction buffer to the cells from more than 0.5 mL normal whole blood.
   C. To minimize shearing the DNA molecules, mix DNA solutions by inversion; avoid vigorous shaking or vortexing.
2. Centrifuge for 10 min at 13,000 *g* at 4-25°C.
3. Following centrifugation, transfer the resulting viscous supernatant to the spin column. Centrifuge at 12,000 *g* for 30 s. Discard flow-through.
4. Add 500 µL binding buffer to the spin column. Centrifuge at 12,000 *g* for 30 s. Discard flow-through.
5. Add 600 µL washing buffer to the spin column. Centrifuge at 12,000 *g* for 1 min. Discard flow-through.
6. Repeat step 5.
7. Centrifuge for an additional 1 min at 12,000 *g* and transfer the spin column to a sterile 1.5-mL microcentrifuge tube.
8. Add 10 to 200 µL TE buffer, and incubate at room temperature for 1 min.
9. Centrifuge at 12,000 *g* for 1 min. The buffer in the microcentrifuge tube contains the DNA.
10. DNA concentrations were measured by running aliquots on 0.8% agarose gel and by reading absorbance at 260 nm with a spectrophotometer.
11. The DNA samples were stored at -20°C until further use.

PCR amplification and gel electrophoresis

PCR was carried out in a 50-µL reaction mixture, which contained 50 ng template DNA (CHO genomic DNA extracted as described above), 0. 25 U Taq DNA polymerase, 2.0 mM dNTPs, 1X Taq DNA polymerase buffer (Mg^{2+} plus) and 20 µM primer. The sequences of the designed primer were as follows: P1: 5'-TTAGTAAGACATCACCTTGCATTT-3', P2: 5'-AGCCATAGTTTGAGTTACCCTTT-3'; P3: 5'-ATATATCCCAATGGCATCGTA-3', P4: 5'-AAATCAAAACTGGTGAAACTC-3'. DNA amplification was done under the following conditions: 95°C for 5 min, followed by 30 cycles of 94°C for 30 s, 50°C for 45 s, and 72°C for 1.0 min, with a final extension at 72°C for 3 min. The PCR products were fractionated on a 1.0% agarose gel using 1X TBE buffer containing 10 mg/mL ethidium bromide and were visualized under UV light, and the gels were photographed using a UV gel documentation system.

RESULTS AND DISCUSSION

A simple protocol for the extraction genomic DNA from different organisms is of high scientific value for a wide range of applications. In the present study, we developed a universal method suited to DNA extraction in cultured cells, animal tissues, plant, and bacteria. We used this protocol to extract genomic DNA from BALB/c mouse liver, CHO cells, *E. coli* JM109, human peripheral blood, and *D. salina.* The results showed that high-molecular weight DNA in large quantities and of good quality was obtained (Table 1; Figure 1), and the purity of the DNA samples was confirmed by absorbance (A_{260}/A_{280}) ratio, which was 1.8-2.0.

Table 1. Quality analysis of genomic DNA by UV absorption at 260 and 280 nm.

DNA sample	OD_{260}	OD_{280}	OD_{260}/OD_{280}
Dunaliella salina	0.296	0.158	1.87
Peripheral blood	0.319	0.164	1.94
Mouse liver	0.279	0.152	1.83
Escherichia coli	0.256	0.138	1.85
CHO cells	0.311	0.165	1.88

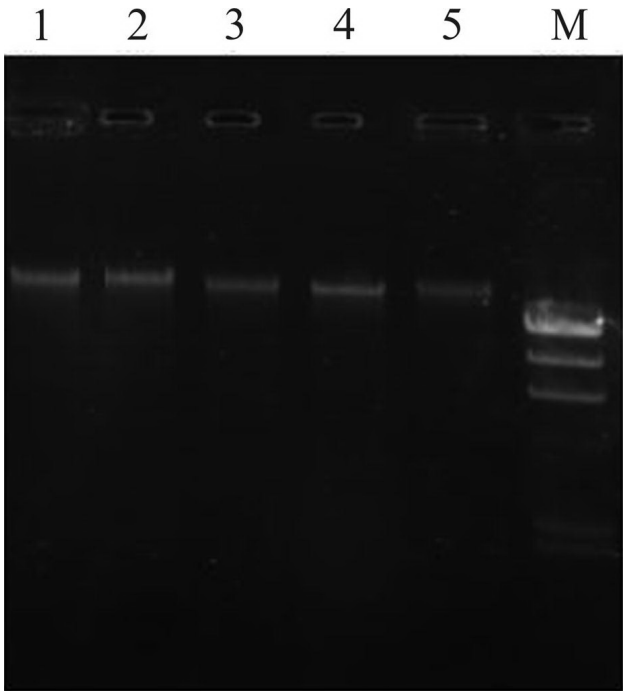

Figure 1. Agarose gel electrophoresis of extracted genomic DNA. *Lane 1* = *Dunaliella salina*; *lane 2* = human peripheral blood; *lane 3* = mouse liver; *lane 4* = *Escherichia coli*; *lane 5* = CHO cells; M = λDNA/*Hin*dIII marker.

In order to further check the quality of the genomic DNA extracted by this method, PCR amplification and enzyme digestion were performed. Agarose gel analysis revealed that 0.8 and 0.5-kb DNA fragments could be amplified from the rapidly extracted CHO cells and

peripheral blood DNA samples (Figure 2), and the extracted genomic DNA could be digested by a restriction enzyme (Figure 3). The DNA obtained was suitable for enzymatic manipulations such as PCR and showed high intensity amplification and enzyme digestion. PCR amplification also indicates that the DNA was of good quality, free from interfering compounds, and that it would be suitable for other DNA analyses such as Southern blotting and RFLP.

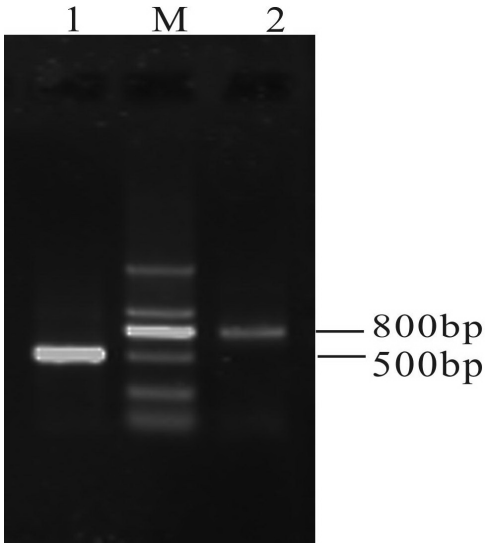

Figure 2. Electrophoresis analysis of PCR products using the extracted genomic DNA as template. *Lane 1* = CHO cells; *lane 2* = human peripheral blood; M = DL2000.

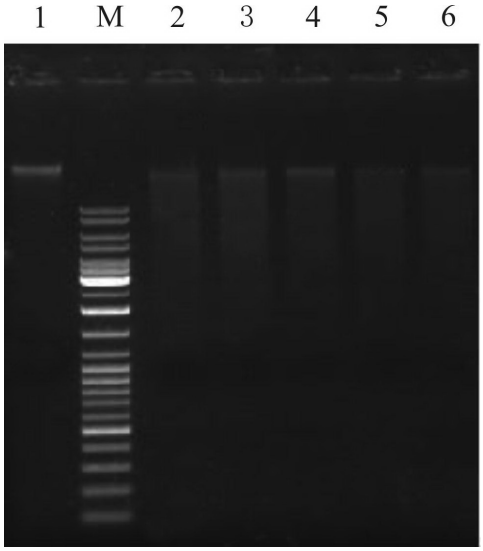

Figure 3. Electrophoresis analysis of enzyme digestion products of genomic DNA. *Lane 1* = Control; *lane 2* = human peripheral blood; *lane 3* = mouse liver; *lane 4* = *Escherichia coli*; *lane 5* = CHO cells; *lane 6* = *Dunaliella salina*; M = Sangon DNA marker.

The extraction of high-quality genomic DNA is a primary and critical step in molecular biology. However, a low-cost and time-efficient extraction protocol is often hindered by two major challenges now. The first challenge is the reduction of secondary chemical reactions (including oxidation) in the initial crude tissue extract, which otherwise could lead to loss of DNA yield; another is the lack of a universal extraction protocol suitable for different organisms because of the differences in compounds between them (Kotchomo and Gachomo, 2009). Previously, genomic DNA purification methods included phenol-chloroform-based approaches, with a popular method described by Sambrook et al. (1989), using Chelex-100 (Walsh et al., 1991; Roberge et al., 1997; Yue and Orban, 2005) and methods based on grinding tissue in liquid nitrogen (Biase et al., 2002). These methods have been used effectively, but are time-consuming, accrue a storage and handling cost, and use hazardous reagents that require special handling and disposal mechanisms. In addition, most of the DNA extraction protocols previously described use proteinase K and RNase to remove protein and RNA contaminations. In contrast, our method does not need these reagents and therefore requires fewer reagents compared to the commonly used methods, and it does not require special chemical handling and disposal procedures.

In summary, we report here a simple, universal, inexpensive, and safe method for extracting DNA from different organisms, which can subsequently be used for PCR amplification. The method needs only a few reagents and is a one-step manipulation, and it could reduce risk of contamination and even be suitable for the high school classroom. This method could be carried out at room temperature and does not require cold-storage facilities. Using this protocol, DNA extraction can be accomplished within 30 min by a non-specialist. The extracted DNA is suitable for other purposes following PCR amplification, enzyme digestion and cloning. It is, therefore, suitable not only for large-scale sample extraction but also for high-quality DNA extraction in laboratories with relatively limited equipment or funds.

ACKNOWLEDGMENTS

Research supported by the National Natural Science Foundation of China (grant #30970035) and Science & Technology Innovation Talents in University of Henan Province (grant #2008A310008).

REFERENCES

Ahmed I, Islam M, Arshad W, Mannan A, et al. (2009). High-quality plant DNA extraction for PCR: an easy approach. *J. Appl. Genet.* 50: 105-107.

Biase FH, Franco MM, Goulart LR and Antunes RC (2002). Protocol for extraction of genomic DNA from swine solid tissues. *Genet. Mol. Biol.* 25: 313-315.

Cheng YJ, Guo WW, Yi HL, Pang XM, et al. (2003). An efficient protocol for genomic DNA extraction from citrus species. *Plant Mol. Biol. Rep.* 21: 177a-177g.

Doyle JJ and Doyle JL (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11-15.

Hoarau G, Coyer JA, Stam WT and Olsen JL (2007). A fast and inexpensive DNA extraction/purification protocol for brown macroalgae. *Mol. Ecol. Notes* 7: 191-193.

Huang X, Zeller FJ, Hsam SL, Wenzel G, et al. (2000). Chromosomal location of AFLP markers in common wheat utilizing nulli-tetrasomic stocks. *Genome* 43: 298-305.

Jobes DV, Hurley DL and Thien LB (1995). Plant DNA isolation: a method to efficiently remove polyphenolics, polysaccharides, and RNA. *Taxon* 44: 379-386.

Kotchoni SO and Gachomo EW (2009). A rapid and hazardous reagent free protocol for genomic DNA extraction suitable

for genetic studies in plants. *Mol. Biol. Rep.* 36: 1633-1636.
Margam VM, Gachomo EW, Shukle JH, Ariyo OO, et al. (2010). A simplified arthropod genomic-DNA extraction protocol for polymerase chain reaction (PCR)-based specimen identification through barcoding. *Mol. Biol. Rep.* 37: 3631-3635.
Roberge C, Pommier SA and Houde A (1997). Rapid DNA purification for Hal gene PCR diagnosis in porcine tissues and extension to other meat species. *Meat Sci.* 45: 17-22.
Sambrook J, Fritsch EF and Maniatis T (1989). Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Nova York.
Walsh PS, Metzger DA and Higuchi R (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 10: 506-513.
Yue GH and Orban L (2005). A simple and affordable method for high-throughput DNA extraction from animal tissues for polymerase chain reaction. *Electrophoresis* 26: 3081-3083.

