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ORIGINAL ARTICLE

An efficient purification and fractionation of genomic DNA from soil by modified troughing method

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DNA purification, DNA size fractionation, genomic DNA from soil, humic acids, metagenomic library.

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Abstract

Aims: The aim of this study was to utilize a modified troughing method for purification of large genomic DNA obtained from microbiota in natural environment and for fractionation of genomic DNA into many size ranges that facilitates construction of metagenomic library.

Methods and Results: Genomic DNA extracted from soil or termite gut was purified by the modified troughing method which utilized gel electrophoresis in the presence of 30% PEG8000. The method performed better than various purification kits and allowed no significant loss in the amount of DNA recovered. In addition, the efficiency of the modified troughing method for DNA size fractionation was investigated. DNA size fractionation was achieved with repetitive rounds of electrophoresis and DNA collection to obtain DNA with many size ranges.

Conclusions: The modified troughing method is a simple and efficient method for purification of genomic DNA and for DNA size fractionation.

Significance and Impact of the Study: The modified troughing method is a straightforward and inexpensive technique readily available for anyone working with environmental genomic DNA. It facilitates cloning of genomic DNA and enhances rapid discovery of useful bioactive compounds from microbial resources.

Introduction

Recently, metagenomic approach is widely used to seek out useful enzymes or bioactive compounds, by identifying genes coding for these substances directly from micro-organisms residing in soils or biological niches (Handelsman *et al.* 1998; Hugenholtz *et al.* 1998; Handelsman 2004). DNA extraction from soil sample is a relatively uncomplicated process (Zhou *et al.* 1996; Yeates *et al.* 1997; Harry *et al.* 1999). However, the extracted DNA usually contains contaminants, the majority of which are humic substances (Yeates *et al.* 1997; Harry *et al.* 1999; Kauffmann *et al.* 2004). Humic acids are natural compounds that present cumbersome problems for DNA analysis, as they almost always inhibit enzymatic reactions (Harry *et al.* 1999; Miller *et al.* 1999). Currently, researchers generally employ purifying techniques [such as gradient centrifugation, chromatography columns, spin columns, hexadecyltrimethylammonium bromide (CTAB), and polyvinylpolypyrrolidone (PVPP)] or purifying kits that are commercially available to remove humic acids from genomic DNA (Miller *et al.* 1999). These methods presumably give sufficiently clean DNA. However, the purifying techniques (without kits) can be labour-intensive and time-consuming. The purifying kits with bind-wash-elute procedures take less time, but they can be highly expensive, especially if large amounts of purified DNA are needed or if DNA recovery is not very efficient.

Another important step for the metagenomic approach is the construction of metagenomic library. The genomic DNA is usually subjected to size fractionation, namely DNA differentiation according to length, before the DNA fractions are cloned into an expression vector. As a pool of genomic DNA with different sizes often has different

efficiency for ligation into vector and transformation into host strain, DNA size fractionation is advantageous for cloning where DNA of specific sizes are desired, especially for obtaining genes with small sizes. Preparative pulsed-field gel electrophoresis (PFGE) was performed to obtain DNA fractions with large sizes (>10 kb) for successful improvement of PAC (P1 artificial chromosome) and BAC (F-factor bacterial artificial chromosome) cloning (Strong *et al.* 1997). Direct isolation of 30–40-kbp fragments from low-melting agarose gel for cloning into fosmid was also reported (Kim and Fuerst 2006). However, high recovery of genomic DNA fractions with small sizes often requires handling with care, as small DNA are easily lost during fractionation. For example, DNA fractionation using sucrose density centrifugation (10–40%) (Yun *et al.* 2004) was reported for the construction of libraries with small inserts (<10 kbp) in a standard sequencing vector. Therefore, searching for a less difficult method for DNA fractionation that allows for high recovery of DNA with small size is indispensable.

To improve utilization of genomic DNA obtained from natural habitat, we present an efficient, inexpensive, and relatively easy technique modified from Zhen and Swank (1993) called troughing method to remove humic acid and other contaminants from genomic DNA. In addition, the modified troughing method was successfully used to fractionate partially digested genomic DNA into various sizes.

Materials and methods

Genomic DNA extraction

Genomic DNA was chemically extracted from soil (e.g. soil in the Jae-sawn Hot Spring in Lampang Province, Thailand) according to the method of Zhou *et al.* (1996). Genomic DNA from termite gut was extracted with IsoplantII (Nippon Gene; Wako, Japan) and Dneasy Tissue kit (Qiagen, Venlo, The Netherland) according to the manufacturer's protocols.

Modified troughing

Procedure for the modified troughing is depicted in Fig. 1. Briefly, genomic DNA extracted from soil or termite gut was subjected to electropheresis in a TAE (Tris-acetate/EDTA) or TBE (Tris-borate/EDTA) agarose gel, which contained 0·1–0·5 μl ml^{-1} ethidium bromide for approximately 45 min at 85 V. The extent of a migration of the genomic DNA was visualized on top of a ultraviolet (UV) transilluminator. Degradation of the genomic DNA by UV ray was minimized by brief exposure (less than 1 min) of the DNA to long-wavelength UV (approximately 360 nm) and the gel was kept on the plastic gel cassette. A sharp scalpel was used to make a rectangular well (trough) approximately 0·5–1 cm in width just below the DNA band directly in front of the path of migration. Excess agarose was removed from the well. The well was then filled with troughing buffer which consists of 30% PEG8000 (polyethyleneglycol, molecular weight 8000) in TAE or TBE buffer. Addition of ethidium bromide into the troughing buffer is not necessary for detection of DNA migration. However, 0·1–0·5 μl ml^{-1} ethidium bromide may enhance visualization of the genomic DNA in the trough. Electrophoresis was continued for approximately 30 min until the DNA band migrated to the middle of the well. The DNA was then collected, extracted once with an equal volume of chloroform/isoamyl alcohol (24 : 1 v/v), precipitated with two volumes of ethanol, washed with 70% ethanol, and resuspended in water or TE (10 mmol l^{-1} Tris, 1 mmol l^{-1} EDTA; pH 8·0) buffer.

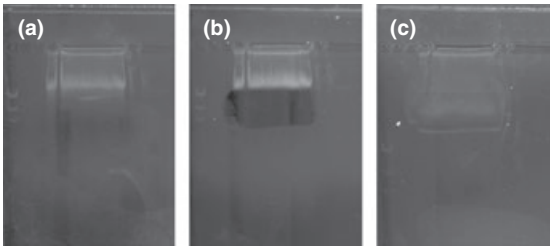

Figure 1 Purification of genomic DNA by the modified troughing method. Procedures of the modified troughing method are depicted. (a) Genomic DNA was subjected to agarose gel electrophoresis for approximately 45 min. (b) A well (trough) was made in front of the DNA band and filled with troughing buffer (30% PEG8000 in Tris-acetate/EDTA or Tris-borate/EDTA buffer). (c) Additional electrophoresis was performed until the DNA band moved to the middle of the well. The DNA was then collected, extracted with chloroform/isoamyl alcohol, precipitated with ethanol, and resuspended in water.

Size fractionation of genomic DNA

Genomic DNA partially digested with *Sau*3AI (or other restriction enzymes) was subjected to electrophoresis for 25 min. The well was then cut at the position of the lowest dye front. After the troughing buffer was added to the well, the first (smallest) DNA fraction was collected after 15 min of additional electrophoresis. Then, the well was filled up again with troughing buffer and the second DNA fraction was collected after 30 min of additional electrophoresis. This process was repeated for the third and fourth (largest) fractions of DNA with 45 and 60 min of additional electrophoresis, respectively.

DNA purification with QIAEXII (Qiagen), QIAquick (Qiagen), and Wizard (Promega, Madison, WI, USA) kits

Purification was performed according to the manufacturer's protocols.

PCR and restriction enzyme digestion

Polymerase chain reaction (PCR) was conducted to amplify a 1·6-kb portion of the 16S sequence of bacterial rDNA using degenerate primers. The reaction is as follows: 3 min of denaturation at 94°C; followed by 30 amplification cycles of 1 min at 94°C, 1 min at 50°C, 1·5 min at 72°C; with a final 10 min of prolonged extension at 72°C.

Approximately 100 μg of purified DNA was subjected to partial digestion by 5 U of *Sau*3AI in an appropriate buffer for 30 min at 37°C. The enzyme was then heat-inactivated at 65°C for 20 min.

Metagenomic library construction

Genomic DNA partially digested by *Sau*3AI was size-fractionated by the modified troughing method. Two DNA fractions with the largest sizes (larger than 7 kbp and *c.* 2·5–7 kbp) were ligated with pZErO-2 plasmid (Invitrogen, Carlsbad, CA, USA), which previously had been digested with *Bam*HI. The ligated plasmids were transformed into the TOP10 *Escherichia coli* strain (Invitrogen) and grown on LB medium containing 25 μg ml^{-1} of kanamycin overnight. The resulting library was stored in 15% glycerol at –80°C.

Results

The modified troughing method utilizes gel electrophoresis and PEG8000 to effectively remove contaminants, especially humic acids, from genomic DNA obtained from soil or termite gut. The genomic DNA is larger than 23 kbp and low-molecular weight humic acids migrate faster than genomic DNA in agarose gels. The troughing method consistently yields 70% recovery of purified DNA (Table 1, Fig. 2). There is no observable difference in the recovery yield when a TAE or a TBE gel was used. It was found that when the level of PEG8000 was increased to 25–30% (instead of 15%) in the buffer, the recovery yield of genomic DNA was greatly improved from *c.* 50% (data not shown) to up to 70–80%. We also compared the recovery rate obtained by the modified troughing method with that obtained by other DNA purification kits available commercially, such as QIAquick Gel Extraction kit, QIAEXII Gel Extraction kit (Qiagen), and Wizard DNA Clean-up system (Promega), which were chosen because they seem to be the most widely used ones (Fig. 2). We consistently obtained a higher or equal yield of DNA purified by the troughing method compared with the Wizard kit purification. In our hands, the Wizard kit did not give a consistent yield (25–60%) (Table 1) and the yield was especially low when the level of humic acids in the soil was high. The modified troughing method also gave a higher yield of purified DNA compared with the QIAquick and QIAEXII purification kits which frequently gave less than 30% and 50% recovery rate, respectively (Table 1). In addition to a high yield of purified DNA, the troughing method also produces DNA with high

Table 1 Comparison of DNA purification by the troughing method with other purification kits

Method	Recovery rate	Time
Troughing	50–70%	*c.* 2.5 h (including electrophoresis and precipitation)
QIAEXII kit	<50%	*c.* 2 h (including electrophoresis)
QIAquick kit	<30%	*c.* 1.5 h (including electrophoresis)
Wizard DNA purification kit	25–60% (varies)	20 min

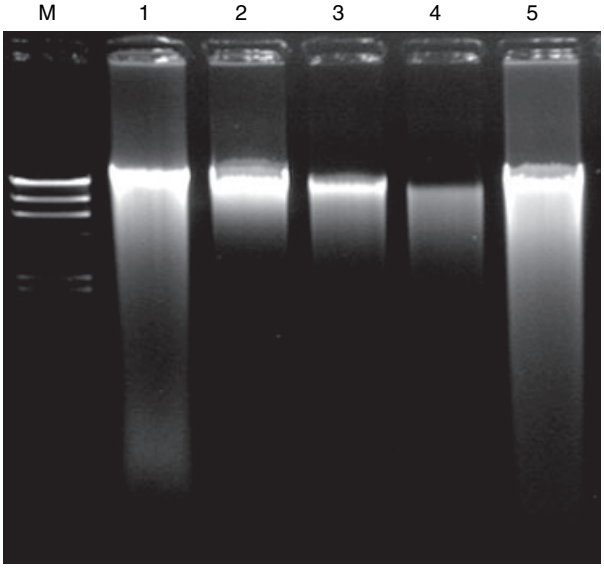

Figure 2 Genomic DNA purified by the modified troughing method compared with DNA purified by several purification kits. The modified troughing method yields *c.* 70% recovery. Equal amounts of genomic DNA were purified by four methods. Equal volumes of purified DNA were loaded into each lane; lane M: DNA markers (λ DNA cut with *Hind*III); lane 1: unpurified genomic DNA; lane 2: genomic DNA purified by the modified troughing method; lane 3: genomic DNA purified by the QIAEX II kit; lane 4: genomic DNA purified by the QIAquick kit; lane 5: genomic DNA purified by the Wizard DNA purification kit.

Method	OD260	OD280	OD230	OD260/OD280	OD260/OD230
Unpurified	0·914	0·672	1·032	1·360	0·886
Troughing	0·633	0·378	0·311	1·675	2·035
QIAEXII kit	0·453	0·285	0·431	1·589	1·051
QIAquick kit	0·259	0·154	0·912	1·677	0·284
Wizard kit*	1·745	1·025	3·398	1·702	0·513

OD, optical density.
*Absorbance values for genomic DNA purified by Wizard DNA purification kit are very high presumably because of the presence of some substances eluted from the columns with genomic DNA. Those values were not considered further.

Table 2 Absorbance values of genomic DNA purified by troughing method compared with those of genomic DNA purified by other purification kits

quality. The 260/280 absorbance ratio of the genomic DNA purified by the modified troughing method was high (1·675), indicating the presence of double-stranded DNA with low levels of protein contamination (Table 2). In addition, a high value (>2) of the 260/230 absorbance ratio obtained by the troughing method indicated low contamination by humic substances (Harry *et al.* 1999). This indicated that the modified troughing method was efficient in removing most humic acids from genomic DNA. This method is also gentle and useful for recovering of metagenomic DNA with little shearing (Fig. 2). Genomic DNA purified by the troughing method (as well as DNA purified by the commercially available kits) was sufficiently clean and could be used successfully for PCR amplification, whereas the unpurified DNA failed to yield a product, demonstrating that the unpurified DNA contained humic acids or other contaminants that inhibited the PCR (Fig. 3a). Furthermore, the purified DNA could be effectively used for other applications such as restriction enzyme digestion, ligation, and cloning (Fig. 3b and data not shown).

In addition to purification of genomic DNA, the troughing method can also be effectively used for DNA size fractionation (Fig. 4). Repetitive DNA collecting coupled with different lengths of electrophoresis during the procedure allows one to separate DNA into many size ranges (e.g. less than 0·75 kbp, *c.* 0·75–2·5 kbp, *c.* 2·5–7 kbp, and larger than 7 kbp). Interestingly, we observed that even with low level of partially digested DNA obtained from termite gut (approximately 25 ng μl^{-1} of genomic DNA), this troughing method could be easily used to recover and concentrate DNA (data not shown).

DNA fractions of different sizes were successfully used for ligation with expression vector and subsequent transformation into *E. coli*, with transformation efficiency of 10^6 CFU μg^{-1} of DNA. The quality of the so-called metagenomic library was ensured by finding that more than 95% of randomly picked clones contain inserts with varying sizes (data not shown). This collection of transformants can be further used to screen for genes encoding useful enzymes or bioactive compounds.

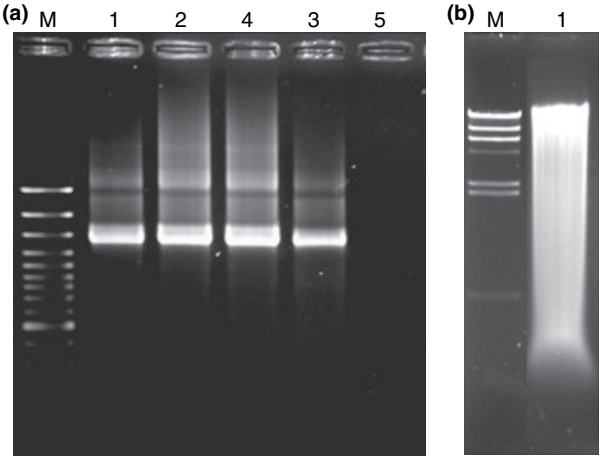

Figure 3 Genomic DNA purified by the modified troughing method can be used for various applications. (a) DNA purified by the modified troughing method can be used for polymerase chain reaction (PCR) to amplify a portion of rDNA genes. Equal volumes of PCR products were loaded into each lane. PCR was performed with the following DNA as templates; lane 1: genomic DNA purified by the modified troughing method; lane 2: genomic DNA purified by the QIAEX II kit; lane 3: genomic DNA purified by the QIAquick kit; lane 4: genomic DNA purified by the Wizard DNA purification kit; lane 5: unpurified genomic DNA. Lane M is DNA marker (λ DNA cut with *Hind*III). (b) DNA purified by troughing method can be digested with restriction enzymes such as *Sau*3AI (lane 1). Lane M is DNA marker (λ DNA cut with *Hind*III).

Discussion

The modified troughing method is a simple and straightforward method to purify genomic DNA, which is usually larger than 23 kbp, away from humic substances and other contaminants. Compared with the various purification kits, the method consistently resulted in higher rate of DNA recovery. The recovered DNA is of good quality and can be further used for many applications. Exactly 30% of PEG8000 was used to prevent the DNA from diffusing in the troughing buffer during electrophoresis. Furthermore, the troughing method can be used to fractionate DNA of various size ranges. For our purpose,

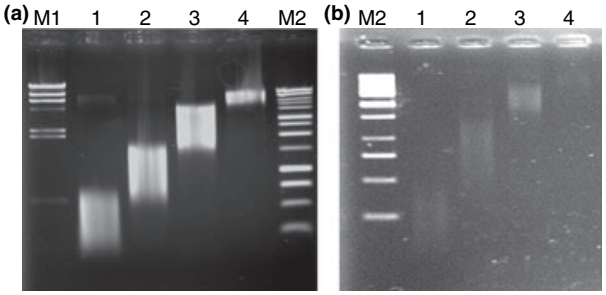

Figure 4 DNA size fractionation by the modified troughing method. Fractionation of genomic DNA sample from soil (a) and termite gut (b). Lane M1: DNA marker (λ DNA cut with *Hind*III); lane 1: DNA fraction 1 (smaller than 0·75 kb); lane 2: DNA fraction 1 (c. 0·7–2·5 kb); lane 3: DNA fraction 1 (c. 2·5–7 kb); lane 4: DNA fraction 1 (larger than 7 kb); lane M2: 1-kb DNA ladder.

the modified troughing method is, thus, a direct and simultaneous procedure used for fractionation and purification of genomic DNA with different sizes. There is no requirement for further DNA extraction from agarose gel. Therefore, in contrast to many methods used for DNA extraction from gel, this modified troughing method requires no concern over the amount (or volume) of the agarose gel presented with the DNA sample, especially when the DNA is purified from a large area of agarose gel in broad size ranges after electrophoresis. This method can also be used to purify several DNA bands with different molecular weights simultaneously. In addition, the method is relatively fast and is not very labour-intensive, with no requirements for special apparatus or expensive chemicals. Although the modified troughing method is slower than various DNA-purifying kits, it offers a simple, low-cost, and high-yield choice readily available for anyone who is working with genomic DNA obtained from micro-organisms in soils or biological niches. The method will facilitate rapid discovery of useful enzymes or bioactive compounds from microbial resources in natural habitat and ultimately lead to advancements in biotechnology.

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