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Isolation of genomic DNA using magnetic nanoparticles as a solid-phase support

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Abstract

In recent years, techniques employing magnetizable solid-phase supports (MSPS) have found application in numerous biological fields. This magnetic separation procedure offers several advantages in terms of subjecting the analyte to very little mechanical stress compared to other methods. Secondly, these methods are non-laborious, cheap, and often highly scalable. The current paper details a genomic DNA isolation method optimized in our laboratory using magnetic nanoparticles as a solid-phase support. The quality and yields of the isolated DNA from all the samples using magnetic nanoparticles were higher or equivalent to the traditional DNA extraction procedures. Additionally, the magnetic method takes less than 15 min to extract polymerase chain reaction (PCR) ready genomic DNA as against several hours taken by traditional phenol–chloroform extraction protocols. Moreover, the isolated DNA was found to be compatible in PCR amplification and restriction endonuclease digestion. The developed procedure is quick, inexpensive, robust, and it does not require the use of organic solvents or sophisticated instruments, which makes it more amenable to automation and miniaturization.

1. Introduction

Isolation of DNA is a prerequisite step for many molecular biology techniques. The separation of DNA from the complex mixtures in which it is often found is frequently necessary before starting other studies and procedures such as sequencing, amplification, hybridization, detection etc. With the completion of the human genome project, the focus is now on evaluating genetic sequences for genotyping (single nucleotide polymorphism analysis) and allelic polymorphism analysis. This requires unprecedented capabilities for DNA purification and sequencing. In addition to DNA analysis, the rapidly growing field of molecular diagnostics and molecular phylogeny requires a need for quick, simple, robust, and high-throughput procedures for the isolation of DNA from diverse organisms and tissues. The process of genomic DNA isolation and purification has evolved considerably within the last decade. The new demands of high-throughput facilities have resulted in the development of new technologies for easier and faster DNA processing than ever before.

Magnetic separation techniques employing magnetic particles coated with different polymers (e.g. agarose, silica) have been used increasingly for molecular biology applications [1, 2]. The purification of genomic and plasmid DNA using magnetizable support (beads or matrix) has already been attempted from different biological sources [3–7]. Furthermore, carboxyl coated magnetic particles (BioMag) have been used as adsorbent for DNA purification under high-salt conditions [8]. In the above-mentioned procedures only coated magnetic particles have been used for various applications. This means only the magnetic property of the particles has been utilized to achieve faster separation. However, [9] have shown that magnetite (Fe_3O_4) particles synthesized by the co-precipitation method specifically adsorbs the amphoteric hydroxyl (–OH) group, which remains on the particles at a pH between 6 and 10 [9]. Under specific conditions, the naked (uncoated) magnetic nanoparticles (Fe_3O_4) permits us to exploit their property to reversibly bind DNA. There are several inherent advantages of the use of such naked particles, where molecules are directly adsorbed to the magnetic support. Due to the absence of polymer coating, the particle size is small ($\leqslant$100 nm), which provides a higher surface area (on a weight basis) for the binding of the biomolecules; this also allows the particles to have a higher magnetic susceptibility to the applied

magnetic field. Moreover, magnetic nanoparticles can exist as stable colloidal suspensions that will not aggregate, allowing for uniform distribution in a reaction mixture. Therefore, we have recently reported the use of naked magnetic nanoparticles as a support for extraction of genomic DNA from mammalian cells [10]. The applicability of these magnetic nanoparticles for elution of DNA from agarose gels was also successfully demonstrated [11]. The aim of the current work was to produce a universal approach for the isolation of genomic DNA using magnetic nanoparticles as a purification medium.

2. Material and methods

2.1. Preparation of magnetic nanoparticles

Magnetic nanoparticles were synthesized by chemical co-precipitation of Fe^{+2} and Fe^{+3} ions in an alkaline solution, followed by a treatment under hydrothermal conditions [12]. Briefly, 100 ml solution of 1 M $FeSO_4 \cdot 7H_2O$ and 2 M $FeCl_3$ (Qualigens Fine chemicals, Mumbai, India) were thoroughly mixed and added to 8 M ammonium hydroxide (Sisco Research Laboratories Limited, Mumbai, India) with constant stirring at 80 °C. The particles thus obtained exhibited a strong magnetic response. Impurity ions were removed by washing the particles several times with hot distilled water. The yield of precipitated magnetic nanoparticles was determined by removing known aliquots of the suspension and drying to a constant mass in an oven at 60 °C. Finally, the magnetic particles were dispersed in TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) and stored at a stock concentration of 10 mg ml^{-1}. The magnetic nanoparticles prepared were stable at room temperature (25–30 °C) without getting agglomerated. The particles were characterized for size using transmission electron microscopy. The mean particle size determined from transmission electron microscopy was about 40 nm.

2.2. Isolation of DNA from mammalian cells using magnetic nanoparticles as a solid support

In the current study, the procedure for isolation of genomic DNA was initially optimized using whole blood as a source of DNA. Once the method was standardized; its applicability was checked for DNA isolation from cultured cells (HCT116) and tissue (rat liver and brain) homogenates. The sample preparation for DNA extraction involved collection of whole blood in a tube containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Cultured cells used in this work were of colon carcinoma cell lines (HCT116), trypsinized, and adjusted to a cell density of 7×10^6 cells ml^{-1} with phosphate buffered saline (PBS, pH 7.4). For tissue DNA extraction, a 10% homogenate of rat liver and brain was prepared in 0.32 M buffered sucrose (pH 7.5).

In a typical extraction, for 30 μl of sample (whole blood, cultured cell suspension, or tissue homogenate), 30 μl of 1% (w/v) sodium dodecyl sulfate solution was added. The tube was mixed by gentle inversion for two or three times and incubated at room temperature for a minute. After incubation, 10 μl of magnetic nanoparticles were added to the cell lysate, followed by addition of 75 μl of binding buffer (1.25 M sodium chloride and 10% polyethylene glycol 6000 [PEG 6000]). The suspension was mixed by inversion and allowed to stand at room temperature for 3 min. The magnetic pellet was immobilized by application of an external magnet and the supernatant was removed. The magnetic pellet was washed with 70% ethanol and dried. The pellet was then completely resuspended in 50 μl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and magnetic particle bound DNA eluted by incubation at 65 °C with continuous agitation. Finally, the supernatant containing the DNA was transferred to a fresh tube. The quality and yield of the DNA was analyzed with agarose gel electrophoresis.

2.3. Elution of DNA from agarose gel using magnetic nanoparticles as a solid-phase support

Gel electrophoresis is an important analytical technique that is also widely used for isolation and purification of specific DNA fragments. The genomic DNA isolation method developed using magnetic nanoparticles as a solid support was checked for its suitability to purify DNA from agarose gel. The optimized procedure is briefly described below.

DNA samples isolated from human blood were electrophoresed on a 0.8% agarose gel (figure 3(a)). The separated DNA was visualized on a UV transilluminator and the band of interest was excised with a sterile blade and transferred to a microcentrifuge tube. Four volumes of SSC (0.75 M sodium chloride, 0.0075 M sodium citrate, pH 7.0) buffer was added to the agarose plug and the tubes were incubated at 80 °C for 5 min to allow the agarose to melt. After incubation, 20 μl of magnetic nanoparticles were immediately added from the stock, followed by addition of 200 μl of binding buffer (1.25 M sodium chloride and 10% PEG 6000). The suspension was mixed by gentle inversion and the tube was allowed to stand at room temperature for 5 min. The magnetic pellet was then immobilized by application of an external magnet and the supernatant was discarded. The magnetic pellet was then washed twice with 70% ethanol and the pellet was dried. The pellet was then resuspended in 30 μl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and magnetic particle bound DNA was eluted by incubation at 65 °C with continuous agitation. Finally, the particles were separated magnetically and supernatant containing the DNA was transferred to a fresh tube.

The current magnetic method was compared with traditional phenol extraction [13] and glass wool spin-column procedures (www.protocol-online.org) that are used for extraction of DNA from agarose gel.

2.4. PCR amplification

All PCRs were performed in a 50 μl reaction volume; 25 μl of PCR 2X master mix (Genetix, USA) was added. Five picomoles each of primers GAPDH-forward (5-ACAGTCCATGCCATCACTGCC-3) and GAPDH-reverse (5-GCCTGCTTCACCACCTTCTTG-3) were added per reaction for amplification of an amplicon in the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene [14]. 100 ng of

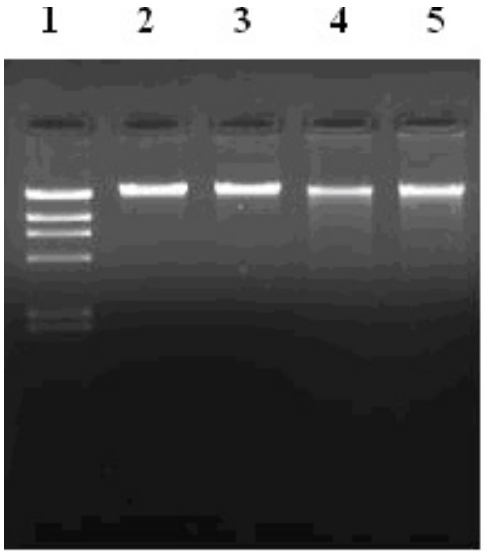

Figure 1. Agarose gel electrophoresis of genomic DNA isolated using magnetic nanoparticles. Lanes: 1 = DNA molecular weight marker (λ phage DNA/*Hind* III digest); 2 = genomic DNA isolated from rat liver homogenate; 3 = genomic DNA isolated from rat brain homogenate; 4 = genomic DNA isolated from cultured cells (HCT116); 5 = genomic DNA isolated from human blood.

template DNA was used in the reaction mixture; PCR was performed on a Techne thermal cycler PCR system (Roche, USA). PCR conditions were 4 min at 94 °C; 34 cycles of 30 s at 94 °C, 30 s at 61 °C, and 1 min at 72 °C followed by 10 min at 72 °C. The PCR products were analyzed on 1.5% agarose gel stained with ethidium bromide.

2.5. Restriction endonuclease digestion of isolated DNA

A 10 μl volume of the eluted DNA solution was mixed with the manufacturer's (Bangalore Genei, Bangalore, India) reaction buffer (1 μl), sterile water (sufficient quantity), and incubated with the restriction endonuclease Eco R1/Hind III (1 μl, 10 units) at 37 °C for 4 h. The digestion mixture was analyzed directly after electrophoresis on 0.8% agarose gel.

3. Results and discussion

3.1. Isolation of DNA from mammalian cells (whole blood, cultured cells or tissue homogenate)

The yields of recovered genomic DNA using magnetic nanoparticles as solid-phase support were up to 1.2 μg per 30 μl of whole blood or 2.0×10^5 cultured cells, while it was about 1.8 μg and 2 μg per 30 μl of liver and brain tissue homogenate, respectively. The DNA yield in each case was estimated fluorimetrically by Hoechst 33258, or preferably, by comparison of intensity of DNA bands in ethidium bromide stained agarose gel. The molecular mass of the extracted DNA was more than 20 kb, as the DNA band migrated at a slower rate than the 23.13 kb band of the λ phage/Hind III molecular mass marker (figure 1).

Further to this, the isolated DNA from all the samples was successfully amplified for a 226 bp glyceraldehyde 3-phosphate dehydrogenase amplicon (figure 2(a)). Also, the restriction digestion of isolated DNA showed no inhibitors of the enzymes present in the sample and the DNA could be used for downstream applications (figure 2(b)). To check the robustness and reproducibility of the method, genomic DNA isolation from blood and cultured cells was performed in ten sets. The recovered genomic DNA from all ten extractions was pooled and spectrophotometric assessment was performed. The yield of DNA was in accordance with that previously estimated and it was about 12–15 μg per 0.3 ml of blood or cultured cells. The average optical density (OD) 260/OD 280 ratio was 1.8 indicating that the DNA was of good quality with negligible protein contamination. Also, as seen in the gel picture (figure 1), no low molecular weight bands or smear were detected, indicating the absence of RNA contamination. This is in agreement with the previous reports; which mention that in the presence of high-salt conditions or chaotropes, the adsorption of double stranded DNA onto a silica support and magnetite (Fe_3O_4) is thermodynamically favored, while the adsorption of proteins and single stranded RNA is not [15, 16].

The current DNA extraction method was tested for its efficiency of DNA extraction and ease of use compared with a commercially available kit. The yield of DNA extracted using magnetic nanoparticles was on average 1.3-fold greater than that using the Qiagen method. Moreover,

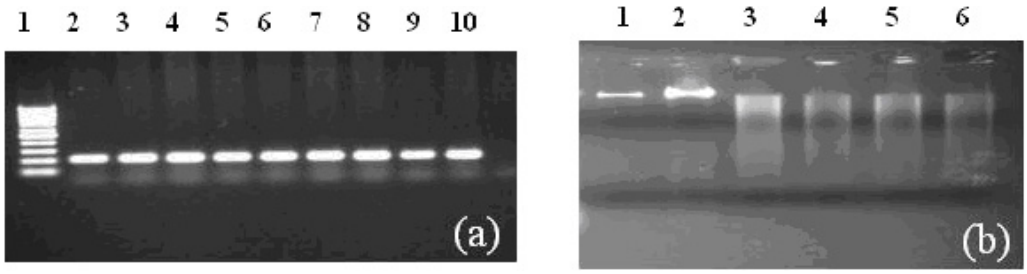

Figure 2. (a) Agarose gel electrophoresis of 226 bp amplicon of the GAPDH gene. Lanes: 1 = DNA molecular weight marker (100 bp ladder); 2–4 = PCR product from genomic DNA isolated from human blood using magnetic particles; 5–6 = PCR product from genomic DNA isolated from cultured cells (HCT116) using magnetic particles; 7–8 = PCR product from genomic DNA isolated from human blood using Qiagen kit; 9–10 = PCR product from genomic DNA isolated from cultured cells (HCT 116) using Qiagen kit. (b) Restriction analysis of extracted DNA. Lanes: 1–2 = undigested genomic DNA isolated using magnetic nanoparticles from human blood and cultured cells (HCT116), respectively; 3 and 5 = *Hind* III digested genomic DNA isolated from human blood using magnetic nanoparticles; 4 and 6 = *Eco R*1 digested genomic DNA isolated from cultured cells using magnetic nanoparticles.

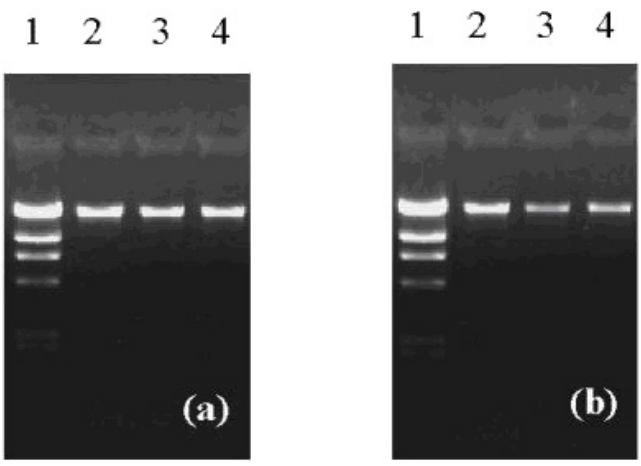

Figure 3. (a) Agarose gel electrophoresis of genomic DNA isolated from human blood cells using magnetic nanoparticles. Lanes: 1 = DNA molecular weight marker (λ phage DNA/*Hind* III digest); 2–4 = genomic DNA (23 kb) isolated from human blood cells (equal volume was loaded from the tube containing the isolated DNA). (b) Agarose gel electrophoresis of extracted DNA eluted from agarose gel. Lanes: 1 = DNA molecular weight marker (λ phage DNA/*Hind* III digest); 2 = DNA eluted with magnetic nanoparticles as solid-phase adsorbent; 3 = DNA eluted using the phenol-extraction method; Lane 4: DNA eluted by the glass wool spin-column method.

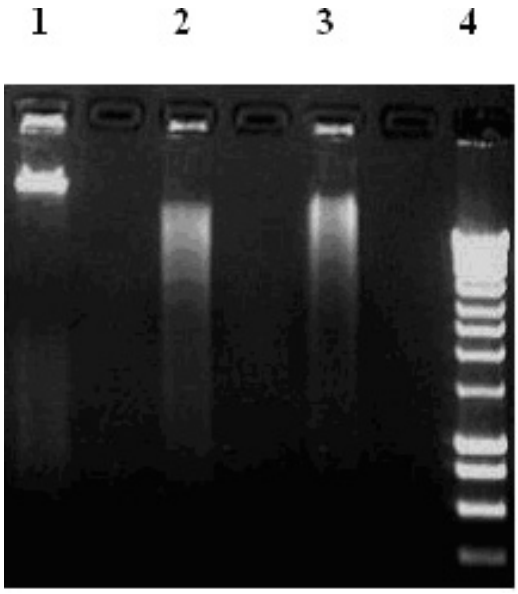

Figure 4. Restriction analysis of DNA extracted from agarose gel. Lanes: 1 = undigested genomic DNA; 2 = *BamH*1 digested genomic DNA extracted from agarose gel using magnetic nanoparticles; 3 = *Hind* III digested genomic DNA extracted from agarose gel using magnetic nanoparticles; 4 = DNA molecular weight marker (1 kb DNA ladder).

the magnetic DNA isolation procedure can be carried out in a single microcentrifuge tube per sample, whereas the Qiagen procedure requires a number of tube transfers.

3.2. Elution of DNA from agarose gel

The yield of DNA was quantified after electrophoresis in 0.8% agarose gel containing 0.5 μg ml^{-1} ethidium bromide, visualized by a UV transilluminator using a gel documentation system (UVP Bioimaging Systems, Cambridge, UK). As observed from figure 3(b), the yield of recovered DNA from agarose gel using magnetic nanoparticles as solid support was on average 80% (80 ± 5%), whereas that obtained with phenol-extraction and the spin-column method was in the range of 50–60%. The DNA yield obtained with the current magnetic method (⩾80%) is similar to that reported by Hawkins and co-workers; where an excess of carboxyl coated magnetic microparticles was used to elute DNA from agarose gels [17]. In order to check the robustness and reproducibility of the current method, DNA fragments were eluted in triplicate and the results were found to be consistent. The yield of recovered DNA of size 23 kb was more than 80%, indicating consistency and applicability of this method. The higher yield with the current method is probably attributed to the nanosize of the magnetic particles and optimum conditions for DNA binding [10].

The isolated DNA was also checked for its compatibility for restriction endonuclease digestion. The results showed a successful restriction digestion (figure 4) indicating the absence of enzyme inhibitors and that the purified DNA can be used for downstream applications.

In summary, the developed procedure for DNA extraction has several advantages. It is quick, inexpensive, robust, and does not require use of organic solvents or centrifuge. Also, the method needs only a magnet and a heating block and can be performed in any laboratory without the requirement of sophisticated instruments. The procedure yields enough DNA for 30 PCR reactions from small (30 μl) quantities of biological specimens in less than 15 min. Furthermore the whole procedure can be accomplished in a single tube, thereby making it more amenable to automation.

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