Magnetic Nanoparticles-Based Extraction and Verification of Nucleic Acids from Different Sources

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In many molecule biology and genetic technology studies, the amount of available DNA can be one of the important criteria for selecting the samples from different sources. Compared with those genomic DNA methods using organic solvents or other traditional commercial kits, the method based on magnetic nanoparticles (MNPs) and adsorption technology has many remarkable advantages like being time-saving and cost effective without the laborious centrifugation or precipitation steps, and more importantly it has the great potential and especially suitable for automated DNA extraction and up-scaling. In this paper, the extraction efficiency of genomic nucleic acids based on magnetic nanoparticles from four different sources including bacteria, yeast, human blood and virus samples are compared and verified. After measurement and verification of the extracted genomic nucleic acids, it was shown that all these genomic nucleic acids extracted using the MNPs method can be of high yield and be available for next molecule biological steps.

KEYWORDS: Magnetic Nanoparticles, Extraction, Nucleic Acids, PCR Verification, Different Sources.

INTRODUCTION

With the growing interest in the genomic characteristics of various diseases and a steep increase in the availability of genomic tests for both clinical and research purposes, the amount of genomic DNA available from biological samples may limit the practicality of genomic analysis.1-4 And what’s more, the process of purification and enrichment of biological samples such as DNA/RNA extraction is crucial for the detection and analysis in the biomedi-cal applications.5-6 There have been various techniques for the extraction and purification of entire genomes that have been developed to avoid the short of genomic DNA samples. Among these extraction methods of nucleic acids, the idea of using magnetic separation techniques to purify biologically active compounds (nucleic acids and proteins) from the cells and cell organelles attracts a fast growing interest over recent years.7-11 Using the pH-sensitive carboxyl-modified magnetic nanoparticles, both cell captures and the subsequent removal of genomic DNA/protein complex after the lysis can be achieved simply by magnetic separation.12-14 More importantly, specifically functionalized magnetic particles were greatly developed for meeting great need of automated DNA extraction and up-scaling application in the near future.15-16 In this paper, using the extraction method of nucleic acids bound to magnetic nanoparticles high yield and relative high purity of respective genomic nucleic acids from four different sources (bacteria, yeast, human blood and virus) were successfully gained, and then were separately verified by PCR method and restriction endonuclease to determine the feasibility of the extraction based on magnetic nanoparticles from different kinds of species.
MATERIALS AND METHODS

Materials

Most chemicals, including ferric chloride hexahydrate (FeCl₃ · 6H₂O), anhydrous ammonium acetate (NH₄Ac), ethylene glycol (EG), ethanol tetraethyl orthosilicate (TEOS) and ammonia were purchased from Nanjing Chemicals (Nanjing, China). All chemicals used in the preparation of Fe₃O₄@SiO₂ microspheres were of analytical grade. All primers were synthesized by Sangon Biotech (Shanghai PRC) Co., Ltd. and Taq DNA polymerase, dATPs, dGTPs, dCTPs, dTTPs, Tris, SDS were also purchased from Sangon Biotech (Shanghai PRC) Co., Ltd. Water used in the experiments was deionized (DI) water.

Sources of Strains and Samples and Their Relative Pretreatment

Both the strain Escherichia coli JM109 and the yeast were bought from Takara Biotechnology Co., Ltd. (Dalian PRC). The E. coli strain was grown in Luria-Bertani medium and prepared by shaking culture at the speed of 200 rpm and 37 °C for 16–20 h. The yeast was grown in YPD (Yeast Peptone Dextros) medium and prepared by shaking culture at the speed of 200 rpm and 28 °C for 18–24 h.

Whole blood (WB) samples and HBV (hepatitis B virus) serum samples were supplied from Second Hospital of Nanjing (Jiangsu province, PRC) after strict pre-treatment procedures.

Preparation of Fe₃O₄@SiO₂ Nanoparticles

The spherical magnetic Fe₃O₄ particles were prepared by hydrothermal method. Typically, 5 mmol FeCl₃ · 6H₂O was dissolved in EG (50 mL) to form a stable orange solution, then 50 mmol NH₄Ac and appropriate amount of the surfactant (R) were added respectively into the above solution under vigorous magnetic stirring until completely dissolved. The mixture was transferred to a Teflon-lined stainless-steel autoclave (50 mL) and sealed to heat at 200 °C for 12 h, followed by cooling to room temperature naturally. The obtained Fe₃O₄ nanoparticles were collected from the solution using a permanent magnet and washed with deionized water for several times. The Fe₃O₄ nanoparticles were dispersed in a mixture of deionized water and anhydrous ethanol (the volume ratio of deionized water and anhydrous ethanol is 1:4). After ultrasonic mixing for about 5 min, the mixture was transferred into a three-neck flask. Next, 2.5 mL of TEOS was added into the Fe₃O₄ nanoparticles solution in batches under vigorous stirring in N₂ ambient. After one and a half hours, 3 mL of ammonia was added slowly and continuously with violent stirring for 3 h. Finally the obtained magnetic product was collected by magnetic separation and washed with deionized water and ethanol for several times till the supernatant was clear completely. The size and morphology of the particles were observed with transmission electronic microscopy (TEM).

Extraction and Measurement of Genomic Nucleic Acids Based on MNPs

Shown in Figure 1 is the extraction process of genomic nucleic acids based on MNPs. In sum, the whole process consists of five major steps: lysis, MNPs-nucleic acids binding, washing, elution and collection. In a typical extraction, for 200 μL of sample (E.coli culture, yeast culture, whole blood, or virus serum), 200 μL of 6 M guanidine hydrochloride lysis solution and 10 μL of Proteinase K (20 mg/mL) was added. The tube was shaken by gentle inversion for two or three times and incubated at room temperature for a minute. After incubation, 200 μL of magnetic nanoparticles (8 mg/mL) were added to the cell lysate, followed by addition of 600 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 keep for 3 min at room temperature. The magnetic pellet was immobilized by application of an external magnet and the supernatant was removed. The magnetic pellet was washed with 70% ethanol twice and then dried thoroughly. The pellet was then completely re-suspended in 200 μL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and magnetic particle bound DNAs were eluted by incubation at 65 °C with continuous agitation. Finally, the supernatant containing the DNA was transferred to a fresh tube. The quality of the DNA was qualitatively analyzed with agarose gel electrophoresis. Then the yield and purification efficiency of DNA samples were measured at UV absorbance by using OneDrop™ OD-1000 Micro Determinator (Gene Company Ltd., USA) according to the instructions by the manufacturer.

Verification of Extracted DNA from Different Sources Based on MNPs by PCR

16S rDNA Gene PCR Amplification for Extracted Genomic DNA from E.coli JM109

All PCRs were performed in a 50 μL reaction volume; 25 μL of PCR 2× master mix (Genetix, USA) was added. Five picomoles each of primers 16S rDNA F27 (5-AGAGTTTGATCMTGGCTCAG-3) and 16S rDNA R1492 (5-TAGGYTACCTTGTTACGACT-3) were added per reaction for the amplification of an amplicon. 100 ng of template DNA was used in the reaction mixture; PCR was performed on a GeneAmp® PCR System 9700 thermal cycler (ABI, USA). PCR conditions were 4 min at 94 °C; 35 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 (EB) and stored at 4 °C.
The universal primers of fungal amplification used for Extracted Genome DNA from Yeast

18S rDNA Gene PCR Amplification for Extracted Genome DNA from Yeast

The universal primers of fungal amplification used for the yeast were as follows: FP (Forward primer): ITS4 5’-TCCTCCGCTATTGATATGC3’ RP (Reverse primer): ITS86 5’-GTGAATCATCGAATCTTTGAAAC-3’.25–27 The 50-μL of PCR mixture contained 10 μL of DNA template, 6 μL of 25 mM MgCl2, 5 μL of PCR buffer without MgCl2; 200 mM each deoxynucleoside triphosphate, 25 pmol of each primer, and 1 U of Taq DNA polymerase. Reactions involved 1 cycle at 95 °C for 5 min, followed by 35 cycles with a denaturation step at 95 °C for 30 s, an annealing step at 55 °C for 1 min, and an extension step at 72 °C for 1 min, followed by 1 cycle at 72 °C for 6 min. PCR was performed on a GeneAmp® PCR System 9700 thermal cycler (ABI, USA). The PCR products were analyzed on 1.5% agarose gel stained with ethidium bromide and stored at 4 °C.

PCR Amplification for GADPH Gene of Extracted Genomic DNA from Whole Blood

All PCRs were performed in a 50 μL reaction volume; 25 μL of PCR 2× master mix (Genetix, USA) was added. Five picomoles of each of primers GADPH F (5′- CGGAGTCAACGGATTTGGTGTATAT-3′) and R (5′- AGCCTTCCATGGTGGTAGAGC -3′) were added per reaction for amplification of an amplicon in the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene.28 100 ng of template DNA was used in the reaction mixture; PCR was performed on a GeneAmp® PCR System 9700 thermal cycler (ABI, USA). PCR conditions were 4 min at 94 °C; 34 cycles of 30 s at 94 °C, 40 s at 55 °C, and 50 s at 72 °C followed by 10 min at 72 °C. The PCR products were analyzed on 1.5% agarose gel stained with ethidium bromide and stored at 4 °C.

PCR Experiment for Extracted HBV DNA

The primers used for HBV amplification were as follows: HBV-FP (Forward primer 1549-1569): 5′-CCGTCTGTGCTTCTCATCTG-3’, HBV-RP (Reverse primer 1641-1669): 5′-AGTCCAAGAGTTCTCTATGYA AGACCTT-3’.29–30 The 50-μL PCR mixture contained 10 μL of genomic DNA template, 6 μL of 25 mM MgCl2, 5 μL of PCR buffer without MgCl2; 200 mM each deoxynucleoside triphosphate, 25 pmol of each primer, and 1 U of Taq DNA polymerase. Reactions involved 1 cycle at 95 °C for 5 min, followed by 35 cycles with a denaturation step at 95 °C for 40 s, an annealing step at 62 °C for 1 min, and an extension step at 72 °C for 1 min, followed by 1 cycle at 72 °C for 6 min. The PCR products were analyzed on 1.5% agarose gel stained with ethidium bromide and stored at 4 °C.

Restriction Enzymatic Verification Analysis of Genomic Nucleic Acids

An analytical scale restriction enzyme digest is performed in a volume of 20 μL on 0.2–1.5 μg of substrate DNA, using a two- to ten fold excess of enzyme over DNA. In a new sterile tube, the components were assembled in order as follows: 2 μL of RE (Restriction endonuclease) 10× Buffer; 0.2 μL of acetylated BSA (10 μg/μL), 1 μL of template DNA (1 μg/μL), and then adding 0.5 μL of EcoR I (10 U/μL) and 16.3 μL of sterile deionized water to 20 μL of final volume. All the above reagents were mixed gently by pipetting, and then the tube was closed and centrifuged for a few seconds. Incubation was conducted at the optimum temperature for 3 hours. All genomic nucleic acids were analyzed on a 0.8% agarose gel stained with ethidium bromide (EB). The condition of gel electrophoresis was as followed: 100 V, 1 h.

RESULTS AND DISCUSSION

TEM Analysis of SiO2/Fe3O4

The surface morphology and particle size of the obtained SiO2/Fe3O4 microspheres were studied by TEM as shown in Figure 2. It was clearly shown that the resulting core–shell structure MNPs coated with silica were controlled at about 500–600 nm with uniform morphology and possessed better disperse character. So the prepared
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Figure 2. TEM images of Fe$_3$O$_4$@SiO$_2$ nanoparticles used for the extraction. It was clearly shown that the prepared core–shell structure MNPs coated with silica were controlled at about 500–600 nm with uniform morphology and better dispersion.

SiO$_2$/Fe$_3$O$_4$ microspheres would be applied in the further extraction.

Extraction of Genomic DNA from Different Sources

The concentrations and purities of all the extraction treatments from four separated sources were calculated from measuring OD (optical density) values of ultraviolet adsorption. The measurement results were shown in Table I. From Table I, it was indicated that from the same initial volume, the yield orders of the extracted genomic DNA from four sources were as follows: E.coli > whole blood > yeast > HBV. The phenomenon was explained that the cells of the first two species possessed a higher nucleic acids content and were easily lysed by the lysis buffer. Meanwhile, the yeast was difficult to be crushed because it contains a thicker cell wall around the cells and the virus content is generally less in the serum. Moreover, the spectrophotometric 260:280 ratios of nucleic acids were respectively as follows: the purity of genomic DNAs were approximately 1.65–1.71 for E.coli, 1.60–1.70 for yeast, 1.45–1.55 for whole blood and 1.25–1.35 for HBV. It indicated that the obtained nucleic acids extracts have a certain high purities and low protein impurities.

Table I. Results of nucleic acids extraction from different sources.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>A260</th>
<th>A280</th>
<th>Purity A260/A280</th>
<th>Concentration (ng/μL)</th>
<th>Nucleic acids yields (μg)</th>
<th>Extraction efficiency (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (E.coli)</td>
<td>0.362</td>
<td>0.212</td>
<td>1.71</td>
<td>18.10</td>
<td>1.81</td>
<td>18.10</td>
</tr>
<tr>
<td>2 (E.coli)</td>
<td>0.339</td>
<td>0.206</td>
<td>1.65</td>
<td>16.95</td>
<td>1.695</td>
<td>16.95</td>
</tr>
<tr>
<td>3 (Yeast)</td>
<td>0.190</td>
<td>0.112</td>
<td>1.69</td>
<td>9.49</td>
<td>0.949</td>
<td>0.95</td>
</tr>
<tr>
<td>4 (Yeast)</td>
<td>0.192</td>
<td>0.147</td>
<td>1.60</td>
<td>9.58</td>
<td>0.958</td>
<td>0.96</td>
</tr>
<tr>
<td>5 (WB)</td>
<td>0.252</td>
<td>0.163</td>
<td>1.55</td>
<td>12.58</td>
<td>1.258</td>
<td>1.26</td>
</tr>
<tr>
<td>6 (HBV)</td>
<td>0.271</td>
<td>0.183</td>
<td>1.48</td>
<td>13.53</td>
<td>1.353</td>
<td>1.36</td>
</tr>
<tr>
<td>7 (HBV)</td>
<td>0.147</td>
<td>0.111</td>
<td>1.33</td>
<td>7.35</td>
<td>0.735</td>
<td>0.74</td>
</tr>
<tr>
<td>8 (HBV)</td>
<td>0.129</td>
<td>0.102</td>
<td>1.26</td>
<td>6.43</td>
<td>0.643</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Notes: A260 means absorption value at 260 nm and A280 means absorption value at 280 nm. Extraction Efficiency is the ratio of nucleic acids yields to the initial sample volume.

Soon afterwards, a qualitative analysis of the obtained nucleic acids extracts were carried out by gel electrophoresis. The result of separated samples was shown in Figure 3 where all genomic nucleic acids have respectively a single bright band. Extracted from E.coli, yeast and whole blood samples, all genomic nucleic acids lengths are near to 12–16 kb yet as for HBV samples, the virus DNA length is about 3 kb consistent with the actual sequence of HBV. In conclusion extracted nucleic acids based on MNPs method from four different sources are all suitable for their corresponding targeted lengths.

Therefore, based on the above quantitative and qualitative analysis of obtained nucleic acids extracts, it is shown that magnetic nanoparticles coated with silane compounds exhibited a great binding efficiency with nucleic acids and the proposed DNA extraction method is widely applicable for a variety of different species samples. It was also demonstrated that the yield and extraction efficiency of genomic DNA was quite better from four different sources.

PCR Verification Experiments of Extracted DNA Samples Based on MNPs from Different Sources

In order to further confirm the extracted nucleic acids from different sources based on MNPs method can be applied
in the following molecular biology experiments or not, the obtained DNA samples are applied then as the templates of PCR verification experiments. Here one point should be necessarily mentioned that selective primers from four different sources for PCR are quite different because different species have their own genomic nucleic acids components. So 16S rDNA gene primer pairs are amplified for E.coli, GADPH gene primer pairs are amplified for whole blood 18S rDNA gene primer pairs are amplified for yeast, HBV gene primer pairs encoding the X protein and DNA polymerase are amplified for the verification of HBV. All the gDNA template was diluted to suitable concentration (10 ng/μL), so as to the same starting template for verification PCR experiments. Detection results of agarose gel electrophoresis of the PCR products were shown in Figure 4.

It is shown in Figure 4(A) that bright band of E.coli PCR products were clearly found at the size of about 1500 bp, indicating that we have successfully gotten the amplified PCR products containing the 16S rDNA gene fragments. At the same time, from Figure 4(B), it can be obviously observed that amplification products yields are very high and specific, and the PCR amplification band is about 350 bp amplified PCR products containing the 18S rDNA gene fragments. Figure 4(C) shows the agarose gel electrophoresis image of PCR products for whole blood. Amplification fragments were clearly presented at about 250 bp in length, which indicated that the amplified PCR products containing GADPH gene fragments was obtained. Finally, from Figure 4(D), it is obviously found that the yields of amplification products are high and specific for HBV, and the PCR amplification band is about 100 bp in length. After the above verification of PCR experiments, it is sure that those products from different sources using the MNPs extraction method can be all available for the basic molecular biology research.

**Restriction Enzyme Digestion Analysis of Genomic Nucleic Acids**

Genomic nucleic acids are further verified and analyzed by restriction enzyme digestion. The results were shown in Figure 5. It was confirmed by restriction enzyme digestion analysis that genomic nucleic acids from MNPs extraction method were successfully applied for restriction enzyme reaction regardless of different biological sources.

**CONCLUSIONS**

We analyzed and compared genomic DNA extraction based on magnetic nanoparticles from E.coli JM109, yeast, whole blood and serum respectively.31–32 After further verification and analysis of both PCR and enzyme digestion experiments, it is sure that those genomic nucleic acids extracted with the MNPs extraction method from different species can be applicable for the basic molecule biology research.
Although the extraction efficiency of nucleic acids is different from different species, it provides a general path for nucleic acid extraction of multi-species. In fact, multifunctional MNPs proved to be a time and cost-effective DNA preparation technique tool independent of centrifugation and hazardous organic solvents. Not only are the pH-sensitive magnetic nanoparticles well suited for routine laboratory use, but also the simplicity of this approach indicates their potential for automated DNA purification in bioseparation, bioanalysis and biomedical detection. In order to extend the application scopes of the MNPs in bioseparation, bioanalysis and biomedical detection, any nucleic acids extraction from the plants and at the same time check the suitability for automated magnetic bead separation instruments and automated workstation. Anyway, since magnetic particles are finding more and more application in biomedical scope, the present will make way, since magnetic nanoparticles are finding more and more separation instruments and automated workstation. Therefore, we can apply the MNPs extraction method into nucleic acids extraction from the plants and at the same time check the suitability for automated magnetic bead separation instruments and automated workstation. Anyway, since magnetic particles are finding more and more application in biomedical scope, the present will make way.

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