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Application of superparamagnetic nanoparticles in purification of plasmid DNA from bacterial cells

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Abstract

The aim of this study was to develop a simple and rapid method for purification of ultrapure supercoiled plasmid DNA with high yields from bacterial cultures. Nanosized superparamagnetic nanoparticles (Fe_3O_4) were prepared by chemical precipitation method using Fe^{2+}, Fe^{3+} salt, and ammonium hydroxide under a nitrogen atmosphere. The surface of Fe_3O_4 nanoparticles was modified by coating with the multivalent cationic agent, polyethylenimine (PEI). The nanoparticles were characterized by transmission electron microscopy, X-ray diffraction, Fourier transformation infrared spectroscopy and superconducting quantum interference device magnetometer. The PEI-modified magnetic nanobeads were employed to simplify the purification of plasmid DNA from bacterial cells. We demonstrated a useful plasmid, pRSETB-EGFP, encoding the green fluorescent protein with T7 promoter, was amplified in DE3 strain of *Escherichia coli*. The loaded nanobeads are recovered by magnetically driven separation and regenerated by exposure to the elution buffer with optimal ionic strength (1.25 M) and pH (9.0). Up to approximately 35 μg of high-purity (A_{260}/A_{280} ratio = 1.87) plasmid DNA was isolated from 3 ml of overnight bacterial culture. EGFP expression was detected by fluorescent microscopy in the transformed *E. coli* cells, indicating the biological activities of DNA fragments were retained after purified from magnetic nanobeads. The protocol, starting from the preparation of bacterial lysate and ending with purified plasmids takes less than 10 min. Thus, the separation and purification qualities of PEI-modified magnetic nanobeads as well as its ease of use surpass those of conventional anion-exchange resins.

Keywords: Plasmid DNA; Purification; Superparamagnetic nanoparticles; PEI

1. Introduction

The need for quick bacterial plasmid DNA preparation methods, free of protein, RNA, salts, and enzyme inhibitors, has increased with the flood of molecular protocols requiring highly purified genetic template [1–3]. Most current methods of plasmid separation are relatively time-consuming and require the use of adsorbents, toxic substances, nucleases, and/or filtration media to separate plasmid from protein, genomic DNA. Several techniques that yield highly purified plasmid DNA in a relatively short time are molecular-exclusion, reversed-phase, hydrophobic interaction, and anion-exchange chromatography [4–10]. Most chromatographic separation processes currently used are limited in terms of speed and generation of waste chemicals. The low capacity of anion-exchange chromatographic sorbents is another pertinent issue in plasmid DNA purification. Thus, the design and development of new matrices with higher binding capacities is one of the most important developments in the area of plasmid processing.

In the recent years, the nanosized magnetic particles receive increasing attention with rapid development of nanostructured materials and nanotechnology in the fields of biotechnology and medicine [11–14]. Due to strong magnetic property and low toxicity, its applications in biotechnology

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and medicine have gained significant attention. It is known that magnetic particles less than about 30 nm will exhibit superparamagnetism. Strictly speaking, the particles are not magnetic but superparamagnetic, meaning they are only magnetic in a magnetic field. This property permits the particles to be redispersed without magnetic aggregate formation [15–17]. Hence, the particles may be reused or recycled. This characteristic prevents clumping and allows for easy dispersion of the particles. Microbial cell lysates are relatively viscous and may present problems in a column operation. On the other hand the magnetic particles respond very rapidly to a magnetic field, typically being adsorbed from a suspension in less than 10 s, leaving a clear supernatant, which can readily be removed by decantation. A further advantage of using magnetic particles for bioseparation applications is their utility with variously-sized starting samples [18–22]. With most commercially available DNA purification systems, the amount of starting material is limited by the fixed size of the purification matrix within plastic columns or centrifugation baskets. Moreover, the magnetically based procedure may present the additional advantage of being suitable for automation systems, minimizing manual labor and time while providing more reproducible results and being useful for large-scale analysis.

As we known, DNA is a polyanionic molecule due to the presence of phosphate groups on the nucleic acid backbone and is therefore conveniently captured on a bead derivatised with positively charged functional groups. For this purpose, a protocol based a modified lysis procedure followed by the specific binding of plasmids to the superparamagnetic nanoparticles with positively charged functional groups was developed. The surface of Fe_3O_4 nanoparticles was modified by coating with multivalent cationic agent, polyethylenimine (PEI). The PEI-modified magnetic nanobeads purification technique is based on the interaction between negatively charged phosphates of DNA-backbone and positively charged PEI groups on the surface of the magnetic nanoparticles. A permanent magnet with a surface magnetization of 2000 G is used to capture the magnetic nanoparticles with bound plasmids, and to carry out a subsequent wash to remove contaminants. Finally, plasmid DNA is eluted from the particles using elution buffer, and is ready for use in downstream applications. The technique does not require any organic solvents and eliminates the need for the repeated centrifugation, vacuum filtration or column separation. In principle, this technique works like other purification systems, but add advantages of increased speed, flexibility and binding capacity.

In this paper, we investigate the suitability of a magnetically driven separation process utilizing PEI-modified magnetic nanobeads for the isolation of plasmid DNA from a bacterial cell lysate. We demonstrated a useful plasmid, pRSETB-EGFP, encoding the green fluorescent protein with T7 promoter, was amplified in DE3 strain of *Escherichia coli*. The salt concentration and pH of the elution buffer used determine whether plasmid DNA is bound or eluted from the magnetic particles. Therefore, the resolution was achieved through careful selection of elution buffer. The yield and quality of purified plasmid DNA was investigated for further downstream application and compared to other currently commercial column devices.

2. Experimental

2.1. Materials

Ferric chlorides, 6-hydrate was purchased from J.T. Baker (Phillipsburg, NJ, USA). Ferrous chloride tetrahydrate was purchased from Fluka (Buchs, Switzerland). PEI and lauric acid were supplied from Sigma Chemical Co. (St. Louis, MO, USA). Agarose L (low electroendosmois) was from Amersham Biosciences (Uppsala, Sweden). Supercoiled DNA ladder was obtained from Invitrogen Co. (Carlsbad, CA, USA). Reagents for use in DNA isolation and analysis were of molecular biology grade. Ribonuclease A was obtained from Sigma. All other chemicals and solvents used were of analytical grade and used without further purification. The water used throughout this work was the reagent-grade water produced by Milli-Q SP Ultra-Pure-Water Purification System of Nihon Millipore Ltd. (Tokyo, Japan). All solutions were freshly prepared.

2.2. Methods

2.2.1. Plasmid construction

An intact enhanced green fluorescent gene (EGFP) was amplified from pEGFP-N1 (BD Bioscience, Franklin Lakes, NJ, USA) using polymerase chain reaction. Upstream and downstream primers (5′-cgggatccACCGGTCGCCAC-3′ and 5′-cggaattcGCGGCCGCTTTACTTGTACAGC-3′) that were engineered to contain *Bam*HI and *Eco*RI cutting sites at their 5′-end, respectively were located at the start and stop codons of EGFP gene, respectively. PCRs were performed in 100 μl volume using a minicycler™ (MJ Research). Each reaction contained 50 mM KCl, 10 mM Tris–HCl pH8.3, 1.5 mM $MgCl_2$, 0.001% gelatin (w/v), 5.0 pmol of each primer, 200 μM of each deoxyribonucleoside triphosphate (dNTP), 2.5 U Ex Tag (Takara) and 0.1 μg pEGFP-N1. Cycling conditions were 20 cycles of 95 °C for 1 min; 60 °C for 1 min and 72 °C for 1 min.

Amplified PCR fragments were linearized with *Bam*HI and *Eco*RI and then purified from the 1% agarose gel using GFX™ PCR DNA and Gel Band Purification Kit (Amersham Bioscience) following the manufacture's protocol. Restricted PCR fragments were ligated with the *Bam*HI/*Eco*RI-digested pRSET B (Invitrogen Life Technologies Ltd.) in frame with the $(His)_6$ tag driven under the T7 promoter to result in the pRSET-EGFP.

2.2.2. Bacteria transformation

BL21 (DE3) *E. coli* (Invitrogen Life Technologies Ltd.) containing a T7 RNA polymerase gene under the control of

IPTG-induced Lac promoter was thawed on ice and then 1 ng of pRSET-EGFP was gently mixed with *E. coli*. After incubation on ice for 30 min, *E. coli* was incubated at 37 °C for 30 s and then kept on ice for 2 min. After heat shock, *E. coli* was cultured in LB broth for 1 h for recovery. Then 100 μl *E. coli* suspension was spread on the LB agar containing 60 μg/ml ampicillin and incubated at 37 °C overnight for the colony formation.

2.2.3. Preparation of bacterial lysates

Bacterial were lysed using a modification of the alkaline method described by Birnboim and Doly [22]. *E. coli* DE3 cells expressing the plasmid pRSETB-EGFP were grown to late log phase in Luira-Bertani broth containing 100 μg/ml ampicillin. The bacterial cells were harvested from 3 ml of cell culture by centrifugation at 3000 rpm for 3 min. The pellet was re-suspended in 0.05 M Tris–HCl buffer, pH 8.0, containing 0.01 M EDTA and 100 μg/ml ribonuclease A (250 μl). Cell lysis was performed by gently mixing the re-suspended cell pellet with 0.2 M NaOH containing 1% (w/v) sodium dodecyl sulfate (250 μl). Genomic DNA and other contaminants were precipitated by addition of 3 M potassium acetate, pH 5.5 (250 μl). The mixture was centrifuged at $10000 \times g$ for 2 min to sediment the precipitated protein, cell debris and denatured chromosomal DNA.

2.2.4. Preparation of PEI-modified magnetic nanobeads

The magnetic nanoparticles Fe_3O_4 were prepared by co-precipitating Fe^{2+} and Fe^{3+} ions in an ammonia solution and treating under hydrothermal conditions. Milli-Q water was re-deionized and deoxygenated by bubbling N_2 gas for 1 h prior to the use. Stock solutions of 1.28 M $FeCl_3 \cdot 6H_2O$ and 0.64 M $FeCl_2 \cdot 4H_2O$ were prepared as a source of iron by dissolving the respective chemicals in Milli-Q water under vigorous stirring. In the same way, stock solutions of 1.0 M NaOH were prepared as alkali sources. A solution of 1.5 M lauric acid at pH 10 was prepared for coating. The imines compound PEI was covalently bound to naked magnetite after hydrolysis of 8% PEI in 50% methanol at 80 °C. The mixture was stirred vigorously for 1 h under a nitrogen stream. The magnetic particles were lightly dispersed using an ultrasonic bath, rinsed successively with acetone, ethanol and deionized water to remove the residual surfactant and unreacted reagents. Finally, the coated superparamagnetic nanoparticles were dispersed into 50 mM Tris buffer (pH 8.5) and stored at 4 °C.

2.2.5. Characterization of magnetic particles

The size and morphology of magnetic nanoparticles were observed by transmission electron microscopy (TEM) using Philips (Eindhoven, The Netherlands) Tecnai G2 F20 FEG-TEM at 200 kV. X-ray diffraction (XRD) measurement was performed on a Rigaku (Texas, USA) D/max III.V X-ray diffractometer using Cu Kα radiation ($\lambda = 0.1542$ nm). The magnetic measurement was done using a superconducting quantum interference device (SQUID) Quantum Design (San Diego, CA, USA) MPMS7 magnetometer. The zeta potentials of PEI-Fe_3O_4 magnetic particles at different pH and salt concentration were measured using a Malvern (Worcs, UK) Brookhaven Zetasizer 3000 analyzer. The coating of PEI to the magnetic nanoparticles was checked using a Perkin-Elmer (Norwalk, CT, USA) Fourier transformation infrared spectroscopy (FTIR) spectrometer Spectrum GX.

2.2.6. Purification of plasmid DNA by PEI-modified magnetic nanobeads

The supernatant of cleared alkaline lysate was placed in a 1.5 ml DNase/RNase free microcentrifuge tube and a 1% (w/v) suspension of PEI-modified magnetic nanobeads in binding buffer (100 μl, 1 M NaCl, 0.05 M Tris–HCl buffer, pH 7.5). The suspension was gently mixed for 2 min at room temperature. The beads were immobilized using a permanent magnet with a surface magnetization of 2000 G and the supernatant removed. The beads were washed by re-suspension in washing buffer (100 μl, 0.05 M Tris–HCl buffer, pH 8.0). After immobilization of the beads, the supernatant was discarded and the plasmid DNA desorbed by addition of elution buffer (100 μl). The beads were immobilized and the supernatant transferred to a fresh DNase/RNase free microcentrifuge tube. The concentration of nucleic acids in solution can be readily calculated from absorbance at 260 nm.

2.2.7. Agarose gel electrophoresis

A 0.8% agarose gel containing 0.5 μg/ml ethidium bromide was run in a Bio-Rad (Hercules, CA, USA) horizontal gel electrophoresis unit and 1 kb molecular mass standards (Amersham Biosciences) were used. The running buffer was TAE (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8.0). Electrophoresis was carried out at 100 V for 40 min. Gel electrophoresis experiments were carried out for plasmid DNA in four different samples, feed solution, supernatants after adsorption, wash, and desorption experiments.

2.2.8. Effects of salt concentration and pH of elution buffer on desorption of plasmid DNA

Effects of salt concentration and pH of elution buffer on desorption of plasmid DNA from PEI-modified magnetic nanobeads was evaluated in the range of NaCl concentration between 0–2.0 M and pH 7.0–9.0, respectively.

2.2.9. Determination of the optimal amount of PEI-Fe_3O_4 magnetic nanobeads

In order to determine the optimal amount of PEI-modified magnetic nanobeads has to add into 3 ml of bacterial culture. Several amounts of PEI-modified magnetic nanobeads were studied at ranges of 100–500 μg. The yields of isolated plasmid DNA were determined by UV spectrophotometry at 260 nm, or preferably, comparison of intensity of DNA bands in ethidium bromide stained agarose gels. The 260/280 nm

absorption ratios of all samples of isolated plasmid DNA were calculated as a first measure of purity.

2.2.10. Restriction endonuclease digestion

A 20 μl volume of the eluted DNA solution was mixed with the manufacturer's reaction buffer (3 μl), sterile water (6 μl) and incubated with the restriction endonucleases (*Bam*HI and *Eco*RI, 1 μl, 10 units) at 37 °C for 18 h. The digestion mixture was analyzed directly by agarose gel electrophoresis.

3. Results and discussion

3.1. Characterization of magnetic particles

Size and morphology of magnetic particles were characterized by TEM. A typical TEM micrograph of magnetic particles is shown in Fig. 1. It shows that the size of magnetic particles is about 11 nm. The XRD patterns characteristic six peaks for Fe_3O_4 (2θ = 30.1, 35.5, 43.1, 53.4, 57.0 and 62.6°), marked by their indices ((2 2 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1) and (4 4 0)), were observed for magnetic nanoparticles. These peaks are in agreement with those in JCPDS file (PCPDFFWIN v.2.02, PDF No. 85–1436) and reveal that the resultant particles were pure Fe_3O_4 with a spinel structure. It is known that magnetic particles less than about 30 nm will exhibit superparamagnetism. The superparamagnetic properties of the magnetic particles were verified by the magnetization curve measured by SQUID. A typical plot of magnetization versus applied magnetic field (M–H loop) at 298 K is shown in Fig. 2. The saturation magnetization of the obtained Fe_3O_4 magnetic particles is 64 emu/g Fe_3O_4. This large saturation magnetization of magnetic particles makes them very susceptible to magnetic fields, and therefore makes the solid and liquid phases separate easily. The very weak hysteresis revealed the resultant magnetic nanoparticles were nearly superparamagnetic. According to the results of FTIR spectra (data not shown), the peak at 1648 cm^{-1} is characteristic absorption band of imines (–C=N–) from PEI.

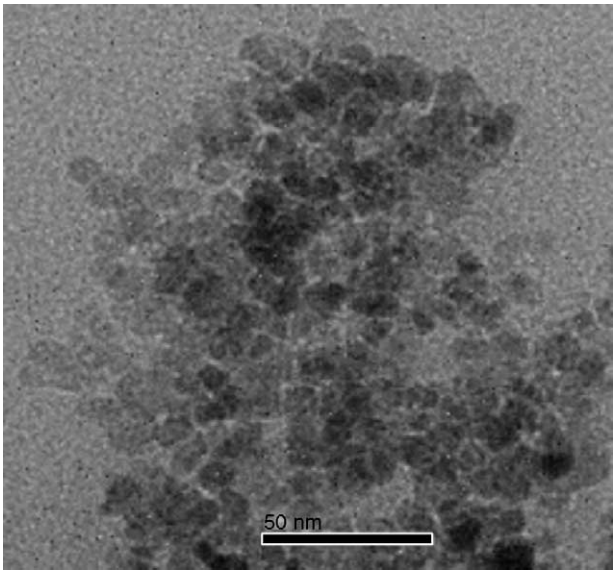

Fig. 1. Transmission electron micrographs of magnetic nanoparticles.

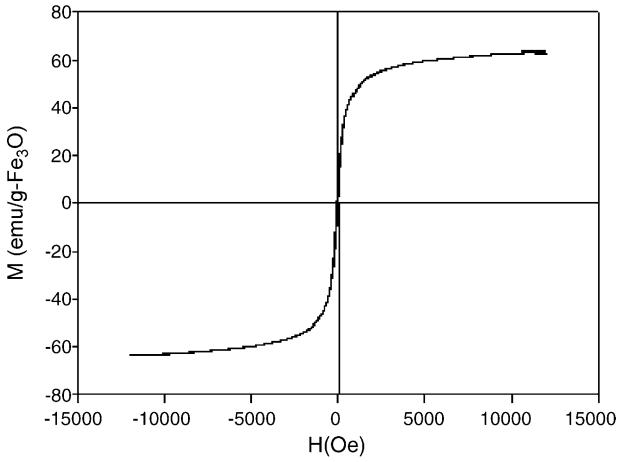

Fig. 2. Magnetization vs. magnetic field for the magnetic nanoparticles.

3.2. Ionic strength effects

The salt concentration and pH of the elution buffer used determine whether DNA is bound or eluted from the magnetic nanobeads. Resolution was achieved through careful selection of the elution buffer. In order to determine the effect of ionic strength on the desorption of plasmid DNA from PEI-modified magnetic nanobeads, elution buffer was studied at several NaCl concentration ranges of 0–2.0 M. Results are shown in Fig. 3, where increasing salt concentration clearly led to an increase in desorption effectiveness. Beyond a NaCl concentration of 1.25 M, the elution of absorbed plasmid increased significantly. The DNA-nanobeads interactions can be modulated through the electrostatic screening afforded by the ionic strength, and through the DNA net charge, as determined by the solution zeta potential. The measured zeta

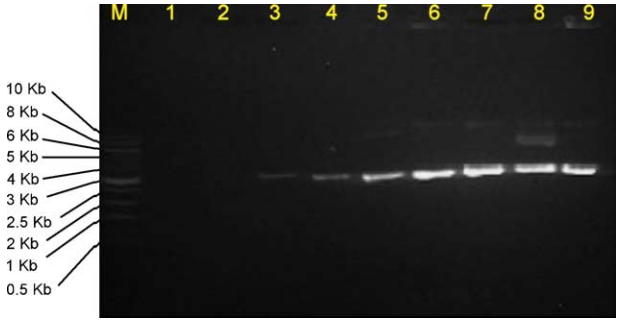

Fig. 3. Effect of salt concentration on elution of plasmid DNA from PEI-modified magnetic nanobeads. Lanes: M, molecular mass marker; 1, [NaCl] = 0 M; 2, [NaCl] = 0.25 M; 3, [NaCl] = 0.5 M; 4, [NaCl] = 0.75 M; 5, [NaCl] = 1.0 M; 6, [NaCl] = 1.25 M; 7, [NaCl] = 1.5 M; 8, [NaCl] = 1.75 M; 9, [NaCl] = 2.0 M.

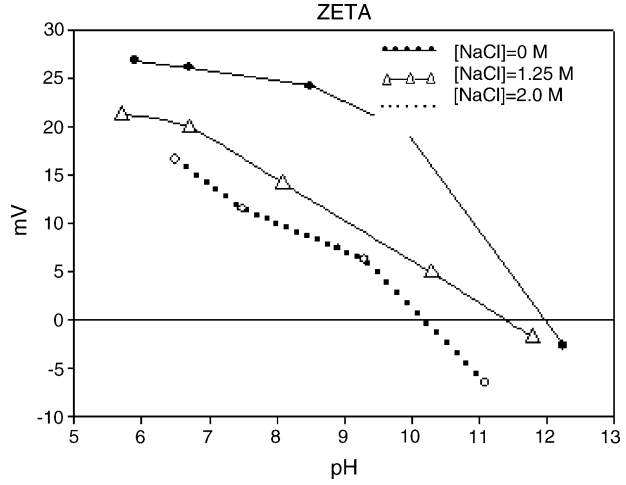

Fig. 4. The zeta potentials of PEI-modified magnetic nanobeads in suspension at different salt concentration and pH.

potentials of PEI-modified magnetic nanobeads in suspension at different salt concentration and pH are shown in Fig. 4. Zeta potential data for our PEI-modified magnetic nanobeads show a steady reduction in positive surface charge as the salt concentration is increased from 0 to 2.0 at a pH range of 6.0–12.0. For example, the zeta potential of PEI-modified magnetic nanobeads decreases from 23 to 7 mV as NaCl concentration increases from 0 to 2.0 M at pH 9.0. This observation was attributed to screening of the electrostatic interactions between the plasmid DNA and the charged PEI layers on the magnetic nanoparticles by salts, which reduced the adsorption of the plasmid DNA on the magnetic particles. As a result, the elution buffer with 1.25 M NaCl was used to conduct all subsequent experiments.

3.3. pH effects

The dependence of plasmid DNA desorption on the pH of elution buffer was studied at several pH ranges of 7.0–9.0. Fig. 5 indicates that pH has appreciably effect on desorption of plasmid from PEI-modified magnetic nanobeads. With the increase of pH of elution buffer from 7.0 to 9.0, the amount of eluted plasmid increased significantly. A possible explanation for pH effect on plasmid DNA desorption may be related to the surface charge of magnetic particles and plasmid DNA. The decrease in the positive potential at the magnetic absorbent surface diminishes the net electrostatic attraction force with the plasmid DNA. These results are consistent with those reported on the zeta potential, as shown in Fig. 4. At 1.25 M NaCl, the value of zeta potential of magnetic particles decreases from 19 to 10 mV as the value of pH increases from 7.0 to 9.0. This behavior again points to the dominant role played by electrostatic interactions in this system. As a result, the elution buffer with pH 9.0 was used to conduct all subsequent experiments.

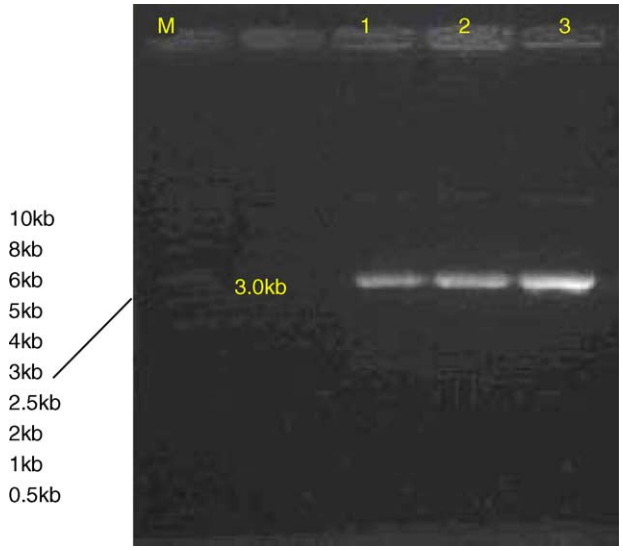

Fig. 5. Effect of pH on elution of plasmid DNA from PEI-modified magnetic nanobeads. Lanes: M, molecular mass marker; 1, pH 7.0; 2, pH 8.0; pH 9.0.

3.4. Determination of the optimal amount of PEI-Fe_3O_4 magnetic particles

The elution of plasmid DNA has now been successfully achieved by exposure to the elution buffer with optimal ionic strength (1.25 M) and pH (9.0). In order to determine the optimal amount of PEI-modified magnetic nanobeads has to add into 3 ml of bacterial culture. Several amounts of PEI-modified magnetic nanobeads were studied at ranges of 100–500 μg. As shown in Fig. 6 and Table 1, the amount

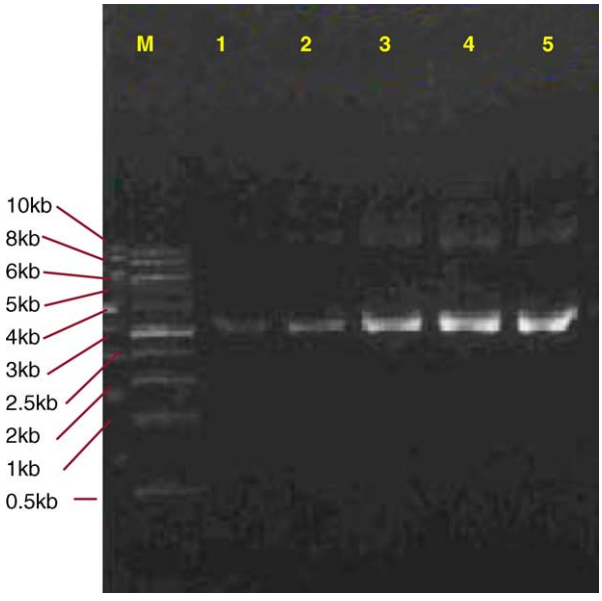

Fig. 6. Effect of the amount of PEI-Fe_3O_4 magnetic particles addition into 3 ml of bacterial culture on elution of plasmid DNA from PEI-modified magnetic nanobeads. Lanes: M, molecular mass marker; 1, 100 μg; 2, 200 μg; 3, 300 μg; 4, 400 μg; 5, 500 μg.

Table 1
Comparison the yield and quality of eluted plasmid DNA from PEI-modified magnetic nanobeads with commercial QIAGEN kits

PEI-Fe_3O_4(μg)	A_{260}	A_{260}/A_{280}	Plasmid (μg)
100	0.013	1.857	4.5
200	0.030	1.764	10.5
300	0.043	1.792	15.1
400	0.073	1.972	25.6
500	0.099	1.867	34.6
QIAGEN	0.108	1.928	18.9

of eluted plasmid DNA increases significantly from 4.5 to 34.6 μg, judged by UV spectrophotometry at 260 nm, as the amount of PEI-modified magnetic nanobeads increases from 100 to 500 μg. This could be attributed to the fact that the amount of plasmid DNA in supernatants after adsorption decreases significantly from 200 to 500 μg, as also shown in Fig. 7. The plasmid DNA was also purified by the QIAGEN (Valencia, CA, USA) plasmid kit according to the manufacturer instruction. In this case, the maximum plasmid DNA elution was about 18.9 μg using QIAGEN plasmid kit from 3 ml of bacterial culture. Therefore, the purification capacity of our PEI-modified magnetic nanobeads is significantly greater than those that can be attained using commercially available anion-exchange resins.

3.5. Dynamic and isothermal plasmid DNA adsorption

The adsorption kinetics of plasmid DNA by PEI-modified magnetic nanobeads is shown in Fig. 8. To confirm a complete equilibrium between plasmid DNA and PEI-modified magnetic nanobeads, these experiments have been examined until the adsorption time was 120 s. All the adsorptions tended toward equilibrium at 60 s. Fig. 9 depicts the influence of the amount of PEI-modified magnetic nanobeads on the equilibrium adsorption capacity. The equilibrium adsorption capacities increased first with the amount of magnetic nanobeads then reached a plateau value at about 500 μg. This is a typical curve of chemical adsorption. The equilibrium adsorption capacities were 70 μg plasmid DNA/mg magnetic nanobeads.

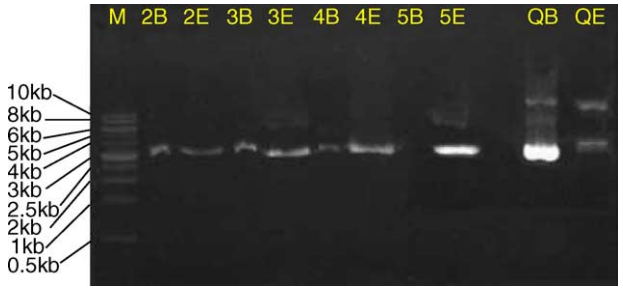

Fig. 7. Agarose electrophoresis of plasmid DNA in supernatants and elution buffer after adsorption on the PEI-modified magnetic nanobeads. Lanes: M, molecular mass marker; 2, 200 μg; 3, 300 μg; 4, 400 μg; 5, 500 μg; Q, QIAGEN; B, supernatants after adsorption; E, elution buffer.

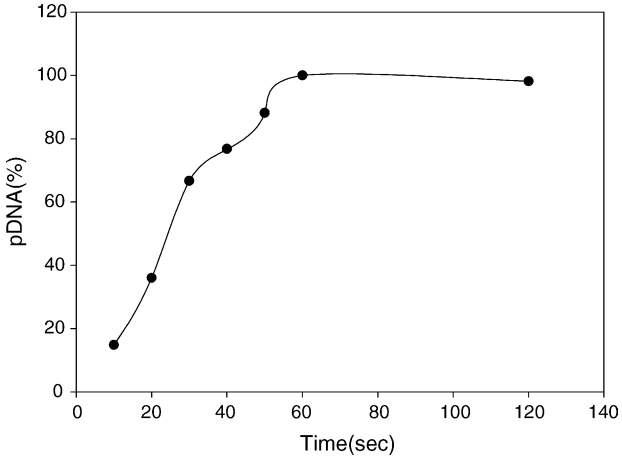

Fig. 8. The adsorption kinetics of plasmid DNA by PEI-modified magnetic nanobeads.

3.6. Expression of purified plasmid DNA

EGFP expression was detected by fluorescent microscopy in the transformed *E. coli* cells, indicating the biological activities of DNA fragments were retained after purified from PEI-modified magnetic nanobeads. Finally, as shown in Fig. 10, the digestion of purified plasmid DNA with two restriction endonucleases (*Bam*HI and *Eco*RI) resulted in the formation of two smaller clusters (approximately 0.8 and 2.9 kb) in agarose gel electrophoresis, which ran faster than undigested purified plasmid DNA. The use of PEI-modified magnetic nanobeads for plasmid DNA purification proved necessary to obtain good quality plasmid DNA and, above all, free of other contaminant molecules of culture origin. Therefore, the yield and quality of plasmid obtained was sufficient to allow further downstream application. The protocol, starting from the preparation of bacterial lysate and ending with purified plasmids takes less than 10 min. Thus, the separation and purification qualities of PEI-modified magnetic

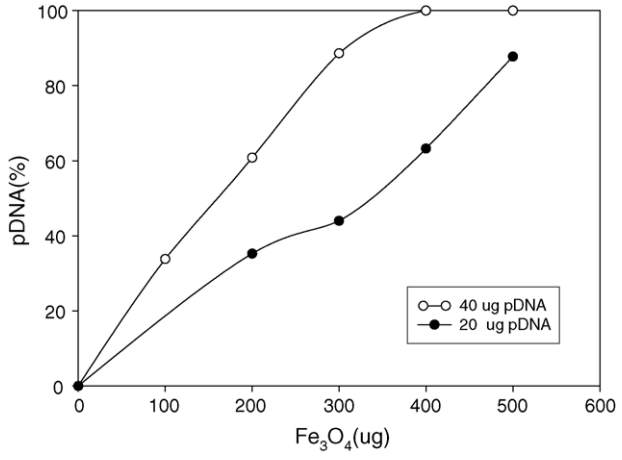

Fig. 9. Effect of the amount of PEI-modified magnetic nanobeads on the equilibrium adsorption capacity.

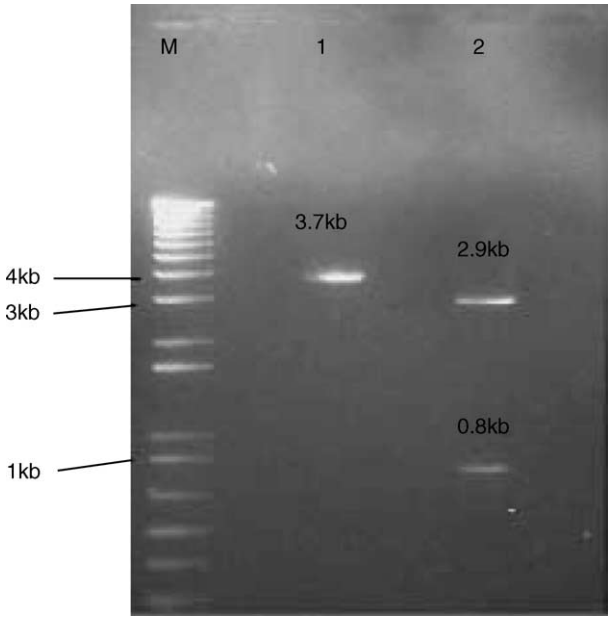

Fig. 10. Agarose electrophoresis of the digestion of purified plasmid DNA with two restriction endonucleases (*Bam*HI and *Eco*RI). Lanes: M, molecular mass marker; 1, purified plasmid DNA without restriction endonuclease digestion; 2, purified plasmid DNA with restriction endonuclease digestion.

nanobeads as well as its ease of use surpass those of conventional anion-exchange resins.

4. Conclusions

In this paper, we have investigated the suitability of a magnetically driven separation process utilizing PEI-modified magnetic nanobeads for the isolation of plasmid DNA from a bacterial cell lysate. We demonstrated a useful plasmid, pRSETB-EGFP, encoding the green fluorescent protein with T7 promoter, was amplified in DE3 strain of *E. coli*. The experimental results show that desorption of plasmid DNA from PEI-modified magnetic nanobeads was affected greatly by the ionic strength and pH of the elution buffer. The elution of plasmid DNA has been successfully achieved by exposure to the elution buffer with optimal ionic strength (1.25 M) and pH (9.0). The sample purification also proved necessary to obtain good quality plasmid DNA and free of other contaminant molecules of culture origin. Moreover, the yield and quality of plasmid obtained was sufficient to allow further downstream application. The protocol, starting from the preparation of bacterial lysate and ending with purified plasmids takes less than 10 min. Thus, the separation and purification qualities of PEI-modified magnetic nanobeads as well as its ease of use surpass those of conventional anion-exchange resins.

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