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Simple and efficient regeneration of silica spin columns for plasmid DNA purification

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Abstract

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Silica column-based DNA purification kits are widely utilized for the isolation and purification of plasmid DNA from bacterial cultures. These kits utilize plasmid purification columns that contain a silica-based matrix, which efficiently binds DNA and enables various downstream applications such as PCR, sequencing, and cloning. While this method offers numerous advantages, it is crucial to consider the associated limitations, including the amount of DNA that can be purified and the cost of purification kits. To address these limitations, our study proposes a new method for reusing silica columns in DNA plasmid purification, based on simple washing of the used columns with ion-free water. The results demonstrated the effectiveness of washing the silica columns with water in cleaning the leftovers of plasmid DNA, resulting in DNA concentrations comparable to the negative control. Importantly, the DNA obtained from the regenerated silica columns remained suitable for downstream applications such as PCR reactions, restriction enzyme digestion, and DNA sequencing, exhibiting no significant differences compared to the newly purchased silica columns. These findings support the feasibility of reusing silica columns for DNA extraction, contributing to sustainable and cost-effective laboratory practices. By adopting this approach, researchers can maximize the utilization of silica columns while reducing the overall cost and environmental impact associated with DNA purification processes. This method provides an opportunity to optimize resources and promote eco-friendly practices in DNA research and analysis.

Keywords: silica column-based DNA purification; reusing silica columns; efficient plasmid purification; sustainable laboratory practices; plasmid purification

Introduction

Silica column-based DNA purification kits are commonly employed for the isolation and purification of plasmid DNA from bacterial cultures. These kits consist of silica membranes made from silica gel, a porous form of silica extensively utilized in chromatography and DNA purification processes. Silica gel, derived from a naturally occurring mineral, is processed into a fine powder and transformed into a gel-like substance to enhance its binding and elution properties for DNA isolation [3, 4, 10]. Plasmid purification columns, filled with a silica-based matrix that binds DNA, offer an optimal method for downstream applications such as PCR, sequencing, and cloning. Silica column-based DNA purification relies on the specific binding of DNA to silica membranes facilitated by chaotropic salts, ensuring that other contaminants like proteins, salts, and detergents do not bind to the column, resulting in the extraction of high-qual-

ity, pure DNA [6, 10]. One notable advantage of utilizing silica column-based DNA purification is its high yield, making it an efficient method for DNA purification. Furthermore, it is a versatile approach that enables DNA isolation from various sources, including blood, tissues, cells, and bacteria. Cost-effectiveness is another notable attribute, making this method an affordable option for researchers operating on constrained budgets. The simplicity and user-friendly nature of the process render it accessible even to researchers with limited technical expertise, as most kits provide step-by-step instructions that are easy to follow [1, 2].

Despite its advantages, it is essential to consider the limitations associated with silica column-based DNA purification. One limitation is the restricted binding capacity for large amounts of DNA, rendering it less suitable for purifying significant quantities. Additionally, the purification process can be time-consuming, especially for larger samples, which can be a drawback when time is a limiting factor [1, 3, 10]. Carryover contamination poses another challenge, as it can lead to false positives in downstream applications. Moreover, there is a limit to the sample size that can be effectively processed using this method due to the binding capacity and elution volume restrictions of the columns. Additionally, the cost of purification kits and associated equipment can accumulate when processing a large number of samples [8, 9]. While silica column-based DNA purification is generally considered environmentally safe, it is important to ensure proper disposal of used kits and waste materials. Moreover, the production and distribution of purification kits can have environmental implications, and it is crucial to make efforts to minimize this impact through sustainable manufacturing practices and the use of recycled materials. Silica column-based DNA purification kits are widely used in DNA research and analysis worldwide, including in low-resource settings and economically disadvantaged countries. However, the cost of these kits can present a barrier to their use in resource-limited settings, particularly for large-scale studies [6, 10].

Although there have been studies on the recycling of silica columns from PCR purification and gel extraction kits, the reuse of silica columns specifically for DNA plasmid purification has not been extensively explored [6, 10]. In this study, we propose a new method for reusing silica columns in DNA plasmid purification. By implementing this approach, purified DNA plasmid obtained from reused silica columns can be effectively utilized in various downstream applications, such as PCR, restriction enzyme digestion, and DNA sequencing. This alternative approach offers a cost-effective and environmentally friendly solution, which is particularly beneficial in resource-limited settings and for large-scale studies.

Materials and Methods

Chemicals

Chemicals used in this study were provided by Sigma-Aldrich (Deisenheim, Germany), Invitrogen GmbH (Karlsruhhe, Germany), and Biobasic inc. (Markham Ontario, Canada). Enzymes exercised in this study were supplied by Thermo Fisher Scientific GmbH (St. Leon-Rot/Schwerte, Germany) and Biolab inc. (Massachusetts, USA).

E. coli strains, plasmids and primers used

The *Escherichia coli* (*E. coli*) strain DH5α transformed with plasmid pGreen3-PR1, harboring the GFP gene and a PR1 gene fused together via a linker (Fig. 1), was provided by the Faculty of Biotechnology and Food technology, Thai Nguyen university of Agriculture and Forestry (Thai Nguyen, Vietnam). Primers PR1-F: TCAGGAGTCCATGACACTCAT/ PR1-R: GATCTTCTTGGGTGGCTTGTAT, primer PR1-P1: ATGCAGATCACCACGTTTCT and primer GFP-R: TTACTTGTACAGCTCGTCCA used in this study were provided by Integrated DNA Technologies inc. (Coralville, USA).

Silica columns regeneration

The silica columns used in this study were obtained from a QIAprep Spin Miniprep Kit (Qiagen, Hilden, Germany). The silica columns were examined at different reuse intervals, ranging from their first use to the 9th reuse with a pGreen3PR1 plasmid. The removal of DNA from the used columns were performed by the following steps: (1) 650 µL ddH_2O was added into the used silica columns from the Qiagen miniprep kit, followed by incubation at room temperature for 1 minute. The number of repetitions of this step from 1 to 5 times was tested. (2) The columns were then centrifuged at 12000 rpm for 15 seconds, and the flow-through in the collection tubes was discarded. (3) The columns were dried by centrifugation at 12000 rpm for 1 minute. (4) The columns were washed by adding 500 µL of absolute ethanol and then centrifuged for 15 seconds at 12 000 rpm. The flow-through was discarded. (5) The columns were dried again by centrifugation at 12000 rpm for 1 minute. The experiment was performed in triplicate.

DNA plasmid purification from *E. coli*

A single colony of *E. coli* DH5α containing pGreen3-PR1 was incubated in 5 mL LB medium supplemented with 100 µg/mL kanamycin on a rotator at 37 C overnight. The pGreen3-PR1 plasmid contains the PR1 gene from *Verticillium dahliae* JR2, which is fused with the GFP gene, using a linker that encodes the GGSGGS peptide (Fig. 1) [7]. The

DNA plasmid was isolated from 4 mL overnight culture using either a new or a regenerated silica columns, following the manufacturer's protocol for QIAprep Spin Miniprep Kit (Qiagen, Hilden, Germany). The concentration of DNA was measured by a Nanodrop ND 1000 spectrophotometer (Peqlab Biotechnologies GmbH, Erlangen, Germany).

PCR amplification and qPCR quantitation of plasmid DNA

The presence or absence of plasmid DNA in the silica columns flow through was checked by PCR reactions with the Taq DNA polymerase (Bio Basic Inc., Toronto, Canada). The PCR reactions were carried out on a Master cycle X50s machine (Eppendorf, Germany) in a total volume of 20 µL, including 0.1 µL of Taq polymerase (5U/µL) (Bio Basic Inc., Toronto, Canada), 2.0 µL of standard Taq reaction buffer (10X), 0.4 µL of dNTPs (10mM), 0.4 µL of each primers (10 µM), 1.0 µL of template DNA, 1.5 µL $MgSO_4$ (20 µM), and 14.2 µL of water. The PCR cycles consisted of an initial denaturation at 95°C for 30 seconds, followed by 40 cycles of denaturation at 95°C for 10 seconds, annealing at 56°C for 20 seconds, and extension at 68°C for 30 seconds. A final extension step was performed at 68°C for 5 minutes. The PCR products were visualized by electrophoresis on a 1% agarose gel under UV light.

The DNA plasmid purified with the reused silica columns was examined by qPCR using the PR1-F/PR1-R primer pair (Fig. 1). The reaction mixture contained 1 µL of each 1.0 mM primers, 1 µL of the column eluate, 10 µL of 5 PRIMER 2X MasterMix (5 PRIMER, Gmb, Hamburg, Germany), and 8 µL of PCR-grade water, resulting in a final volume of 20 µL. The reactions were run on a Light Cycler 2.0 System (Roche, Manheim, Germany) under the following conditions: initial denaturation at 95°C for 3 min, followed by 42 cycles at 95°C for 10 seconds, 56°C for 15 seconds, and 72°C for 25 seconds, and a final step of 63°C for 15 seconds, followed by a continuous increase of the temperature by 0.1°C per cycle up to 95°C to obtain a melting curve. Standard curves were generated using pure pGreen3-PR1 plasmid at concentrations ranging from 0.1 to 100 ng/µL. The absolute amount of plasmid DNA in the column eluate was calculated using a correlation coefficient formula generated from the standard curve. All qPCR experiments were performed in triplicate. The data were analyzed using One-way ANOVA and the Tukey-Kramer post hoc test with $\alpha = 0.01$ in Microsoft Excel.

Restriction digestion of plasmid DNA

One microgram of plasmid DNA purified with a reused silica column was digested overnight with *XhoI* an *KpnI* restriction enzymes using Neb 1.1 buffer (New England Biolabs, Ipswich, MA, USA). The digested DNA samples were loaded onto a 1% agarose gel and electrophoresed for 45 minutes at 120 volts (V), then visualized under UV light. The results were compared to the calculated sizes of fragments using SnapGene Viewer 4.2.11 (GSL Biotech LLC), which was used to calculate the sizes of fragments following digestion with endonucleases (Fig. 1).

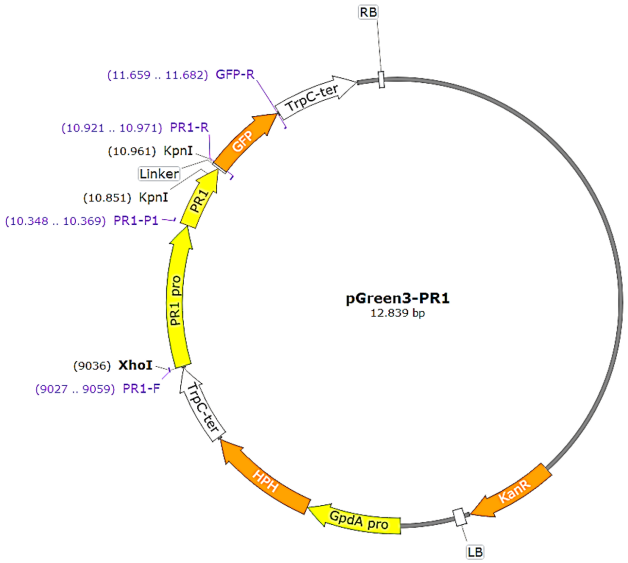

Fig. 1. The map of the pGreen3-PR1 plasmid

Gene sequencing

Eight hundred nanograms of plasmid DNA purified with a reused silica column was sequenced with either primer PR1-P1 or primer GFP-R. DNA plasmid sequencing was performed by PhuSa Genomics Co. (Can Tho, Vietnam).

Results

Silica columns regeneration by washing with water

DNA is a polar molecule, in which the sugar and phosphate moieties exhibit hydrophilic properties, while the nitrogenous base groups exhibit hydrophobic properties. Because of this, DNA molecules are infinitely soluble in water. Therefore, we used water to wash away and clean the leftovers of plasmid DNA from used silica columns. In this experiment, we tested the treatment of silica columns that had been previously used to purify the pGreen3-PR1 plasmid applying different number of cycles of washing the columns with water and final elution with elution buffer. The obtained eluates were tested for presence of leftovers of plasmid DNA by qPCR and conventional PCR (Fig. 2). Pure plasmid DNA was used as a positive control, while a TE solution was used as a negative control.

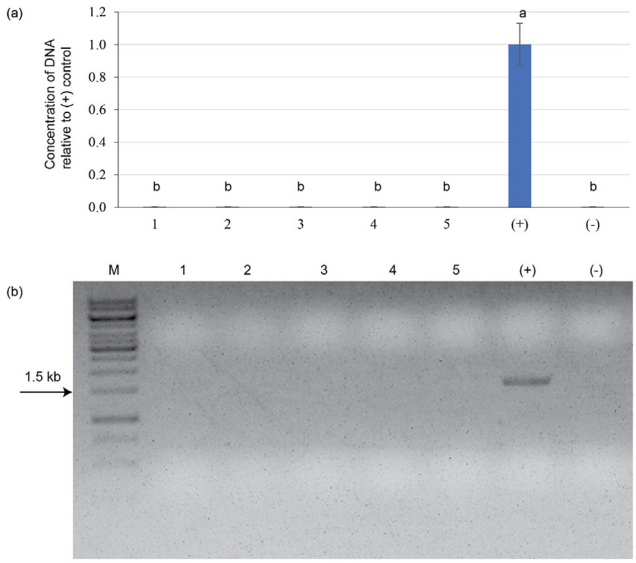

Fig. 2. Detecting traces of plasmid DNA following silica column regeneration by PCR and qPCR

(a) Mean DNA plasmid concentration and standard deviation in the elution buffer relative to the positive control based on qPCR data. **(b)** Agarose gel electrophoresis of PCR products with primers PR1-F and PR1-R using eluates from the regenerated column as templates. (+) indicates positive control – pure DNA plasmid, (–) indicates negative control – fresh TE buffer, 1–5 indicate the number of washing repetitions during the silica column regeneration process, M indicates GeneRuler 1 kb DNA ladder (Thermo Fisher Scientific). Letters "a" and "b" indicate groups which are significantly different as calculated by Tukey-Kramer multiple comparison test with α = 0,01

The results in Figure 2a shows that washing the silica column with water can effectively remove the plasmid DNA from the column. Using 1–5 cycles of washing produced results comparable to the negative control. In the positive control, where purified plasmid DNA was used, the presence of the plasmid DNA could be easily detected using both qPCR (Fig. 2a) and conventional PCR (Fig. 2b) reactions. These results demonstrate that removal of plasmid DNA from the silica column can be achieved through washing the columns with water. Once the silica column has been washed with water and dried with alcohol, it can be reused for isolation of new plasmid DNA without concerns of contamination from previous experiments.

Impact of the number of regeneration cycles on the yield of plasmid DNA

To assess the feasibility of multiple reuses of the silica columns, we conducted plasmid DNA isolation experiments using reused silica columns following 1 to 9 cycles of column regeneration. The experiment aimed to assess the reusability of silica columns in plasmid extraction for genetic engineering studies. We conducted plasmid DNA isolation experiments using reused silica columns with different regeneration cycles each including one washing step during the regeneration process. New silica column was used as a separate control group. The experiment involved extracting DNA plasmids from a strain of *E. coli* DH5a containing the pGreen3-PR1 vector. Four milliliters of *E. coli* bacterial cultures with an OD value of 1.0 were used in the experiment. The results of the plasmid DNA extraction are shown in Fig. 3a.

The results showed that the amount of extracted plasmid DNA decreased, when using reused silica columns with different number of regeneration cycles compared to the new silica column control group. The DNA concentration obtained from the new silica column reached 295.0 ng/µL. In contrast, the DNA concentration obtained from the reused

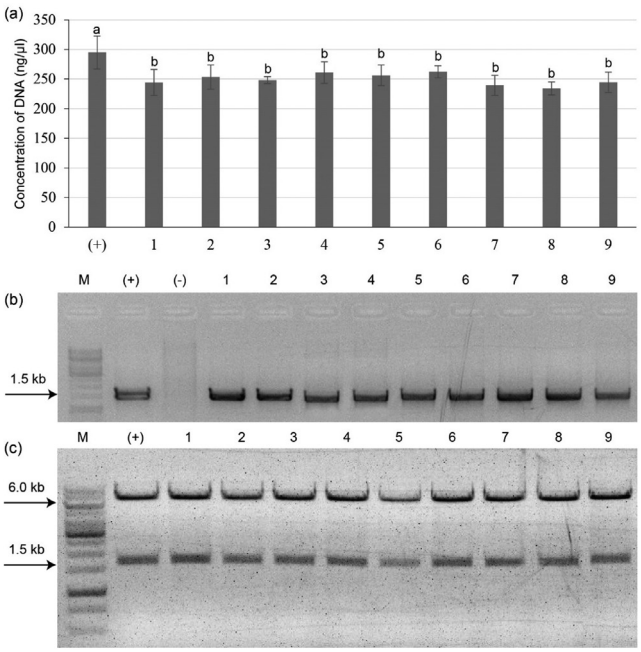

Fig. 3. Analyzing the effect of the number of regeneration cycles on the capacity of the silica columns and the quality of the purified plasmid DNA.

(a) Mean concentration of the purified plasmid DNA following 9 cycles of column regeneration**. (b)** Agarose gel electroporesis of PCR products with primers PR1-F and PR1-R. **(c)** Agarose gel electrophoresis of plasmid DNA digested with XhoI and KpnI.M indicates1 kb DNA maker, (+) shows the positive control (new column), (–) specifys negative control (TE fresh buffer), numbers 1-9 indicate the number of regeneration cycles of the silica columns. Letters a and b reveal groups which are significant different as caculated by Tukey-Kramer multiple comparison procedures with α = 0,01

silica columns ranged between 240–280 ng/μL. The maximum decrease in DNA concentration was 20.56 % compared to the control group with the new silica column. However, increasing the number of regeneration cycles of the silica column did not significantly affect the concentration of the obtained DNA (Fig. 3a). To ensure that the obtained DNA was sufficiently pure for further studies such as PCR, restriction enzyme digestion, and DNA sequencing, we conducted PCR reactions, restriction enzyme digestion, and DNA sequencing using the plasmid DNA obtained from above.

Impact of the silica column regeneration on the PCR amplification of plasmid DNA

In the experiment assessing the impact of the silica column regeneration on the PCR amplification of plasmid DNA extracted by the reused columns, 100.0 ng of plasmid DNA was used for PCR amplification with primers specific for the PR1 gene. DNA plasmid obtained from a new silica column was used as a positive control, while ion-free water was used as a negative control. The electrophoresis results of the PCR products are shown in Fig. 2b. The results showed that in the positive control group, only one DNA band appeared, equivalent to the size of the amplified DNA fragment of approximately 1.5 kb. In contrast, the negative control did not show any DNA bands. In the PCR reactions using plasmid DNA extracted with reused silica columns with different number of regeneration cycles, only one DNA band similar to the positive control result appeared (Fig. 2b). The intensity of the DNA band obtained on the agarose gel was not significantly different between the PCR reactions using DNA from the reused silica columns and the positive control. This indicates that reusing the silica columns for plasmid DNA extraction following their regeneration does not affect the efficiency of gene amplification in PCR reactions.

Impact of the silica column regeneration on the digestion of plasmid DNA with restriction enzymes

To further evaluate the quality of plasmid DNA obtained by reused silica columns, we used the extracted plasmid DNA in restriction enzyme digestion reactions. The plasmid DNA obtained by reused silica columns was subjected to restriction enzyme digestion with the enzymes *Xho*I and *Kpn*I. The results showed that in the new silica column control group, the pGreen3-PR1 plasmid DNA was cut into two DNA fragments with sizes of 1.6 kb and 11.0 kb, respectively, which corresponded to the calculated sizes on the vector map. The preps using reused silica columns with different regeneration cycles produced identical results that matched the positive control. This indicates that the quality of the DNA plasmid extracted with reused silica columns is not affected by the regeneration procedure. This DNA plasmid can be used for genetic engineering experiments such as PCR and restriction enzyme digestion.

Impact of the silica column regeneration on the sequencing of plasmid DNA

Plasmid DNA from samples following 1, 5, and 9 cycles of silica column regeneration were selected for test sequencing and comparision with control plasmid DNA purified using a new silica column. The results of the DNA sequence comparison are shown in Table 1.

The comparison results indicate that the DNA sequences obtained from the new silica column and the regenerated silica columns are not different. For the DNA obtained from the new silica column, a DNA segment of approximately 40% of the compared DNA sequence was identified, with a similarity rate of 99.72%. The DNA sequence obtained from the regenerated silica columns also had a length ranging from 43–44% of the compared DNA sequence, with a similarity rate ranging from 99.72% to 99.81%.

The results above indicate that the silica columns can be easily regenerated by washing with ion-free water. Following the regeneration procedure, no DNA was detected in the column eluates. The regenerated silica columns can be reused for at least 9 times. The DNA extraction efficiency may decrease up to a maximum of 20% compared to new silica columns, but the DNA extraction efficiency remains unchanged when increasing the number of regeneration cycles. The DNA obtained from the regenerated silica column can be used for PCR reactions, restriction enzyme digestion, and DNA sequencing. The analysis results show no difference compared to the results from new silica columns.

Table 1. DNA sequencing results for plasmid DNA extracted with regenerated silica columns. The symbol (+) indicates positive control sample (plasmid DNA extracted with a new column)

Samples	Max score	Total score	Query cover (%)	Percentage identify (%)	Accesion length (bp)
(+)	1960	1960	44%	99,72%	1070
1	1958	1958	44%	99,72%	1069
5	1925	1925	43%	99,81%	1051
9	1925	1925	43%	99,81%	1053

Nguồn NCBI BLAST nucleotid [5]

Discussion

The results of our study demonstrate that the silica columns for plasmid DNA purification can be effectively regenerated by washing with ion-free water. This cleaning process ensures the complete removal of DNA, as evidenced by the absence of detectable DNA in the TE solution used for elution of the regenerated columns. These findings align with previous studies on the efficient cleaning of silica columns [2, 4, 10].

We also observed that the silica column can be reused for a minimum of 9 times without compromising its functionality. This is in line with previous studies that have reported the reusability of silica columns in DNA extraction protocols (Brown et al., 2017; Martinez et al., 2019). However, it is important to note that the DNA extraction efficiency may decrease by up to a maximum of 20% compared to a new silica column. Similar observations have been reported by other researchers when assessing the impact of column regeneration on the DNA extraction efficiency [6, 10].

Interestingly, we found that the DNA extraction efficiency remains consistent even when increasing the number of regeneration cycles. This suggests that the decrease in efficiency is primarily attributed to factors other than the number of regeneration cycles, such as column material degradation or adsorption saturation [2, 3, 10].

Importantly, the plasmid DNA obtained from the regenerated silica columns was suitable for various downstream applications, including PCR reactions, restriction enzyme digestion, and DNA sequencing. The analysis results showed no significant differences between the DNA extracted from the regenerated silica columns and the new silica columns. These findings are consistent with previous studies highlighting the suitability of DNA extracted from regenerated silica columns for downstream applications [2, 6, 10].

In conclusion, our study demonstrates that the silica column can be effectively cleaned and reused multiple times without compromising the DNA extraction efficiency or downstream application compatibility. These findings contribute to the body of knowledge supporting the practical and cost-effective use of regenerated silica columns in DNA extraction protocols, thereby reducing plastic waste and promoting sustainable laboratory practices [2, 6, 10].

Significance Statement

The study has revealed a simple method for regeneration of silica columns for plasmid DNA purification. As a result, the regenerated silica columns can be reused for plasmid DNA extraction from bacteria without compromising the extraction efficiency or the quality of the extracted DNA. The research findings provide students and scientists in economically disadvantaged countries with a more efficient, cost-effective, and environmentally friendly method of using silica columns for DNA extraction, reducing plastic waste released into the environment.

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