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Research paper

A Simple and Efficient Method of Genomic DNA Extraction and Purification from Diverse Biological Samples

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ABSTRACT

Genomic DNA (gDNA) is the heritable, genetic information contained in each cell that provides the information needed to develop, maintain, and reproduce an organism. Organized into chromosomes, gDNA is protected by proteins and partitioned into the nucleus in eukaryotic cells. The ability to extract and purify gDNA from samples of interest is an essential first step in the study of many biological processes. There are different methods to extract and purify gDNA such as solution-based, spin column-based, and magnetic beads methods. In this study, gDNA has been extracted and purified from diverse biological samples such as plants, bacteria (gram-negative and gram-positive), human blood, scorpion tissues, and soil sample using a simple and efficient mini-spin column method. The concentration of DNA was determined using a fluorimeter and Nanodrop. The integrity of DNA was determined by gel electrophoresis.

KEYWORDS DNA Extraction; DNA Purification.

INTRODUCTION

DNA extraction is a method to purify DNA by using physical and/or chemical methods from a sample separating DNA from cell membranes, proteins, and other cellular components. For the first time, Friedrich Miescher isolated DNA in 1869 [1]. The use of DNA isolation techniques leads to efficient extraction and purification of high-quality DNA. Purified DNA should be pure and devoid of contaminants, such as RNA and proteins. Manual DNA extraction/purification methods as well as commercially available kits can be used for DNA extraction followed by purification. Many commercial kits are available to isolate DNA from a variety of biological materials [2, 3]. In this study, mini-spin-column method was used for the extraction and purification of genomic DNA. DNA extraction procedure (s) involves lysing the cells and solubilizing DNA, followed by chemical or enzymatic methods to remove macromolecules, lipids, RNA, or proteins. In this study, genomic DNA has been extracted from diverse biological samples including human blood, plant tissues (leaves), bacteria (both gram-positive and gram-negative), soil, and scorpion *Buthus sindicus* tissues. After extraction and purification of DNA, gel electrophoresis was carried out to check whether the extraction has occurred successfully and to determine the integrity of the genomic DNA [6]. After gel electrophoresis, samples containing intact DNA band are processed further for molecular biology studies. The concentration of the DNA was

determined using Quantus Fluorimeter and Nanodrop [4]. The purified DNA samples were stored at -25 °C for further use [7].

MATERIALS AND METHODS

DNA Extraction and Purification from Human Blood

Fresh blood was collected from three individuals in EDTA vacutainer. The DNA was extracted and purified from fresh blood using a GJC® DNA Purification kit according to the user manual. The 1% agarose gel was made in TBE (Tris borate EDTA buffer). The agarose gel electrophoresis was run at 90 volts for 30 minutes and then the gel was visualized using a transilluminator. The concentration of purified DNA was determined using Quantus Fluorimeter by adding 1ul of purified DNA in 200ul of working solution.

DNA Extraction and Purification from Plant

The plant leaves were washed with distilled water to remove the soil particles and then washed with 70% ethanol to remove any microbial contamination. The plant leaves were cut and weighted using a weighing machine, and multiple concentrations of leaves were used. Mortar and pestle and hand homogenizer were used to break the cell wall of the plant and the DNA was extracted and purified using a GJC® DNA purification kit according to the user manual provided.

DNA Extraction and Purification from Bacteria

The four bacterial species *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Salmonella typhi* were collected. The DNA was extracted and purified from the overnight cultures and old cultures, by picking a handful amount of bacterial culture from the agar plate using a wire loop and was added in 1.5ml Eppendorf containing the S1 lysis solution. The bacterial DNA was extracted and purified using a GJC® DNA purification kit according to the user manual.

DNA Extraction and Purification from Scorpion

Two male and two female scorpions (*Buthus Sindicus*) were collected from Sindh, Pakistan. The scorpions were washed with distilled water three times to remove the soil particles [5]. The pedipalp and metasoma were cut and washed with absolute ethanol three times to remove the microbial contamination. Different concentration of the tissues was used, and the DNA extraction and purification were carried out using a GJC® DNA purification kit according to the user manual.

DNA Extraction and Purification from Soil

The soil sample was collected from a field and 5gm of soil was added in 50ml LB broth. The broth was incubated overnight at 37ºC in a shaker at 120 rpm. 1.5 ml of broth was transferred to two separate 1.5 ml Eppendorf and the bacterial DNA from the soil sample was extracted and purified by using a GJC® DNA purification kit, using the bacterial DNA purification protocol.

RESULTS

DNA Extraction and Purification from Human Blood

The DNA was extracted and purified from all three samples and the bands of purified DNA extracted from fresh blood using a GJC® purification kit are shown in Figure 1. The estimated concentration of the purified DNA is shown in Table 1.

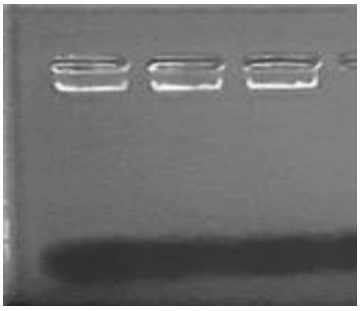

Figure 1: Human genomic DNA purified by GJC Genomic DNA Purification kit. From left lanes 1-3 represent samples A, B, and C respectively.

Table 1: The Concentration of Purified DNA Extracted from Human Blood.

Sample	DNA (ng/µl)
Sample A	156
Sample B	158
Sample C	90

DNA Extraction and Purification from Plant:

The band of purified DNA is present only in sample Pl-03 (as shown in Figure 2.), which was treated with S1 lysis solution during homogenization. There were no bands in the other samples. The process is repeated to check the efficacy of the method and all the samples were homogenized in the presence of an S1 lysis solution. The agarose Gel electrophoresis was performed and the bands were visualized using a transilluminator (as shown in Figure 3). The concentration of DNA is shown in Table 2.

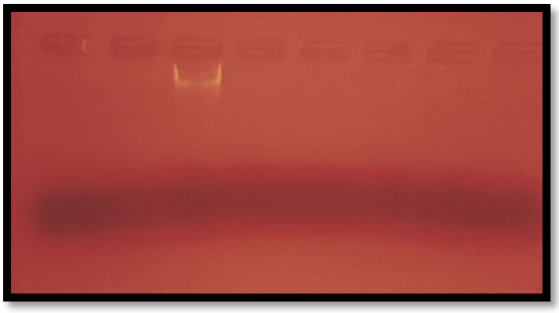

Figure 2: The well 03 from the left-hand side represents bands of sample Pl-03 that was treated with s1 lysis solution during homogenization.

Table 2: Concentration of purified DNA extracted from Plant tissue.

Sample ID	DNA (ng/µl)
Sample PL-01	49.7
Sample-PL1-01	42.8
Sample-PL1-02	51.2
Sample-PL1-03	44.1

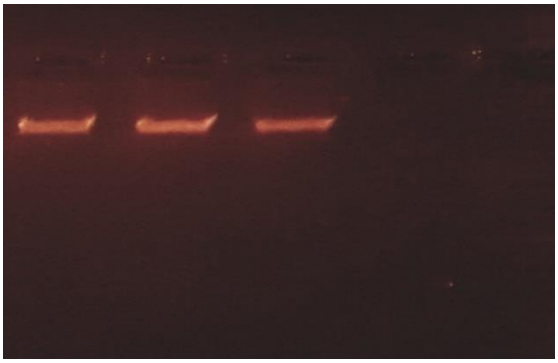

Figure 3: The well 01,02,03 from left-hand side represent bands of sample PL1-01, PL1-02, and PL1-03 respectively.

DNA Extraction and Purification from Scorpion

The DNA was extracted and purified from the scorpion tissues of both males and females. The agarose gel electrophoresis was performed, and bands were present in

all the samples as shown in Figure 4. The concentration of purified DNA extracted from scorpion tissues in mentioned in Table 3.

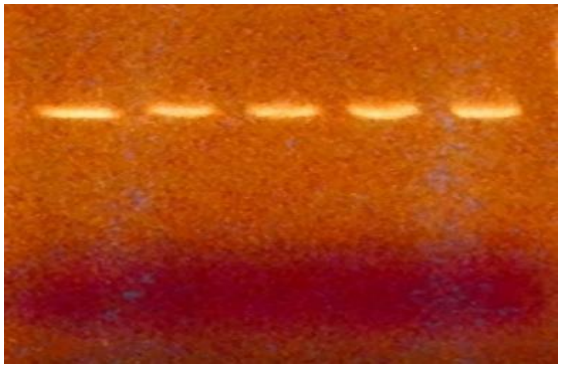

Figure 4: The well 01,02,03,04,05 from the left-hand side represent bands of sample SC-01, SC-02, SC-03, SC-04, and SC-05 respectively.

Table 3: The concentration of purified DNA extracted from scorpion tissues

Sample ID	DNA (ng/µl)
Sample-SC-01	199.5
Sample-SC-02	219.4
Sample- SC-03	285.4
Sample- SC-04	253.4
Sample- SC-05	211.9

DNA Extraction and Purification from Bacteria

The agarose gel electrophoresis of purified DNA was performed and bands were present in all the samples as shown in Figure 5 and the DNA concentration of the purified DNA is shown in Table 4.

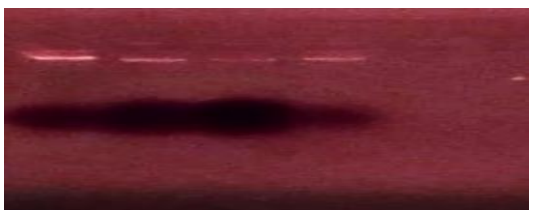

Figure 5: The band 01,02,03,04 from the left-hand side represents DNA purified from *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Salmonella typhi* respectively.

Table 4: The concentration of purified DNA extracted from scorpion tissues

Samples	Bacterial specie	DNA (ng/µl)
Sample A	*Escherichia coli*	120
Sample B	*Bacillus subtilis*	135
Sample C	*Staphylococcus aureus*	80
Sample D	*Salmonella typhi*	149

The bacterial DNA was extracted and purified from two samples. The bands of both the samples are present on Agarose gel as shown in Figure 6. The concentration of purified DNA is shown in Table 5.

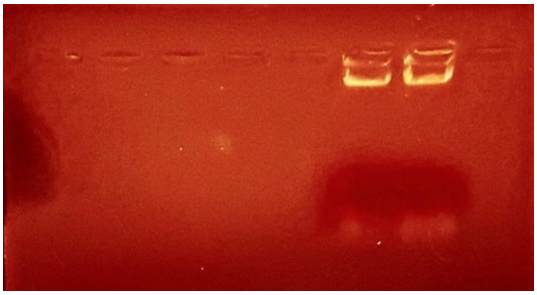

Figure 6: The well 06 and 07 from the left-hand side represent Bands Sample S-01 and S-02 respectively.

Table 5: The concentration of purified DNA extracted from Soil samples.

Sample ID	DNA (ng/µl)
Sample S-01	363.5
Sample S-02	354.2

CONCLUSION:

There are multiple methods to extract genomic DNA from biological samples. Many commercial kits are available for the

extraction and purification of DNA. In this study, the Genomic DNA from diverse biological samples has been extracted and purified using GJC® DNA purification kit. It is based on the mini-spin column method which is a simple and efficient method for the extraction and purification of genomic DNA.

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