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Isolation of DNA from Saliva and Cheek Cells Using Household Chemicals

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Abstract: Saliva is a feasible specimen used in sample collection for research purposes. It is reported that saliva typically contains ~430,000 epithelial cells per mL. There are various cost-effective methods and commercial kits available which are used to extract DNA from saliva. However, this study aims to explore a simple, time-saving and cheap method of DNA extraction using daily life household chemicals without the help of any electronic equipment. This protocol utilizes pineapple juice which contains bromelain enzyme as a proteolytic agent and dishwashing liquid as a detergent to rupture the cell membrane and remove the contamination. It takes approximately 10-15 min for the completion of extraction procedure. Results have indicated the good quality of DNA by gel electrophoresis and good yield with purity of 1.7-1.8 optical density. Thus, this method serves as a simple, convenient, easy-to-handle, time-saving, cheap and less laborious way for the extraction of DNA in daily routine practices with a good precision of quality and quantity that could be used for diagnostic purposes.

Key words: Saliva, DNA extraction, dishwashing liquid, bromelain enzyme.

1. Introduction

Genomic DNA is a vital form of nucleic acid that is isolated for research purposes and forensics [1]. Once DNA is extracted in its integrated form, it could be preserved for long-term storage. In past, well defined protocols for the extraction of genomic DNA have been reported from a variety of specimens such as cigarette butts, human skin, blood, muscles and saliva [2-4].

Saliva is the most feasible form of sample that is used in sample collection to achieve the research objectives. In comparison to blood, saliva has several benefits: no special personal protective measures and anticoagulant vacutainers are required, permits remote collection from patients of different pathological diseases, no suffering of needle injection pain and fear, no chances of needle prick injury for researcher, easily accepted by patients for their volunteer participation in research, low risk of direct transmission of infectious diseases, contains no clotting factors. It is reported that saliva typically contains ~430,000 epithelial cells per mL [5].

Therefore, saliva serves as an ideal specimen to handle and store for the extraction of DNA. This study will demonstrate the isolation of DNA without the utilization of any electronic equipment and expensive research grade chemicals. Thus, the aim of this study is to present the simple, time-saving and cheap method of DNA extraction using daily life household chemicals.

2. Materials and Methods

Reagents: NaCl (Oxide), absolute ethanol (Sigma-Aldrich), pineapple juice, dishwashing liquid.

This protocol was performed in following steps:

For sample collection, approximately 80 mL of H_2O splashed into the oral cavity for 2 min and spitted into a jar.

After that, 2 g of NaCl was added into the sample containing jar, gentle stirring was performed and left for 5 min.

Approximately 80 mL of H_2O was mixed with 25 mL of dish washing liquid into a separate jar.

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Then 10 mL of this solution was added in salted specimen containing jar and gentle stirred.

Then this mixture was transferred into a clean test tube.

Pineapple juice of 0.1 mL was added and gently stirred.

Chilled absolute ethanol of 10 mL was added into the test tube and slight stirring was performed.

The solution was allowed to stand at room temperature for 1-2 min.

DNA thread becomes visible after the precipitation.

The DNA threads were separated out into a 1.5 mL Eppendorf using micropipette and dissolved in 50 µL of H_2O at 55 °C for 5-10 min.

Quantitative assessment was performed by measuring optical density at 260/280 ratio using Nanodrop (IMPLEN NanoPhotometer®, Germany).

Qualitative assessment was performed by gel electrophoresis at 0.7% agarose gel. Then 1 µL sample was mixed with 1 µL of DNA gel loading dye 6× (ThermoFisher Scientific). And 0.3 µL of SYBR safe DNA gel stain (ThermoFisher Scientific) was used as a visualizing dye.

3. Results and Discussion

Table 1 represented the purity of DNA by means of the optical density at 260/280 ratio and concentration in ng/µL. Total replicates n = 7 were extracted by following this protocol. A range of concentrations from 117-337 ng/µL were observed. Though, purity was evident in all samples with 1.7-1.8 O.D. which indicates no remnants of RNA or protein contamination. However, in Fig. 1, the integrity of DNA was characterized by genomic DNA banding pattern in gel electrophoresis. Fig. 2 shows the precipitation of DNA after the extraction using above protocol.

The saliva consists of several facets that ideally contribute to making it a suitable candidate for extracting DNA [6]. This study has explored the pure and integrated DNA extracted from saliva and cheek cells in a short period of time by a handy protocol for instant practices using household chemicals. Pineapple juice contains a proteolytic enzyme bromelain which helps to degrade protein contamination [7] whereas dish washing liquid acts as

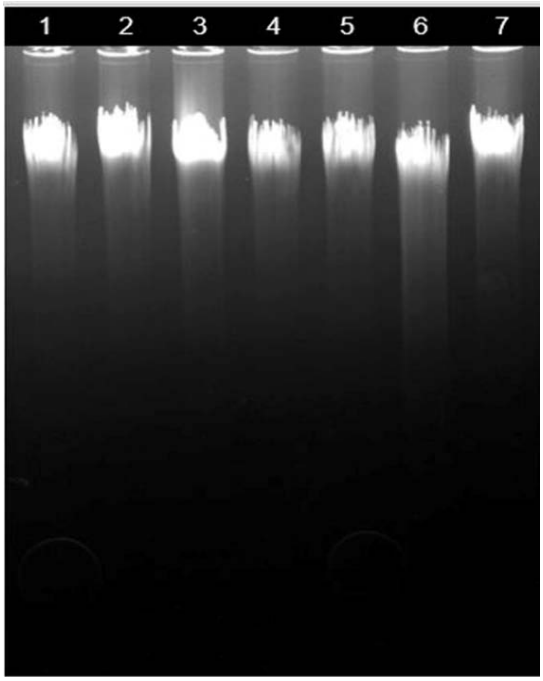

Fig. 1 Genomic DNA resolved at 0.7% agarose gel extracted from the saliva and cheek cells.

Fig. 2 Precipitated DNA threads.

a detergent to disrupt cell membrane [8].

Table 1 The optical density and concentration of DNA samples.

DNA sample No.	O. D at 260/280	Concentration (ng/µL)
Replicate 1.	1.7	237
Replicate 2.	1.8	243
Replicate 3.	1.8	337
Replicate 4.	1.8	117
Replicate 5.	1.7	324
Replicate 6.	1.8	221
Replicate 7.	1.7	200

DNA: deoxyribonucleic acid, D: optical density.

In general, there are many protocols that explained the DNA extraction from fresh saliva [6]. Precisely, various DNA extraction kits are also available which followed the standard methods for obtaining the high quality of DNA for long-term storage with varying levels of amount and purity [4, 9, 10]. Limitations of this study include that it is applicable for beginners with limited resources in a facility but is not recommended for long-term cohort studies conducted at large scale as it provides inadequate yield.

4. Conclusions

This study suggests a simple, convenient, easy-to-handle, time-saving, cheap and less laborious method for the extraction of DNA in daily routine practices for primary learners with a good precision of quality and quantity which could be used for diagnostic purposes.

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