RESEARCH ARTICLE

Frugal Method for Extracting DNA from Saliva Suitable for Low-Budget Labs and Usable in PCR Assays

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ABSTRACT

Blood is the preferred source of DNA as it yields large quantities of high-quality DNA. However, there are challenges associated with this process, including difficulty in finding small and thin veins, rigid veins due to aging, and individuals with phlebotomy aversion, especially children. Therefore, obtaining DNA from saliva is considered a suitable alternative that requires minimal resources and is usable in DNA assays without the need for a phlebotomist, at a lower cost compared to most DNA extraction kits that require expensive materials. The suggested frugal method relies on providing a new solution for extracting DNA from human saliva without the need for enzymes or high grade reagents or columns, which makes suitable for low budget laboratories in developing countries to manufacture it locally, it uses a phase separation with technical skills to extract the DNA from the saliva achieving safety by not using hazardous reagents, and it is a sustainable eco-friendly method that gives decent yield of good quality DNA in ~20 minutes to be used in PCR applications with a cost that is estimated to be 10 times cheaper than current used methods.

Key words: DNA extraction, saliva, sustainable, frugal, economic, PCR

Introduction

DNA is the primary genetic material in humans and living organisms. It was first discovered in 1869 by the Swiss scientist Friedrich Miescher (Dahm, 2005). Its main components are sugar, nitrogenous base, and phosphate group. Sources for DNA extraction include blood, hair, tissues, and saliva (Lamm et al., 2020).

DNA has numerous clinical diagnostic applications, including screening newborns for treatable genetic diseases early in life, confirming diagnoses in suspected medical conditions, detecting mutations, pre-implantation testing, and is widely used in forensic medicine to identify crime and disaster victims and suspects (Cottrell, 2004).

Saliva is secreted by the sublingual, submandibular, and parotid glands (Alhajj & Babos, 2023). Additionally, the shedding of the superficial layer of epithelial cells in the mucous membrane of the human mouth, which occurs approximately every 2.7 hours (Dawes, 2003), ultimately leads to saliva composed of 75% squamous cells and 25% lymphocytes, depending on an individual's oral health. A study by Endler in 1999 found that the average proportion of viable epithelial cells containing intact genomic DNA in collected saliva samples is at least 58% (Chacon-Cortes & Griffiths, 2014). Other components in saliva, such as enzymes, hormones, antibodies, and other bioactive molecules, may also affect the quality and quantity of extracted genomic DNA (Song et al., 2023). Generally, caution should be exercised in DNA extraction and saliva preservation (Gong & Li, 2014). DNA extraction and purification are crucial steps in the field of biotechnology and medical molecular assays. It serves as a starting point for numerous applications, ranging from basic research to routine diagnostic and therapeutic decision-making (Garbieri et al., 2017).

Materials and Methods

The method of DNA extraction from saliva samples involves collecting a saliva sample and processing it through simple steps to extract high-quality DNA within a short time frame estimated at 15-20 minutes by the individual themselves, without the need to send the sample or handle the kit by a specialist. These extraction steps do not require complex equipment or technical expertise, distinguishing this method by its ease of use. The components of the extraction kit can be maintained effective for up to 18 months at room temperature.

Tools and equipment used for the extraction:

This method of extraction only needs the following tools and materials:

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• Micropipette: for withdrawing samples and its own plastic tips for consumption.

• 50ml glass beaker: for collecting liquid solutions.

• Plastic falcon tubes: 5-10 ml tubes for collecting saliva samples.

• centrifuge: to precipitate contents as needed in the protocol.

• Refrigerator: for storing samples and for putting the ethanol.

• 1.5ml centrifuge tubes: for sample collection and to preserve the DNA.

•a solution that contains 5% sodium lauryl sulfate (SLS) or sodium dodecyl sulfate (SDS) (Bondi et al., 2015), it can be obtained in its pure state or from dishwashing solutions, it serves as a surfactant to break cellular membrane to free the DNA.

• Distilled water.

• Cold Ethyl alcohol (ethanol) 70%

• Sodium chloride (NaCl), it provides a suitable ionic environment for the DNA (Heikrujam et al., 2020)

Preparation of solutions used in the extraction method:

Salt Solution for DNA Preservation Buffer1: add 5 grams of sodium chloride to 200 ml of distilled water. The salt is dissolved in the distilled water until complete dissolution to obtain a mixture that is sterilized by autoclaving at 121 degrees Celsius for 15 minutes to maintain sterilization and prevent the growth of halophilic bacteria. The purpose of the saline solution is to stabilize the DNA upon release and prevent its degradation (Yakovchuk et al, 2006) Saliva contains cheek cells from which DNA is extracted. After the subsequent extraction steps, the cell membranes will be broken, exposing the DNA to solutions and acidity levels that can damage it. Therefore, sodium chloride ensures the viability of the DNA by providing a suitable osmotic environment for it.

Surfactant preparation **Buffer2**: prepare 5 ml of surfactant solution containing 5% sodium lauryl sulfate or sodium dodecyl sulfate and dilute it by adding it to 40 ml of distilled water. Sodium lauryl sulfate or sodium dodecyl sulfate is the active ingredient that removes surface tension to dissolve the cell membrane and release DNA into the solution (Parsi, 2015).

Protocol of DNA extraction:

The frugal saliva DNA extraction method involved the following steps:

The Saliva DNA Extraction Protocol requires the following steps:

I. 3000 µl of Buffer 1 is taken and placed in the mouth for gargling for two minutes. The aim of this step is to dislodge as many cheek cells as possible. Teeth can be used to gently scrape the cheeks and tongue while moving the saline solution in the mouth, and the tongue can also be used to separate cells from the buccal mucosa.

II. The mixed saliva sample with salt is then spat into an appropriate tube, it will have an approximate volume of 4-5 ml from the sum of buffer1 volume with the saliva sample, from this sample 500 µl is taken and placed inside a 1.5 ml centrifuge tube.

III. centrifuge for 2 minutes at a speed of 5000 revolutions per minute in a standard centrifuge (Olatunde et al., 2022) to collect the precipitate composed of cheek cells at the bottom of the tube, while saliva and other components remain suspended.

IV. We discard the supernatant and add 200 µl of Buffer 2. use the micropipette to mix the pellet with Buffer 2 through repeated pipetting up and down, and wait for 2-5 minutes until the solution takes effect in order to break the cell membrane.

V. add 400 microliters of pre-chilled 70% ethanol at -4°C very carefully on the wall of the centrifuge tube above the sample by attaching the micropipette tip head on the wall and pushing slowly the emptying button. After waiting 30-60 seconds, the DNA becomes clearly visible in the supernatant portion of the solution, where the solution will divide into three distinct phases. The ethanol will draw the layer of surrounding water layer of the DNA (Oda et al., 2016). The alcohol being less dense than the lower phase solution containing the cell

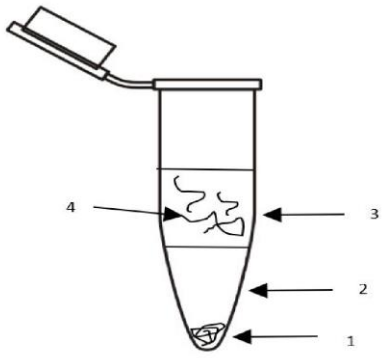

Figure 1. *It shows phase separation in the tube after centrifugation.*
The drawing includes a 1.5 ml tube containing the following phases: 1- Cellular aggregate composed of broken cell membranes found in saliva 2- Surfactant phase with dissolved cellular aggregate components containing proteins and other compounds 3- 70% alcohol phase containing condensed DNA 4- DNA is in the form of a white cloud.

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pellet and Buffer 2, the DNA will float to the upper phase (Figure 1).

VI. gently and steadily pipette 300 microliters of the supernatant containing the concentrated DNA and alcohol, taking care not to draw the lower phase containing the surface tension remover and pellet. Then, transfer the withdrawn liquid to a new 1.5 ml tube.

VII. centrifuge the sample in the new tube for two minutes at a speed of 5000 revolutions per minute. The DNA will appear as a milky precipitate, and it should be free from impurities.

VIII. The supernatant is discarded, and the milky DNA precipitate is preserved.

IX. After discarding the supernatant alcohol, the tubes should be left open in a horizontal position in a sterile environment for 5-15 minutes to allow the alcohol to evaporate. The readiness of the sample and the disappearance of alcohol can be determined by smelling and detecting the absence of alcohol odor from the sample.

X. 50 microliters of distilled water are added to preserve the extracted DNA samples. The extracted sample is then placed in a refrigerator at a temperature of 2-8 degrees Celsius for preservation for one month, or in a freezer at -20 degrees Celsius for preservation for 2-4 years.

To check the quality and the quantity of the extracted DNA we performed agarose gel electrophoresis, and a spectrophotometer check of the purity of extracted DNA. For the electrophoresis 1% TAE solution was used with agarose form vivantis (Malaysia) with Biorad (USA) horizontal electrophoresis apparatus and voltage supply, followed by staining with ethidium bromide Vivantis (Malaysia) at a concentration of 0.5 micrograms/ml for 20 minutes. The stained results can then be visualized under ultraviolet light as a distinct and intact DNA band near the hole. Due to the high molecular weight of the sample, it was found through several experiments that this DNA extraction protocol yields DNA concentration averaging 150 micrograms/ml, as determined by spectrophotometric assays, with good quality and purity as evidenced by the results of gel electrophoresis.

After checking the quality and quantity of DNA samples, a PCR test was conducted to examine feasibility of PCR assays using this DNA extraction method, and the primers were obtained from Macrogen, inc. (Korea) dNTPS and MgCl2 provided from vivantis (Malaysia), Taq polymerase from Genedirex (Taiwan), the thermocycler used was Applied biosystems 9700 (USA), ladder marker 50bp from Genedirex (Taiwan).

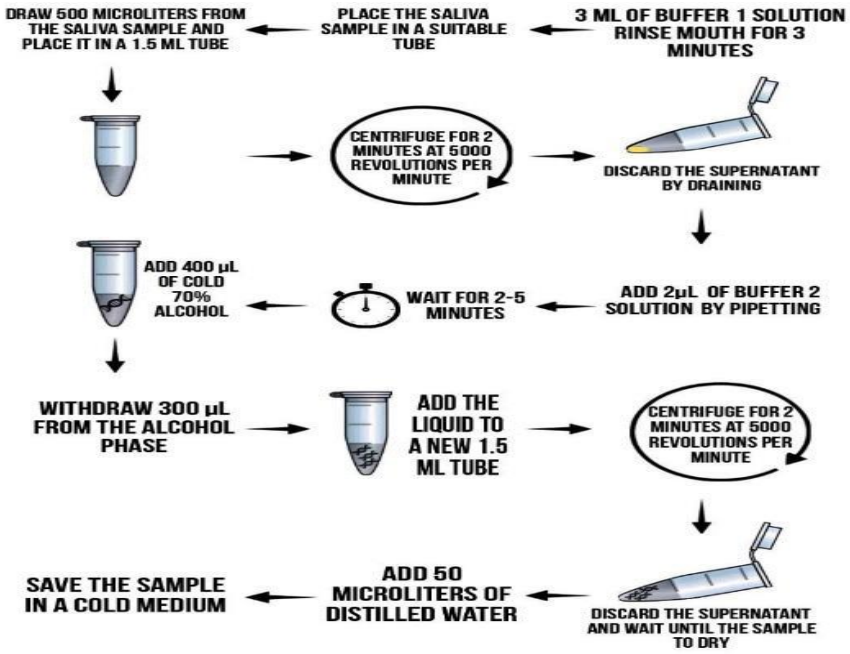

Figure 2. *Basic Steps of the Saliva DNA Extraction Method described in this article. Assessment the quality and quantity of the DNA sample*

Table 1. *The primers used in the PCR reaction. (Shayah et al. 2019).*

Genotype	primer	Nucleotide sequences	Length (bp)	Annealing Temperature	Content CG
VKORC1-1639 G>A	Forward	5′-GAGCCAGCAGGAGAGGGAAATAT-3′	23	62.4	54.5%
	Reverse	5′-CTTCGAAAACATGGAGTTGCAGT-3′	22	64	43.5%

Table 2. *Thermal cycles protocol of polymerase chain reaction*

Thermal Stages	Temperature	Time	Number of Cycles
Stage 1:**Initial denaturation**	94°C	10minutes	1
Stage 2: **Denaturation**	94°C	1minutes	35
Annealing	68°C	1minutes	
Extension	72°C	1minutes	
Stage 3:**Final extension**	72°C	10minutes	1
Storage	4°C		

Table 3. *Polymerase chain reaction reagents and its volumes.*

Series	Reagents	Quantity Used in µL
1	dH_2O	36.2
2	10 X PCR Buffer	5
3	50 mmol/L MgCl2	4
4	10 mmol/L dNTPs	1
5	20 Pmol/L forward primer	0.75
6	20 Pmol/L reverse primer	0.75
7	SU/µL Taq DA polymerase	o.3
9	40 ng sample genomic DNA	2
.	Total reaction volume	50

Results

Sample Measurement Results on the Spectrophotometer:

Spectral analysis was conducted using optical spectrophotometry (Eppendorf 6131 BioPhotometer) at wavelengths of 260 nanometers and 280 nanometers to measure and analyze the state of each sample of DNA extracted from saliva. This device provides two important metrics:

(1) Concentration (nanogram/microliter)

(2) Purity (through the relative absorbance ratio at 260 nanometers/280 nanometers)

Regarding proteins and ribosomal DNA. Samples with a ratio closest to 1.8 indicate relatively pure DNA sample (Adhikary & Kumar, 2022). Ratios below 1.8 significantly indicate higher protein contaminants. Samples are considered pure if the absorbance ratio is between 1.8 and 2.0 (Garbieri et al., 2017). This analysis requires 10 microliters of each sample diluted in 1 ml of distilled water.

Therefore, the extraction of genomic DNA from saliva was stable and effective at each time point tested after 1 day and after 2 months. The quantity of DNA obtained was good, while the purity of the DNA was moderate.

Gel Electrophoresis Results:

To determine the quality and state of the extracted DNA, electrophoresis was performed using agarose gel 1% to further characterize the extracted DNA's state. The following figure (Figure3) provides a representative example of a gel containing 5ul of extracted DNA samples with 1ul of 6x loading dye, where it showed no breakage or contamination in the saliva DNAextraction method suggested in this study.

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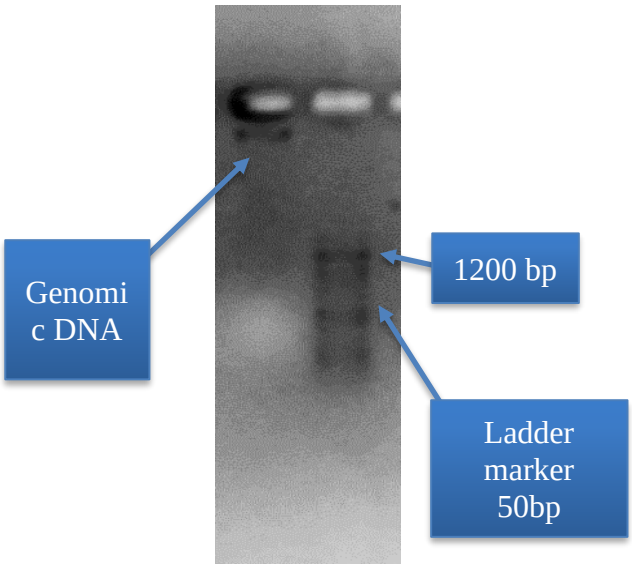

Figure 3. *Results of the Gel Electrophoresis 1% agarose of the extracted DNA, It shows a band near the well representing genomic DNA extracted from a saliva sample, next to it a 50 bp ladder marker as positive control.*

PCR Sample Testing Results:

Traditional PCR was also employed to verify the presence of human DNA. (Figure 4) The extracted DNA was analyzed using polymerase chain reaction (PCR) and electrophoresis to determine the percentage of samples that could be amplified using human DNA-specific primers.

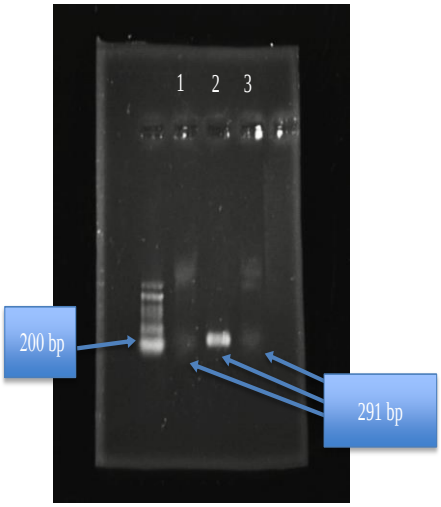

Figure 4. *Results of Samples After PCR Testing, the 291 bp band in sample2 line is an amplicon from a PCR of the extracted DNA using the here mentioned method, it shows a clear amplification in the desired length.*

This program is taken from a study (Shayah et al., 2019).

Extracted DNA using this method was functional to conduct a PCR reaction as shown in figure (4) where the desired band of 291bp was obtained from a DNA sample used by our saliva extraction and sample 2 shows the optimal result while samples 1&3 seems to need a better enhancement, therefore this frugal phase DNA extraction method showed a promising result to be applied for PCR and could be tested for other downstream applications to DNA.

Table 4 illustrates the differences between extracting DNA from saliva and blood using methods such as chemical phenol chloroform extraction, columns, magnetic beads, and designed kits (Endle et al., 1999) (Gupta, 2019) (Hung et al., 2017) (Saiyed et al., 2008), the addressed features in the table are relative to the protocols and materials used in these methods, and the need of experience to do the extraction.

Discussion

It is evident from the foregoing that the designed kit requires less or equal time compared to other saliva and blood extraction methods. Additionally, it is relatively cost-effective due to minimal use of expensive chemicals and utilization of inexpensive household materials, and it achieves many ecological standards, it uses minimum electrical energy and very available and safe chemicals that can be discarded without harm to the environment.

Recommendations

It is recommended to conduct further studies to improve the efficiency of the methods used in DNA extraction prescribed in this research. However, sometimes the available resources are limited, which poses some challenges for low-income countries and low budget laboratories. Therefore, better results have been achieved by performing the extraction process multiple times in 2 repeats, to enhance efficiency.

This method could be applied in molecular biology labs in middle schools, or in a university research lab that uses extracted DNA for downstream assays, such as PCR or RFLP.

Still, this method could be further enhanced and optimized to be more efficient by designing a DIY centrifuge to use in case there is a lab has no functional centrifuge device.

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Table 4. *A comparison between types of human genome DNA extraction methods*

	Time	Cost	Efficiency	Yield	Ease	Quality	Purity	Purification principle	Safety	Need for enzymes	REFERENCES
The designed saliva kit in this article	20 min	Very cheap	Effective	Good	Easy	Good	Good	Separation Phases	Safe	No	
Blood: Chemical	1 hour	Relatively inexpensive	Effective	~ same as beads	Difficult	Good	Good	Phenol chloroform Phases	Toxic materials	Yes	Carpi et al. 2011
Blood: Columns	30 min	Medium	Effective	Medium	Medium	Good	Higher than chemical	Filters	Safe	Yes	Ayoib et al. 2017
Blood: Magnetic bead granules	15 min	High	Highly effective	7.21 micrograms	Difficult	Good	Good	Beads Magnetic	Safe	Yes	Saiyed et al. 2008
Saliva: Chemical	1 hour	Inexpensive	Effective	High	Difficult	Good	Good	Phenol Chloroform Phases	Toxic materials	Yes	Polgárová et al. 2010
Saliva: Columns	30 min	Medium	**Effective**	Medium	Medium	Good	Higher than chemical	Filters	Safe	Yes	Kalyanasundaram et al. 2013
Saliva: Magnetic bead granules	30 min	High	Effective	High	Easy	Good	Good	Beads Magnetic	Safe	Yes	Bhati et al. 2021

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