Optimization ofDNA Extraction Protocol from Banana Fruits

ABSTRACT

Background: DNA extraction is the process by which the DNA is purified from the living or dead cells or tissues of organisms. There are many different kits ~~with~~ commercially available for DNA extraction, with varying protocols. This study aimed at determining the optimal conditions for the extraction of high yield of DNA from banana fruits using different precipitation methods.

Methodology: DNA was extracted from banana fruit by different precipitation methods. The DNA yield was determined using the ~~nanodrop~~ Nanodrop DNA quantification technique.

Results: The optimal sodium chloride salt concentration for DNA extraction from banana fruits was 4mM and the amount of DNA extracted increased as the temperature decreased with the highest yield obtained at 4 ^{0}C to 0 ^{0}C. The pH fluctuation was registered in this study as an extraneous variable.

Conclusion: Optimal DNA extraction from plant living tissue by the precipitation method requires~~,~~ 4mM of sodium chloride ~~salt~~and temperature below the melting temperature~~would yield higherconcentration of DNA~~. Further studiesare required to determine the optimal pH, organic solvents~~,~~ andsample freezing temperature during the DNA extraction protocol.This protocol should be reproduced using other plant parts to be approved for DNA extraction from plant samples.

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Keywords: DNA extraction; banana fruits; sodium ion salt concentration; temperature.

1. INTRODUCTION

DNA extraction is the process by which the DNA is purified from the living or dead cells of~~r~~ tissues of org

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Currently, DNA extraction is a routine procedure in molecular biology[1]. These biomolecules can be isolated from any biological material for subsequent downstream p~~rocesses,~~ analytical~~,~~ or preparative purposes[1, 2].There are many different kits commercially available for DNA extraction[2]. Varying amounts of DNA are obtained with these kits due to the differences in the optimization of concentrations of their reagents and conditions during DNA extraction[3]. It is upon this gap that this study aimed at determining the optimal conditions for the extraction of high yield of DNA from banana fruits using different precipitation methods. In the precipitation method of DNA extraction from plant materials, there are three basic and two optional steps involved. The basic steps include cell lysis, DNA precipitation and ~~Washing~~ washing of DNA pellets and elution of DNA while the optional steps include protein hydrolysis and RNA removal.

Detergents are a class of molecules whose unique properties enable manipulation (disruption or formation) of hydrophobic-hydrophilic interactions among molecules in biological samples. In nucleic acid research, detergents are used to lyse cells in order to release DNA [4]. Moderate concentrations of mild (non-ionic) detergents compromise the integrity of cell membranes, thereby facilitating lysis of cells and release of solubleDNA [4].

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Water is a polar molecule that has a partial negative charge near the oxygen atom due to the unshared pairs of electrons, and partial positive charges near the hydrogen atoms[5]. Because of these charges, polar molecules like DNA can interact electrostatically with the water molecules, allowing them to easily dissolve in water[5]. DNA is hydrophilic due to the negatively charged phosphate (PO_4^-) groups along the sugar-phosphate backbone hence being soluble in water [5].
The role of the salt is to neutralize the charges on the sugar-phosphate backbone[5]. In this study, sodium chloride (NaCl) was used because of its ability to keep sodium dodecyl sulfate (SDS) in the detergent soluble in 95% isopropanol so that it doesn't precipitate with the DNA[5]. In solution, sodium chloridedissociates into Na^+ and Cl^- ions. The positively charged sodium ions neutralize the negative charge on the PO_4^- groups on the nucleic acids, making the molecule far less hydrophilic and, therefore, much less soluble in water[5]. Varying concentrations of salts are used when extracting DNA using different protocols. Therefore, this study assessed the optimal salt concentration that yield high amount of DNA.

The electrostatic attraction between the Na^+ ions in solution and the PO_4^- ions are dictated by Coulomb's Law, which is affected by the dielectric constant of the solution[5]. Water has a high dielectric constant, which makes it fairly difficult for the Na^+ and PO_4^-on DNA to come together. Absolute (95%) Isopropanol, on the other hand, has a much lower dielectric constant, making it much easier for Na^+ to interact with the PO_4^-[5]. This shields the negative charge on the DNA molecule and makes the DNA less hydrophilic, thus causing it to precipitate out of the solution[5].
Incubation of the nucleic acid/salt/ethanol mixture at low temperatures (–20° or –80°C) is commonly cited as a necessary step in protocols. However, according to Maniatis et al this is not required, as nucleic acids at concentrations as low as 20 ng/mL will precipitate at 0–4°C, so incubation for 15–30 minutes on ice is sufficient[6]. This study addressed these differences in the incubation temperatures particularly when extracting DNA from plant materials.
PrecipitatedDNA is normally separated from the rest of the solution by centrifugation. The DNA pellet can then be washed in cold 70% isopropanol and allowed to dry before resuspending in a clean aqueous buffer or DNase free water.
This study reported data on the optimal salt concentrations and temperatures that could be used to extract high yield of DNA from ~~plant~~ banana fruit ~~materials~~. Therefore, the findings of this study may inform on the optimal conditions required to purify high yield of DNA from this specific plant material~~s~~ for further molecular analysis and applications.

2. METHODOLOGY

2.1. Determination of the effect of ~~salt~~NaCl concentration on the DNA yield

A mixture of 25g of peeled banana (*Musa acuminata)*fruit and 50 ml of nuclease free water (Cat.W4502, Sigma Aldrich, Darmstadt, Germany) was blended to dissociate cells and strained to yield a banana fruit solution. Ten (10) ml of 10% Sodium Dodecyl Sulfate SDS (10) (Cat. MB-015, Rockland, Inc., USA) was added into the strained banana fruit solution to lyse the cells and release the DNA. The solution was stirred for 10 minutes at room temperature.The mixture was centrifuged for 5 minutes to separate the debris from the solution.The supernatant was aliquoted into 20 eppendorf tubes each containing 1ml of the supernatant.Different amounts of sodium chloride salt were calculated, weighed, and dissolved in each of the solutions to make different salt concentrations (0.25mM, 0.50mM, 0.75mM, 1.00mM,1.25mM, 1.50mM, 1.75mM, 2.00mM, 2.25mM, 2.50mM, 2.75mM, 3.00mM, 3.25mM, 3.50mM, 3.75mM, 4.00mM, 4.25mM, 4.50mM, 4.75mM, 5.00mM). Each solution was stirred for 5 minutes.Then, 2ml of 99.5mMisopropanol (Cat. I9516, Sigma Aldrich, Darmstadt, Germany) kept at a given temperature was addedgently down the side of the respective tubes to precipitate DNA in the solution. The solution was applied into the QIAamp Mini column and ~~spun~~ centrifuged at 8,000 rpm for 1 minute to separate the DNA precipitate from the solution. The DNA was washed using 500uL of Qiagen DNA wash buffers. The DNA was eluted in 100uL of nuclease free water. DNA concentration was determined using NanoDrop™ 2000 Spectrophotometer (Cat. ND-2000, ThermoFisher) ~~hence~~ and the total amount of DNA extracted was determined.

2.2. Determination of the effect of temperature on the DNA yield

The DNA extraction was performed as described in section 2.1 while varying the temperature of 99.5% absoluteisopropanol from -10°C to 100°C in 5°C increments~~(-10°C, -5°C, 0°C, 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C, 70°C, 75°C, 80°C, 85°C, 90°C, 95°C, 100°C)~~. These temperatures were achieved by cooling and heating isopropanol using fridge/freezer and oven respectively while monitoringusing a thermometer.

2.3. Data analysis

The Pearson's product moment correlation coefficient was used to determine the relationship between the concentration of the sodium chloride salt used and the amount of DNA extracted and the relationship between the temperature and the amount of DNA extracted because the variables are bivariate, and the salt concentrations and isopropanol temperatures were measured on a fixed interval.

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3. RESULTS AND DISCUSSION

3.1. Effects of salt concentration on the DNA yield

Four (4mM) was the optimal salt concentration at which the maximum yield of DNA was obtained. This can be rationed as the mass of salt to mass of sample solution~~as~~ of1:25(Figure 1A). There was a very strong positive and significant correlation between the sodium chloride salt concentration and the amount of DNA extracted before the optimal concentration of sodium chloride salt (Figure 1B; r = 0.998; *P* = *.001*). There was also a very strong negative and significant correlation between the sodium chloride salt concentration and the amount of DNA extracted after the optimal concentration of sodium chloride salt (Figure 1C; r = -0.995; *P* = *.001*).

The strong correlation between the DNA yield and the sodium chloride concentration shows that ~~that~~ the amount of DNA extracted highly depend on the ionic strength [7]. Sodium chloride is added as the negatively charged DNA molecules bind Na^+ and this encourages the free strands of DNA to group together and form larger strands of DNA[8]. The steady increase in the DNA yield along the increasing sodium chloride concentration before the optimal salt concentration (4mM) could be due to the formation of larger DNA toroids [7]. Further increase in the sodium chloride concentration beyond the optimal salt concentration forms fiber-like condensates instead of toroids hence leading to a sharp fall in the DNA yield [7].

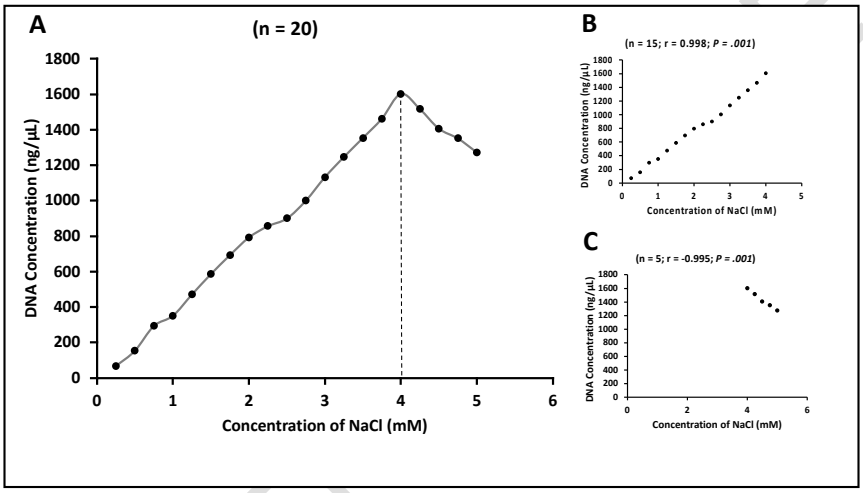

Figure 1. Effects of salt concentration on the DNA yield: **A** *is the DNA yield across various salt concentrations,* **B** *is the correlation between the sodium chloride salt concentration and the amount of DNA extracted before the optimal concentration of sodium chloride salt (r = 0.998; P = .001) and***C** *is correlation between the salt concentration and the amount of DNA extracted after the optimal concentration of salt (r = -0.995; P = .001).*

3.2. Effect of temperature on the DNA yield

There was a very strong negative and significant correlation between the temperature and the amount of DNA extracted (Figure 2; r = -0.997; *P* = *.001*).

Higher DNA yield is expected at a temperature lower than its melting temperature compared to the DNA yield obtained at a temperature higher than the DNA melting temperature. However, these optimal temperatures may vary depending on the melting temperature and DNA abundance in a given sample [9]. However in a study by Markegard et al (2016) on the effect of DNA concentration on the complementary base pairing of the DNA strands, it was found ~~out~~ that DNA abundance accounts for only about 2% of the total DNA molecules in a given sample[9]. At higher temperatures, there is excessive thermal energy that destabilizesDNA helix and double strand structureshence giving a negative activation energy to complementary base pairing [10] and this could negatively affect DNA precipitation. This is consistent with the notion that as the temperature approaches melting temperature (T_m), the strands favor the melted state hence promoting dissociation of the DNA strands [11].

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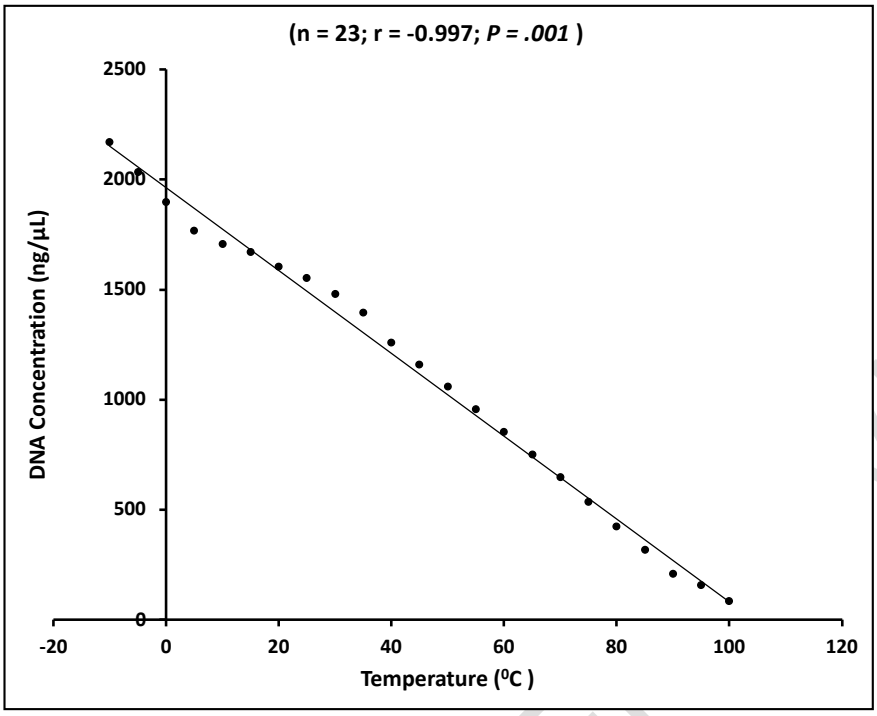

Figure 2. **Effect of temperature on the DNA yield:** *Correlation between the temperature and the amount of DNA extracted (r = -0.997; P = .001)*

4. CONCLUSION

In the extraction of DNA from ~~Banana~~ banana fruits, by the precipitation method, 4mM of sodium chloride and temperature below the melting temperature of DNAyielded the highestconcentration of DNA. However, the DNA yield is not solely dependent on the salt concentration and temperature. There are also other factors like size of the DNA fragment, pH, cationic polymers, organic solvents, freezingand crowding agents that could influence the amount of DNA extracted from a given sample. Therefore, there is a need for extensive studies on these parameters for optimization in order to overcome the burden of low yield DNA extracts. This protocol should be reproduced using other plant parts for the protocol to be approved for DNA extraction from plant samples.

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