

# MODIFYING AND ADAPTING A PLANT-BASED DNA EXTRACTION PROTOCOL FOR HUMAN GENOMIC DNA EXTRACTION: A COST EFFECTIVE APPROACH

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## ABSTRACT

This research involved modification of a standard protocol for DNA extraction in plants for its suitability as an extraction method for DNA in human whole blood in which some of the plasma was removed. Human blood samples were obtained from a 100 apparently healthy individuals residing in Calabar. The modified DNA procedure yielded good quality genomic DNA which was used in carrying out allele specific polymerase chain reaction which also yielded good quality amplicons. This method is simple and suitable for the extraction of DNA from human red cell.

**KEYWORDS:** Genomic DNA, Human red blood cells, amplicons

## INTRODUCTION

The extraction of human genomic DNA is obviously a crucial step in molecular techniques such as genotyping, DNA fingerprinting, diagnostics and forensic analyses. Undoubtedly, the process can be tedious, time consuming and may involve multiple steps in order to obtain high quality DNA (Maurya et al., 2013).

The poor research facilities and scientific development in Sub-Saharan African countries orchestrated by poor research funding as well as difficulty in assessing test kits has placed heavy economic burden on many patients whose samples are to be analyzed. The implication of the above is that a cost effective, safe, rapid and reliable DNA extraction protocol becomes imperative for use, especially in smaller laboratories.

Understandably, many economic and in-house methods for DNA extraction from mammalian peripheral blood exist namely salt-out technique (Miller et al., 1988), phenol-chloroform extraction (Sambrook et al., 1989) and QIAamp DNA Blood Kits methods (Maurya et al., 2013). Maurya et al. (2013) comparing salt-out as modified, phenol-chloroform and DNA extraction kit method showed that modified salt-out protocol gave higher human genomic DNA yield of 40.8µg/ml followed by phenol-chloroform methods (38.5 µg/ml) and kit method (35.3µg/ml).

They also observed that the amplification products from the extracted DNA were of high quality for amplification-based diagnostic techniques.

These thrilling results for the modified salt-out method (Maurya et al., 2013) notwithstanding, the cost effectiveness is doubtful bearing in mind the society to which the technique is to be adapted. The DNA extraction method by Dellaporta et al. (1983), a plant-based technique produces high quality DNA in plant species (Madhan-Shankar et al., 2009) and can be modified for use in the extraction of human genomic

DNA. The beauty in Dellaporta's technique modification is that its reagents are cheap, easily available and can be adapted in any laboratory with high quality and quantity of DNA products.

This research is aimed at modifying Dellaporta's plant-based DNA extraction methods for use in the extraction of human genomic DNA for molecular diagnostic purposes.

## MATERIALS AND METHODS

### Collection of blood samples

Human whole blood samples of a 100 apparently healthy individuals who reside in Calabar and formed part of a larger study population for molecular studies. Ethical approval was granted for the molecular studies by the University of Calabar Teaching Hospital, Calabar. The blood was initially collected into EDTA bottles, 2/3 of the plasma was removed for other tests but the buffy coat was not removed. The samples were kept frozen at -80 ° C and thawed only when DNA extraction was to be carried out.

### DNA extraction process

One hundred and fifty microlitres (150µl) of human whole blood sample was transferred into 1.5ml eppendorf tube. Three hundred and fifty microlitres (350µl) of extraction buffer was added. The tubes were inverted 3-4 times and incubated in water bath at 65 C for 10 minutes. This mixture was allowed to cool at room temperature. 160µl 5M potassium acetate was added to the tubes, inverted and centrifuged at 10,000 r/min for 10 minutes. 400µl of the supernatant was carefully transferred into new eppendorf tubes and 200µl of cold isopropanol was added, inverted 5-6 times gently to precipitate DNA and kept at 4 C for 15-20 minutes.

The tubes were removed and centrifuged at 10,000 r/min for 10 minutes to sediment the DNA. The supernatant was decanted gently to ensure that DNA

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pellets are not distorted. The pellets were washed using 500µl cold ethanol and centrifuged at 10,000 r/min for 5-10 minutes. The ethanol was decanted and the DNA was air-dried at room temperature. The extracted DNA was re-suspended in 50µl of Tris-EDTA-TE buffer. DNA quantity was estimated by running an aliquot of the DNA on agarose gel electrophoresis (100ml 1xTAE + 0.8g agarose powder + 0.8µl ethidium bromide) with 110V for 45 minutes.

The amount of DNA extracted in the sample was quantified by checking the intensity of the bands on the gel and was scored as faint, low +, medium 2+ and high 3+ with the 3+ indicating a larger amount of DNA than 2+ and a + fig 1.

### Polymerase chain reactions

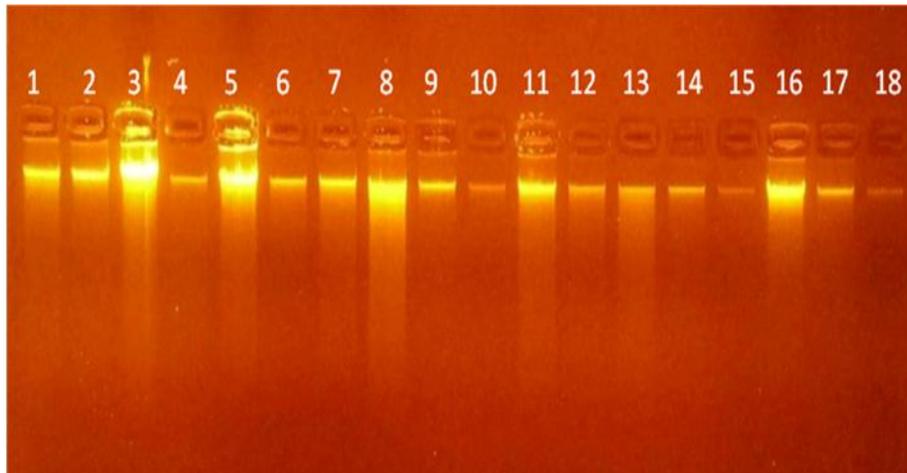
DNA samples were diluted to 1/10, 1/50 and 1/100 depending on the amount of DNA extracted. Two microliters of genomic DNA was amplified in a 15µl reaction mixture containing 7.5µl GoTaq<sup>®</sup> green master mix (Promega), 0.30µl each upstream and downstream oligonucleotides primers and 4.9µl nuclease-free water. For testing A1166C polymorphism of the angiotensin II type I receptor gene, the primer sequence used (La Pierre et al., 2006) was; 5'-AATGCTTG TAGCCAAAGTCACCT - 3' and 5' - GGCTTTGCTTTGTCTTGTTG - 3' with cycling conditions as follows – initial denaturation at 94 C for 2

minutes followed by 40 cycles of further denaturation at 94 C for 1 minute, annealing at 60 C for 1 minute, extension and final extension at 72 C for 2 minutes and 72 C for 10 minutes, respectively. However, the primer specific PCR for M235T allele of the angiotensinogen gene was 5'-CAGGGTGCTGTCCACACTGGACCCC-3' and 3'-CCGTTTGTGCAGGGCCTGGCTCTCT - 5' with cycling conditions as follows- initial denaturation at 95 C for 10 minutes followed by 35 cycles of further denaturation at 94 C for 1 minute, annealing at 56 C for 1 minute, extension and final extension at 72 C for 1 min 30 secs and 72 C for 10 minutes.

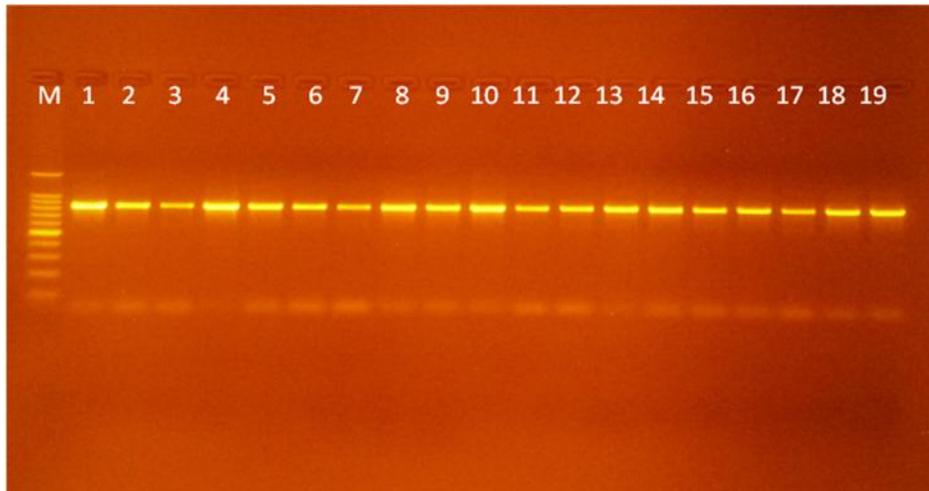
### RESULTS

Our result showed that the modification of the Dellaporta et al.'s protocol for use in the genomic DNA extraction from packed human red blood cells yielded good quality DNA. It was also observed that the amount of DNA extracted as quantified by the banding intensity was high, especially in individual samples on lanes 3, 5, 8, 11 and 16, respectively (Fig. 1) having 3+.

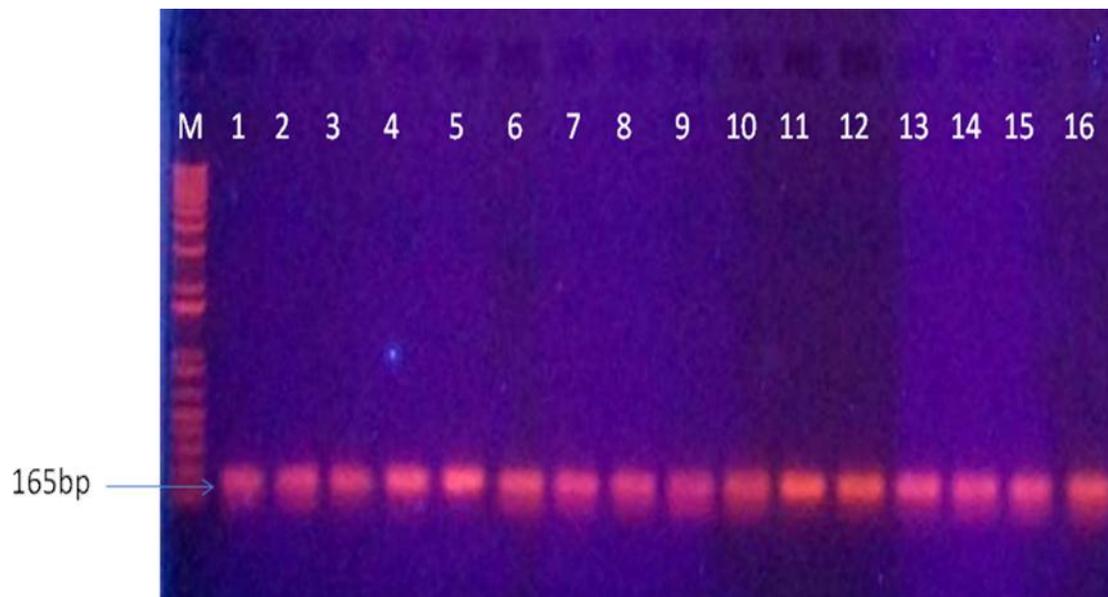
Gel electrophoresis result after amplification of the angiotensin II type I receptor gene and angiotensinogen gene revealed that polymerase chain reaction produced good quality amplicons (Figures 2 and 3).



**Fig.1:** Gel electrophoresis showing DNA quality after extraction and the amount of DNA [(Lanes 3, 5, 8, 11 and 16 as high 3+); (Lanes 1, 2, 7 and 13 as medium 2+); (Lanes 4, 6, 9, 12, 14 and 17 as low +); (Lanes 10, 15 and 18 as faint)]



**Fig. 2:** Agarose gel electrophoresis showing the 850bp PCR product of the Angiotensin II type I receptor gene. (Lane M is the 100bp DNA ladder Lane 1-19 are the amplified PCR product)



**Fig. 3:** Gel electrophoresis showing 165 bp PCR products after amplification of the angiotensinogen gene. (Lane M is the 1kb plus DNA ladder while lanes 1-16 contain the amplified PCR product)

## DISCUSSION

A successful biomarker genotyping, DNA fingerprinting, diagnostics and forensic analyses depends majorly on the quality and quantity of PCR products. Any DNA extraction technique to be canvassed for and adapted for use, especially in the Sub-Saharan African countries that are synonymous with poor infrastructural capacity and decay, poor research funding culture and high poverty level should be cost effective without compromising quality and quantity.

Maurya et al. (2013) reported a modified salt-out DNA extraction method previously used by Miller et al., (1988) that gave higher DNA yield when compared with phenol-chloroform method (Sambrook et al., 1989) and

QIAamp DNA Blood Mini Kits method. Our observation was that the processes and reagents were almost the same. In our report, we modified a plant-based DNA extraction by Dellaporta et al, 1983 protocol by substituting chloroform-isoamyl alcohol with isopropanol and eliminating the addition of pancreatic Ribonuclease A (RNase A) in extraction process. Dellaporta's method produced high quality DNA in plant species (Madhan Shankar et al., 2009). After the modification, good quality genomic DNA was also produced from packed human red blood cells, which were confirmed by the intensity of the bands produced. According to Hu et al. (2012), blood samples have been reportedly used as the best source of genomic DNA.

The quality of DNA extracted is directly proportional to the extent to which the products are degraded and their amplifiability using PCR. Our result corroborates the report of Maurya et al. (2013) who observed high unique amplification products, which demonstrated excellent performance of the isolated DNA for amplification-based diagnostic techniques. This was tested by checking the amplification PCR products of angiotensin II type I receptor gene and angiotensinogen gene. Kooffreh *et al.* (2013a) adapted the modified Dellaporta's protocol to extract DNA from human whole blood to study the M235T variant of the angiotensinogen gene. The DNA extracted from this method showed good amplification products and were cleanly digested, suggesting high quality DNA products. Kooffreh et al. (2013b) also reported its use to extract DNA for amplification in PCR to establish the relationship between Angiotensin II type 1 receptor polymorphism and hypertension, with thrilling results.

## CONCLUSION

This is explicitly a prove of concept that modifying and adapting Dellaporta's plant-based DNA extraction protocol to extract genomic DNA from human whole blood yielded sufficient amount and good quality genomic DNA, which could be used for human genetic studies.

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