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Microsatellite (mEgCIR3808) Analysis in Oil Palm: An Optimisation of a Direct PCR-based using FTA Card as a Storage Medium

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Abstract

This paper explores the effectiveness of Flinders Technology Associates (FTA) card, with a focus on the role of FTA card application in storing oil palm DNA for PCR analysis. The study underscores the importance of optimising the washing steps of FTA card and validation of PCR amplification of using optimised washing steps towards oil palm leaves. Therefore, the study specifically examines practices for FTA washing method for oil palm sample which is crucial and necessary in obtaining promising results. The optimised washing steps such adding centrifugation while washing the FTA cards matters and has proven to enhance the band intensity of the PCR amplification results. An optimised, proper and accurate first-basis fundamental steps will contribute the value of SSR fingerprinting as a molecular detection for *Elaeis guineensis,* highlighting the potential of genetic markers in oil palm research.

1. Introduction

The oil palm (*Elaeis guineensis* Jacq.) exhibits significant genetic variation present in both among and within oil palm population that has a direct impact on plant's productivity and adaptation towards environment [1]. Genetic analysis allows early identification of oil palm planting material and plays a vital role in understanding the genetic diversity, population structure and inheritance patterns of oil palm species [2], [3], [4].

Understanding the effectiveness of DNA molecular markers is a prerequisite in plant characterisation since DNA extraction method is a major element of PCR analysis of plants [5]. Among these markers, microsatellite obtained prominence due to its prevalence, highly polymorphic and co-dominance inheritance [6]. Traditionally, microsatellite analysis time-consuming and costly procedures, including DNA extraction from fresh or frozen tissue samples [7].

However, difficulties arise when preserving and transferring plant tissues, particularly from remote oil palm plantations [8]. Moreover, the DNA extraction process can be prone to DNA degradation and contamination may compromised the results [9].

To overcome these challenges, alternative methods for DNA preservation and storage has been explored where the FTA (Flinders Technology Associates) card has gained popularity among researchers due to its simplicity and effectiveness [7], [4]. However, the contribution of the FTA card as a medium in storing the DNA has received little attention within oil palm studies. A paper-based technology called FTA that contains chemically treated filter paper lyses cells upon contact, preserving DNA in a stable form for subsequent analysis [10], [11]. It was developed to directly fix and store nucleic acids from freshly pressed mature oil palm tissues. Besides, the stored DNA in FTA card's matrices does not need prior purification and DNA extraction steps required [12].

The unique design and composition of FTA card enables efficient and stable preservation of genetic material, such as DNA, without the need for low temperature storage or specialised equipment [4], [12]. Due to that, FTA card is a highly transferable, compact, not involving harsh chemicals useful for fieldwork, remote sampling, and the transportation of samples from locations with limited access to lab facilities [13]. Not only that, FTA card also has simpler method in sample processing such collecting samples in form of FTA punch from FTA card, and FTA washing as recommended by the manufacturer's protocol [14]. However, since oil palm used as DNA sources, the modification of FTA washing and additional step which is elution step and incubation of FTA elute are necessary to be included in washing steps part as it is lies within the basic principles of the DNA extraction [15].

This paper highlights on optimising a direct PCR-based method via FTA cards towards oil palm samples onto microsatellite markers; mEgCIR3808. The main to enhance the integrity of the results of PCR amplification of direct PCR-based method. The findings contribute to the optimised FTA washing steps in microsatellite analysis in oil palm through direct PCR-based using FTA as a source of medium storage. The attempt of using optimised washing steps able to be successful applications of FTA card in plant studies involving huge number of samples that suggest their potential suitability for plant conservation research [16] and plant population genetics [15] where the analysis of enormous number of samples are often required.

1.1 Overview of The Oil Palm

The *E. guineensis* species, also known as the African oil palm, is indigenous to the southwest and west of Africa that becomes primary source of oil palm and widely planted for commercial purposes [17]. Oil palm tree composed of five main parts such leaves, trunk, adventitious root system, spikelet; both male and female flower and fruit. Leaves were commonly to be used as source of DNA. In comparison to other plant taxonomies, the polysaccharides and secondary metabolites were notably greater in oil palm leaves, causing the leaf structure to develop bulky, open leaves with high tearing resistance and yet low tensile strength [8], [1]. Oil palm leaves have distinct characteristics, making it essential to choose leaves for sampling based on their maturity stages to extract DNA with high quality.

Fig. 1 *The structure of an oil palm morphology*

Fig. 1 shows the structure of oil palm morphology and the types of leaves. There are three levels of oil palm leaves which are young, mature and oil leaves. Young leaves located at the centre and top of the oil palm trunk (labelled as 1 to 8; containing high quality DNA due to lower in starch deposition, numerous cells and low levels of secondary of metabolites. However, accessing these leaves requires significant efforts that makes mature

(labelled 9-40) and old leaves (labelled 41+) as an alternative although they yield slightly lower in DNA quantities [18]. Mature leaves are considered a good source of high-quality DNA as compared to old leaves that contain higher concentrations of secondary metabolites that may interfere with assays [8]. Despite of the difficulty in achieving young leaves, the use of mature and old leaves is justified. Although slightly challenging to extract DNA from mature leaves, it able to provide enough quantities suitable for PCR amplification [19]. Therefore, each of oil palm leaves part have able to give good results of PCR amplification.

1.2 Storage Medium for Oil Palm Leaves

Choosing a storage medium requires consideration in available resources and specific requirements of the further analysis [20]. The collection and handling samples has posed significant considerations prior to plant molecular analysis. It is vital to find effective and dependable transportation techniques that minimise the loss of DNA quality [21]. Therefore, DNA extraction method is the commonly used in preserving the DNA quality and integrity. There are five standard principles of DNA extraction that remain unchanged across all experiments. Fundamentally, genomic DNA isolation include five central steps: (i) cell lysis- removal of cellular structure such as cell walls or cell membranes around the DNA allowing the release the DNA, (ii) separation and precipitation, (iii) purification, (iv) washing and (v) elution steps [22].

It begins with the lysis method where cell lysis three different methods such as physical method, chemical and enzymatic method. Then, precipitation of DNA can be performed using precipitation using isopropanol, filtration, centrifugation or even bead based [9]. Afterwards, the purification matrix binding can be done through ion exchange, cellulose binding, solution-based or silica-based then proceeds DNA washing using alcohol before eluting the DNA in low -ionic strength buffer that could be either nuclease-free water or TE buffer [8].

In plant molecular biology research, DNA extraction is a critical step, and the PCR method such direct or indirect can have an impact on the subsequent amplification method. Direct PCR speeds up the DNA extraction procedure by enabling the use of unprocessed samples directly in the PCR reaction and this technique saves time but may reduce sensitivity because of the possibility of impurities [23]. Contrarily, according to [23], indirect PCR requires an additional DNA extraction step to obtain purified DNA templates, ensuring higher sensitivity and specificity but at the expense of more work and time.

Application of direct PCR-based using a paper-based, ultra-rapid technology called FTA has filter matrices that have been saturated with a proprietary chemical formulation that results in cell lysis and make it less complicated to collect and immobilise nucleic acids [24]. The FTA card was developed as an optional for commercial DNA extraction techniques that may be difficult and time-consuming for large-scale field sampling [5], which may require a lot of harsh chemicals that can degrade DNA over time, and need more storage space, such as a storage room at -80°C or -20°C [25]. It can apply up to 500 ul of sample volume and has two to four areas [10]. The card includes chemical denaturants, and a free radical scavenger are both present in the two main chemistries that make up FTA technology, they can both directly lyse cells, denature proteins, and shield DNA from deterioration [26]. When using FTA, the proteins and inhibitors in the matrix are washed away while the DNA is kept firmly bound. This helps to inactivate life forms and stop the growth of bacteria and other microorganisms [10].

Table 1 described the comparison between direct PCR-based and indirect PCR-based based on basic DNA extraction principles. Based on the Table 1, in direct PCR-based, it requires rapid oil palm tissue leaves homogenisation using pestle and mortar with the presence of DNA extraction buffer [12]. According to the [9], direct PCR-based also known as physical DNA extraction method, or paper DNA extraction as it is a cellulose-based paper that use physical method in cell lysis. The cellulose binding mechanism has resulted the FTA matrix's fibres physically trap the large nucleic acids, which are then preserved intact, while the cellular debris can be quickly removed by giving the inoculated card an instantaneous washing [24], [26].

The first step of the extraction protocol has already been fulfilled as cell lysis takes place on the card itself that consisting simple elution and purification that sufficient enough to isolate genomic DNA. The FTA card does not need preservative reagent and only uses elution buffer only [24]. The involvement of centrifugation in important in removing any unwanted substances and debris [22]. According to [2], [24], the centrifugation and elution steps are important in obtaining the genomic DNA. Besides, the manufacturer's protocol requires further modification especially in washing and elution steps since it is critical in successfully obtained results for downstream analysis when applying oil palm leaves as DNA sources as it is considered as high-level polysaccharides plant [19], [27].

1.3 Microsatellite Marker (mEgCIR3808)

DNA molecular marker have revolutionised in the genetic study and remains to be crucial in clarifying the genetic basis of oil palm [28]. These markers demonstrate heritable genetic variations that are easier to identify and examine since they are derived from specific DNA sequences. Since the 1980s, molecular marker implementations have been on increasing popularity. An important turning point in plant genomic research

came ten years later with the creation of PCR-based DNA markers [6]. Since that time, a wide range of genetic markers have been acknowledged for use in plant genomics and molecular breeding [29]. Microsatellites, single nucleotide polymorphisms (SNPs), and amplified fragment length polymorphisms (AFLPs) are only a few of the various DNA molecular markers that have been developed and used in oil palm molecular identification due to the development of molecular techniques like the polymerase chain reaction (PCR) [30].

Table 1 *Comparison DNA extraction principles between direct PCR-based and indirect PCR-based [11], [24]*

Direct PCR-based	DNA extraction principles	Indirect PCR-based
Physical method using pestle and mortar	Lysis	1)Physical method such liquid nitrogen or grinding 2)Chemical method such SDS and chaotropes 3)Enzymatic method
Centrifugation	Precipitation	1)Precipitation using isopropanol/ alcohol 2)Filtration 3)Centrifugation 4)Bead-based
Cellulose binding	Purification (Purification matrix binding)	1)Ion exchange 2)Cellulose binding 3)Solution-based 4)Silica-based
QIAcard FTA wash buffer and TE buffer	Washing	Alcohol
No stated	Elution	Low ionic-strength buffer (e.g.: nuclease-free water and TE buffer)

The molecular foundation for DNA fingerprinting is provided by simple sequence repeats, commonly known as microsatellites, which use a set of primers to amplify repetitive DNA [31]. In the genomes of different species, microsatellites are a class of one to six nucleotide tandem repeat motifs that are widely distributed, multi-allelic [32] distributed at random, and polymorphic [29]. Microsatellites have numbers of advantages such as it is highly polymorphic even in closely related lines, it only requires minimal amount of DNA, capable of being exchanged between laboratories, easily automated for high throughput screening, and are highly transferable between populations that makes microsatellites have emerged as the marker of choice in many areas of plant molecular identification [4]. Besides, the distinctive features of microsatellites which is a type of co-dominant marker that able to distinguish between homozygotes and heterozygotes had become microsatellites widely employed in various oil palm genetic studies such as genetic diversity analysis, species identification, and genetic linkage mapping.

2. Methodology

2.1 Materials and Reagents

The reagents and chemicals that were used in this study were distilled water (BioPure, Malaysia), molecular biology grade water (Lonza, USA), QIAcard FTA wash buffer reagent (GE Healthcare, Buckinghamshire, UK), 1x TE buffer (EMD Millipore Corporation, Germany), 10x TBE buffer (50mM Tris-base; pH 8 (Thermo Fisher Scientific, USA), Boric acid (Merck, Germany) and Na_2EDTA (J.T. Baker, USA), Agarose powder (Thermo Fisher Scientific, USA), Gelred nucleic acid staining (Merck, Germany), Quickload 100 bp DNA ladder (New England Biolabs, Ipswich, MA), SSR primer (Next Gene Scientific Sdn Bhd, Malaysia), 6x DNA loading dye (purple) (Transgene Biotech, Beijing, China), 5x Hot FIREPol Blend master mix (Solis Biodyne, Estonia).

2.2 Plant Materials and Sampling Location

A total of 25 samples of fresh oil palm leaves were collected from oil palm plantation labelled P1S1_A1, P1S2_A2, P1S3_A3…P1S25_A25. The samples were kept in tight zip lock plastic bag individually, labelled, placed in the box and brought back to the laboratory. The samples were immediately prepared within a 24-hour collection period.

2.3 Sample Preparation Using Direct PCR-Based

Each of the leaf tissues used was wiped to remove any presence of dust, dirt that will interrupt the collecting process of DNA. The 0.4g of leaf was weighted using a weighing balance. A few drops of water were added to

allow some moisture and crushed using mortar and pestle. The crushed leaf was placed onto the FTA card (on the circle part) by using a pair of tweezers. To prevent leaf from embedded onto the FTA cover, a parafilm was cut into small sizes and placed on top of the pasted crushed leaves on the circle. A pestle was used to press and roll the FTA card to ensure obtaining green extract of the leaves. Upon completion, the FTA card containing the sample was labelled and kept in the zipper plastic bag and stored at room temperature.

2.4 FTA Preparation for Direct PCR-Based

2.4.1 Manufacturer's Standardised of FTA Washing Steps

According to manufacturer's protocol [11], a punch of desired spot of FTA card was collected using punching device and was placed in PCR tube. A 200 ul of QIAcard FTA wash buffer was added into the PCR tube and was let to be incubated for 5 minutes at the room temperature. The QIAcard FTA wash buffer removed and discarded using a micropipette. The washing steps using QIAcard FTA wash buffer were repeated twice that makes a total of three washes. Then, a 200 ul of TE-1 buffer containing 10mM Tris-HCl, 0.1mM EDTA, pH 8.0 into the same PCR tube and let it incubate for another 5 minutes. The TE-1 buffer was removed and discarded using micropipette. The TE-1 buffer was added for once that makes a total of two washes. The punch was allowed to be dried at room temperature approximately about three hours or with heat assist drying for about 10 minutes at 56°C. The dried FTA punch was ready to be used in PCR.

2.4.2 Modified FTA Washing Steps

Table 2 summaries the comparison modification FTA washing sample with manufacturer's protocol and previous studies. According to the previous studies [12], [15], [27], the FTA washing method has been modified several times to ensure the FTA card can be unbound and used as a DNA source especially for the high-level polysaccharide plants. With the purpose to standardise the FTA washing method, we developed FTA elute as product for FTA washing step using oil palm as DNA source. From the FTA elute itself, the DNA content that has been used in PCR component can be measured and standardised the volume and assumed that the PCR product able to successfully amplified compared to the directly used washed FTA disc. Therefore, several modifications have been made. The most significant modifications where we introduce the centrifugation steps after every washing step and the usage of two FTA discs in direct-PCR based that has not yet been reported. While other modifications such (1) two times washing using FTA purification reagent, (2) necessary to air-dried FTA disc before elution step, (3) involve usage of 80ul of TE buffer as elution buffer and (4) incubate FTA elute at 95°C for 5 minutes have been supported by [15], [27]. Other than that, modification made on (1) no incubation step after both FTA purification reagent and (2) no transferal of washed FTA disc into new PCR tubes have been fortified by [32].

Table 2 *Comparison of modification direct PCR-based method using FTA card with previous studies and manufacturer's protocol*

Steps	Source 1 (manufacturer's recommended)	Source 2 [12]	Source 3 [27]	Source 4 [15]	Modification
Type of FTA card	Based on manufacturer's protocol	FTA classic card	Whatman FTA card	FTA Plantsaver card	QIAcard FTA card
Number of FTA disc	1 (both 2mm or 6 mm disc)	1 (2.0mm disc)	1 (6mm disc)	8 (2.0mm disc)	2 (2.0mm disc)
Washing step using FTA purification reagent	3 times	3 times	2 times	2 times	2 times
Require incubation after washing	No	No	Yes (Incubate for 4 minutes after washing)	No	No
Washing step using TE buffer	2 times	2 times	2 times	2 times	2 times
Transferal of FTA disc	No	No	Yes	Yes	No
Need to be air-dried	Yes (Air-dried and	Yes (Air-dried and use	No	No	No

	use directly)	directly)			
Elution step	No elution buffer	No elution buffer	80ul of TE buffer	80ul of TE buffer	80ul of TE buffer
Vortex step included	Yes	Yes	Yes	Yes	Yes
Centrifugation (after done/ every washing process)	No	No	Yes (Only after done washing steps)	Yes (After every washing steps, remove discs, centrifuged	Yes (After every washing steps)
Incubation time for 5 minutes at 95°C	No	No	Yes	Yes	Yes

Fig. 2 illustrate the 16 possibilities that have been chosen to be used on the FTA washing step that includes (i) centrifugation speed, (ii) clarity of vortexed FTA disc, (iii) incubation of FTA elutes and (iv) usage of TE buffer. Firstly, for centrifugation speed consist of two different speeds which are 2500rpm and 6200rpm. The centrifugation step helps to separates particles between their size and density [33] and also ensure that the DNA will not be discarded during removal of both FTA purification reagent and TE buffer. Then, for the clarity of vortexed FTA disc whether it should be vortex for one minute that results in clear state of FTA disc or vortex for about 10 secs to produce semi-clear state of FTA disc. Afterwards, involvement of incubation period of FTA elutes and usage of TE buffer as elution buffer were tested on the washing troubleshooting.

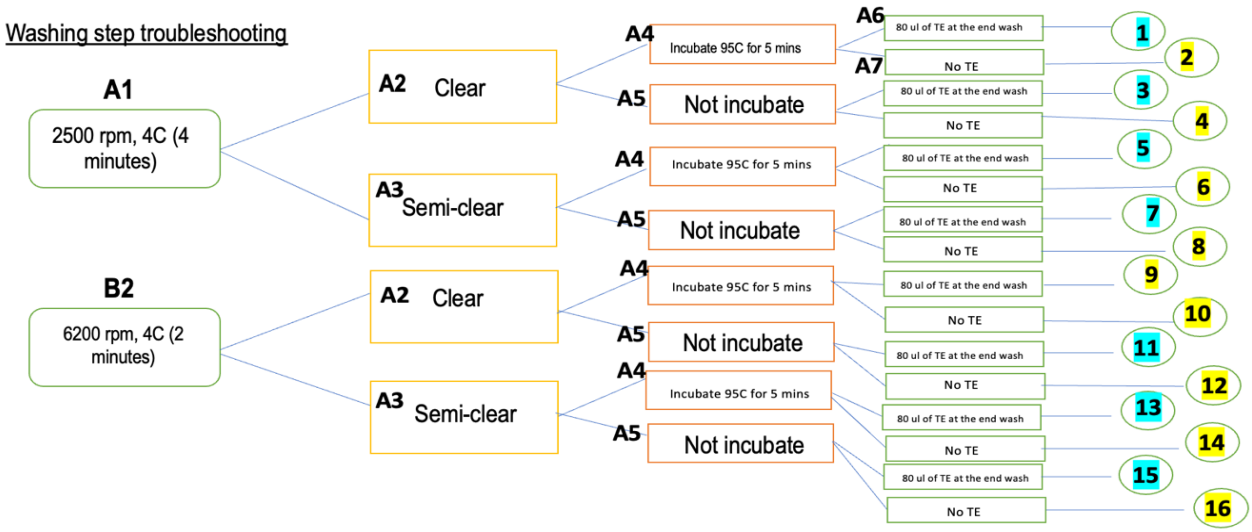

Fig. 2 *Tree diagram of 16 possibilities of washing step troubleshooting*

Based on the washing step troubleshooting, the modified washing step begins with the clean and sterile PCR tubes were placed on the PCR tube rack and labelled according to PCR gradient temperature. Example: for plantation 1 denoted as P1S1_A1, P1S2_A2, P1S3_A3…P1S25_A25 and for plantation 1 denoted as P2S1_A1, P2S2_A2, P2S3_A3 until P2S25_A25. Harris MicroPunch was sterilised by punching to a clean paper. About 2 disc of FTA card was punched using Harris MicroPunch and inserted into a labelled PCR tube. A 200 ul of QIAcard FTA wash buffer was used to wash the FTA sample. The samples were vortexed and centrifuged at 6100 rpm, 2°C for one minute. Discard the QIAcard FTA wash buffer. Repeat for once that makes a total of two washes using QIAcard FTA wash buffer. Then, a 200 ul of TE buffer was pipetted and inserted into the PCR tubes. The samples were vortexed and centrifuged at 6100 rpm, 2°C for one minute. Discard the TE buffer. Repeat for once that makes a total of two washes using TE buffer. The FTA elute was allowed to be incubated for 5 minutes at 95°C. The FTA elute then was allowed for air-dried for an hour at room temperature or 20 minutes under drying cabinet.

2.5 PCR Profile

Table 3 shows the standardised PCR profile by manufacturer, while Table 4 shows optimised PCR profile.

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Table 3 *Standardised PCR profile by manufacturer*

PCR components		Volume (ul)	
The two disc of FTA card		-	
Reverse primer (10mM)		0.2–0.6	
Forward primer (10mM)		0.2-0.6	
5x Hot FIREPol Blend master mix		4.0	
Sterile distilled water		Up to 20 ul	
	Total volume	25.0	
Steps	Temperature (°C)	Time (Min)	Cycle
Initial denaturation	95	12-15	1
Denaturation	95	10–20 s	25-30
Annealing	Ta	0.5-1	
Extension	72	20s-4 min	
Final extension	72	5-10	1

ul- microlitre; mM- millimole; min-minutes; °C- degree Celsius

Table 4 *Optimised PCR profile*

PCR components		Volume (ul)	
FTA elute		4.0	
Reverse primer (10mM)		1.0	
Forward primer (10mM)		1.0	
5x Hot FIREPol Blend master mix		4.0	
Sterile distilled water		16.0	
	Total volume	25.0	
Steps	Temperature (°C)	Time (Min)	Cycle
Initial denaturation	94	4	1
Denaturation	94	0.5	35
Annealing	Ta	1.5	
Extension	72	1	
Final extension	72	10	1
Holding	10-12	∞	1

ul- microlitre; mM- millimole; min-minutes; °C- degree Celsius

2.6 Primer Optimization Via FTA Card

PCR was conducted in a 25 ul reaction volume that containing 4ul FTA elute, 1 ul reverse primer (5'CCGCTAACTTGGTATAC-3'), 1 ul of forward primer (5'ATTTCCAGCAGCTATTC-3'), 4 ul of 5x Hot FIREPol Blend master mix and 19 ul of sterile distilled water. Gradient temperature was set at temperature between 41.0 to 51.0°C. PCR cycling conditions begins with initial denaturation 94°C for 4 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, optimised annealing temperature gradient for 90 seconds at 41.3°C and elongation steps for 1 minutes at 72°C. Final extension at 72°C for 10 minutes and PCR was hold at ∞ for 10 to 12°C. The PCR amplification was performed using thermal cycler (Next Gene Scientific Sdn Bhd, Malaysia). Later, PCR products were separated in 1.5% agarose gel electrophoresis using 1x TBE buffer.

2.7 Gel Electrophoresis for Primer Validation

About 2 ul of DNA ladder (Quickload DNA ladder) was used as marker and 2.0 ul loading dye + 5.0 ul of PCR product was pipetted into the well of the 1.5% agarose gel. SSR products were electrophoresed on 1.5% agarose gel in a 1x TBE buffer at 90V, 200mA for 75 minutes or 150V, 250mA for an hour. The gel was stained in 130 ul

gel red nucleic acid staining in 130 ml agarose gel and was allowed it to be solidified. The gel was photographed using a gel documentation system (Bio-rad gel documentation system).

3. Findings

From the PCR amplification obtained, only FTA disc that have been eluted in TE buffer at the end wash able to amplify produce band result during gel visualisation. Both centrifugation speed does not affect much on the result as both speed still under low centrifugation speed. Besides, the clarity of the vortexed disc does not really matter due to the centrifugation step has been added after washing using both FTA purification reagent as well as TE buffer. Subsequently, the involvement of incubation step is optional, and it is recommended to incubate to allow the samples completely dissolved in the TE buffer.

Fig. 4 shows the successful PCR amplification of 25 oil palm samples when using modified and optimised washing steps. Despite the lower DNA concentration observed in samples extracted using FTA cards through gel visualisation, this modification can serve as a viable alternative for field sampling especially when optimised primer pairs capable of amplifying low DNA concentrations are employed. If the direct PCR-based sample prepared strictly followed as modified protocol the DNA concentration in FTA elute can be controlled without undergo DNA quantification and therefore able to successful amplified and gives promising result as in Fig. 4.

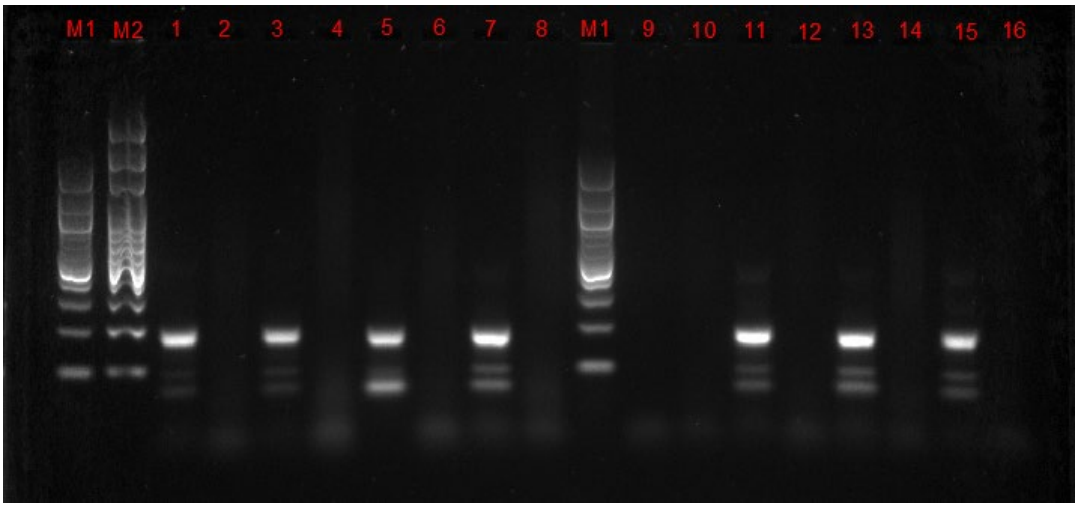

Fig. 3 *PCR amplification of 16 possibilities of FTA washing troubleshooting*

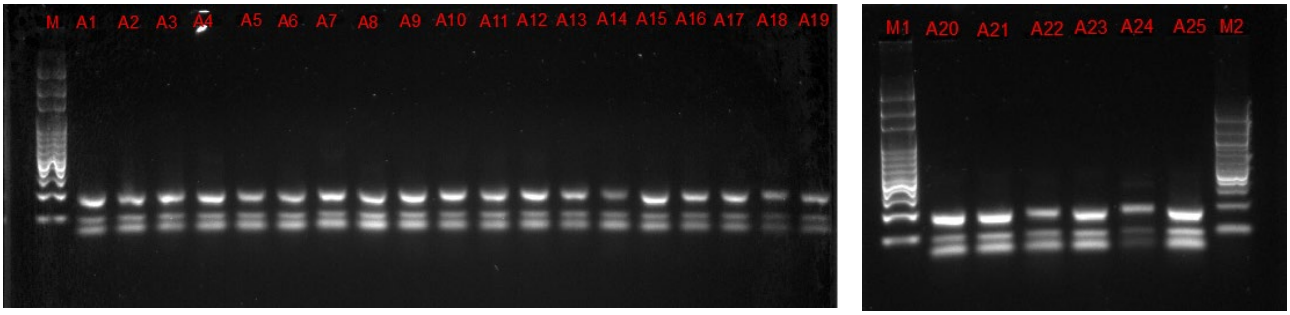

Fig. 4 *Optimised washing steps applied on 25 samples of an oil palm plantation*

4. Discussion

Following the basic principles of DNA extraction is not only necessary, but essential to ensure the quality, reliability, and validity of your genetic research. It helps maintain scientific protocols that contributes to the advancement of knowledge and ensures the ethical handling of biological samples. The FTA card is a specialised tool for collection and preservation of DNA that follows basic principles of common DNA extraction method [26]. It contains a specialised DNA binding matrix that is treated with chemicals that lyse and denature proteins that allows for DNA immobilised and absorbed renders it available for binding [12], [15], [26]

Centrifugation, incubation and elution steps are vital in laboratory procedure as they serve crucial functions that allows the isolation, purification and molecular analysis [9]. After several modifications, the centrifugation step has been finalised to be applied after every washing step as it used to separate differences in density, size and concentrations of molecules and nucleic acid and easier to collect targeted material. Furthermore, it helps in

pelleting dense particles at the bottom PCR tube that assist in purifying target molecules from contaminants as well as to accelerate chemical reactions [8].

Subsequently, incubation is an optional and recommended to be implemented in FTA washing step as incubation period used to facilitate hybridisation of DNA probes bind specifically to the target sequences and allows sufficient time for DNA probe and target sequences to interact [15], [12]. In addition, the elution step really important to be applied at the end of FTA washing [15]. Because it enables the recovery of preserved nucleic acids, the elution step in FTA card-based sample processing is crucial for their use in various molecular biology applications. It ensures the integrity and accessibility of genetic material, making FTA cards a valuable tool in sample collection, transport, and long-term storage for oil palm DNA analysis [10].

Based on the result obtained in Fig. 3, only samples that have been applied with centrifugation and elution step able to produce band on the gel visualisation. Then, the modification protocol made has been validated on the 25 oil palm samples and produce successfully amplified PCR product that shows in Fig. 4. Therefore, it is advisable to implement centrifugation after every washing step and elution step with TE buffer especially for high-level polysaccharides plant.

5. Conclusion and Recommendation

In this study, with two punches of FTA discs sufficient to obtain DNA and successfully amplified. Subsequently, the additional centrifugation after every washing and eluting the FTA discs in TE buffer could be life-changing especially for oil palm samples as this is a first attempt to be used in FTA card [15]. As conclusion, the modifications made from previous studies capable to show promising result. The optimisation of FTA card protocol holds great potential for expanding its application in oil palm studies. The FTA card addresses some of the drawbacks of other DNA extraction methods when it comes to sample collection and transport [12]. On-site collection would allow more samples to be collected as it is not required transporting kilograms of silica to preserve samples. Other than that, FTA card can be stored at room temperature, more stable, does not require refrigeration, less laboratory expertise, fewer exposure towards toxic substances [13], [14]. Although isolating oil palm DNA using FTA card had a much lower DNA concentration, the method is a good alternative for field collection if well-optimised primer pairs that can amplify low concentrations of template DNA are used [18].

By refining the protocol, future researcher may boost the reliability and effectiveness of DNA extraction from oil palm samples using FTA card as suggested using modification made FTA washing steps. The broader application of the optimised FTA card protocol in oil palm studies can significantly contribute to many research areas such as assessment of genetic diversity and population structure analysis [34]. The ability to preserve large numbers of samples of oil palm using FTA cards capable of accelerating large-scale studies and allow to conduct comprehensive analyses across various oil palm populations as well support conservation efforts by providing reliable and effective methods in DNA storage and retrieval. Also, this optimized protocol is potentially to be used with other microsatellite markers in oil palm genetic analysis that are crucial in targeting regions and amplify specific DNA sequences interest [35]. This allows in gaining a thorough understanding of genetic basis plants especially high-level polysaccharides plants.

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Conflict of Interest

Authors declare that there is no conflict of interest regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: Nurul Ain Shahira ***conceptualized the central research idea, while*** *Wan Nurhayati* ***provided the theoretical framework and managed funding acquisition****. Nurul Ain Shahira and Amirul Akmal* ***designed the research,*** *with Wan Nurhayati and Mohd Razik* ***supervising the research progress.*** *Nurul Ain Shahira* ***wrote the manuscript,*** *and all authors contributed to the review, revision, and approval of the article for submission.*

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