SHORT COMMUNICATION

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# A SIMPLE AND RAPID PROTOCOL FOR ISOLATION OF GENOMIC DNA FROM OIL PALM LEAF TISSUE

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**ABSTRACT**

*Molecular genetic studies in oil palm require a cost and time effective method to extract sufficient amount of genomic DNA from a large number of samples. A simplified protocol was developed to isolate high quality DNA from immature spear leaves of* Elaeis guineensis*. The method was modified from a previously described protocol. The modifications involved increasing the amount of starting material to 2 g, grinding the samples in liquid nitrogen, and inclusion of 2% PVP-40, ascorbic acid, DIECA, and 1% β-mercaptoethanol into the lysis buffer. The RNAse treatment was also included to improve the quality of DNA. This method successfully yielded higher amount of DNA that was similar in quality to that prepared using the conventional protocol. The DNA was also suitable for simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) analyses.*

**Keywords:** DNA extraction, PCR, oil palm, *Elaeis guineensis.*

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## INTRODUCTION

*Elaeis guineensis* is a commercial oil palm species originating from West Africa and has been an important source of vegetable oil for many decades (Carter *et al*., 2007). To cope with the increasing demand, efforts to increase the palm oil yield and quality have been initiated. Research strategies involving biotechnological techniques have been implemented since 1990s (Maria *et al*., 1995; Rahimah *et al*., 2006; Billotte *et al*., 2010). The prerequisite for successful application of these techniques is genomic DNA of good quality and purity. Thus, it is important to have a fast and reliable method for extracting DNA from oil palm tissues.

A number of commercial genomic DNA extraction kits are available to speed up the extraction process. However, the use of commercial kits to isolate oil palm DNA is mostly expensive and often does not give satisfactory results compared to the conventional protocol (Ying and Faridah, 2006). The hexadecyltrimetyhlammomnium bromide (CTAB) protocol described by Doyle and Doyle (1987; 1990) is one of the conventional methods commonly used for the isolation of DNA from plant species (Borges *et al*., 2012) including oil palm. In contrast to commercial kits, this protocol is time-consuming and laborious and as such, can be a problem if DNA is to be extracted from hundreds of samples. Therefore, there is a need for a rapid and simple extraction procedure that yields good quality and quantity of genomic DNA. Several protocols for rapid preparation of DNA from plant tissues have been reported (Ausubel *et al*., 2003; Dhakshanamoorthy *et al*., 2009; Arif *et al*., 2010) and can be exploited for extracting DNA from oil palm.

The existing modified CTAB-based protocol applied in our laboratory is a combination of methods described by Saghai-Maroof *et al*. (1984), Rogers and Bendich (1985) and Doyle and Doyle (1987) which, was published by Weising *et al*. (1995). This method

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has sucessfully produced high quality and quantity of DNA but only allows a small number of samples to be processed at a time. The current method yields about 200 – 680 $\mu$g DNA $g^{-1}$ leaf tissue (Rahimah *et al*., 2006). Since the oil palm tissue is very fibrous, approximately 1 to 2 hr is spent grinding four to six samples using mortar and pestle in liquid nitrogen. As such, one laboratory technician can only handle a limited number of samples per day. Additionally, four days are needed to complete the entire extraction protocol. Due to these drawbacks, initiative was taken to test a published DNA extraction protocol that can be completed within a day, and gives the required quality and quantity.

This study explored the DNA extraction protocol described by Arif *et al*. (2010). The published protocol of Arif *et al*. (2010) suggests that grinding the tissue in the extraction buffer (with NaCl) and sterile sand provides acceptable DNA yield suitable for routine molecular biology analysis including PCR amplification. The protocol omits the use of liquid nitrogen ($N_2$), polyvinylpyrrolidone (PVP) and lithium chloride (LiCl) and reportedly produces on average 70 $\mu$g DNA $g^{-1}$ sample. The protocol as described was tested on oil palm tissues, but did not produce sufficient amount of DNA for certain applications and the quality was also slightly below expectation. As such, this study describes minor modifications to the extraction protocol of date palm described by Arif *et al*. (2010) for routine isolation of acceptable quality and quantity of DNA from oil palm tissues.

## EXPERIMENTAL

### DNA Extraction

The genomic DNA was extracted from frozen, young leaves of *E. guineensis* collected from a field experiment established at Kluang, Johor, Malaysia. Initially the method described by Arif et al. (2010) was applied without modification. A total of 10 samples were evaluated. DNA from the same sample was then re-extracted using the modified method described below:

The 2X CTAB lysis buffer (2% cetylmethylammonium bromide, 100 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 1.4 M NaCl, and 2% PVP-40), 7.5 ml, was pre-heated in a glass beaker to 60°C in a water bath. While the buffer was heating, 2 g of frozen leaves was ground using a sterile mortar and pestle with gradual addition of liquid $N_2$. The 250 mg sterile acid sand was added into the mortar prior to the grinding process. The frozen fine powder was left at room temperature for 5 min.

Seventy-five $\mu$l each of 0.5 M ascorbic acid, 0.4 M DIECA, and 1% β-mercaptoethanol were added to the pre-heated 2X CTAB lysis buffer (this step was carried out in the fumehood). The powdered tissue was further thawed by immersing the mortars in warm water. Following this, 7.5 ml of the lysis buffer was added into the mortar and gently mixed using the pestle. The mixture was then transferred into a 15 ml Falcon tube, vortexed briefly and incubated at 60°C for 30 min in a shaking water bath. The samples were then allowed to cool at room temperature for 30 min. This is followed by centrifugation at 4000 rpm for 30 min at 25°C in a swing-bucket rotor of the Eppendorf Centrifuge 5810 R. Six millilitres of the upper aqueous phase was carefully transferred into a new Falcon tube using wide bore pipette. An equal volume (6 ml) of chloroform: isoamyl alcohol (24:1) was added and the tube was vigorously shaken for 5 min to mix the solution thoroughly. DNA was precipitated by centrifugation at 4000 rpm, 30 min, 25°C. About 5 ml of the supernatant was again transferred to a new Falcon tube and treated with 6 $\mu$l of RNAse at 37°C or room temperature for 30 min. The 500 $\mu$l of 3.0 M sodium acetate and 1 volume (5 ml) of cold isopropanol were added gently, mixed and kept in -20°C freezer for at least 30 min. After centrifugation at 4000 rpm, 4°C for 30 min, the supernatant was discarded. The pellet was resuspended in 2 ml of wash buffer (76% ethanol, 10 mM ammonium acetate) and kept at 4°C for at least 30 min. The wash buffer was carefully poured off and the pellet was dried in a speed vacuum for 10-15 min. Two millilitres of 70% cold ethanol was added and kept at room temperature for 15 min. The ethanol was discarded and the tubes were placed in a speed vacuum to allow complete drying of the pellet. The dried DNA pellet was then carefully transferred into a 1.5 ml eppendorf tube and dissolved in 300-350 $\mu$l of TE buffer, depending on size of the pellet, followed by incubation at 50°C in a shaking water bath. After the pellet was fully dissolved, a small aliquot of the DNA samples was electrophoresed in 0.8% agarose gels at 100V for 1.5 hr. The results were visualised after staining the gels in 0.5 $\mu$g $ml^{-1}$ ethidium bromide (EtBr) solution.

### DNA Quantification

The total DNA yield and purity index ($A_{260}$/ $A_{280}$ ratio) were determined using Multiskan Go (Thermo Scientific). Five $\mu$g of each of the extracted DNA was also digested with six (BglII) and four (HaeIII) base pair cutter restriction enzymes to check its digestibility. The DNA was also tested to evaluate its suitability for simple sequence repeat (SSR) analysis and single nucleotide polymorphism (SNP) genotyping.

### SSR Analysis

Primer mEgCIR2332 ($GA_{14}$) (Billotte *et al*., 2010) was used in PCR amplification. Fifty ng $\mu l^{-1}$ genomic DNA was incorporated to a

8 μl reaction mix containing 1× PCR buffer, 0.2 mM dNTPs, 1.5 pmol forward primer [F:CACGACGTTGTAAAACGACGAAGAAGA GCAAAAGAGAAG] 0.5 pmol reverse primer [R: GCTAGGTGAAAAATAAAGTT], and 0.05 U of Taq polymerase. Thermocycling was performed on a GeneAmp PCR System 9700 (Applied Biosystem) at 95°C for 3 min, followed by 35 cycles of 95°C for 30 s, 52°C for 30 s, and 72°C for 30 s; final extension was 72°C for 35 min. PCR products were visualised on 4% super fine resolution (SFR) gels stained with 0.5 μg $ml^{-1}$ EtBr.

**SNP Analysis**

Ten samples that were extracted using the modified method were subjected to genotyping with 4451 SNP markers using the Illumina Infinium assay. The SNP calls were made using GenomeStudio software (Illumina, Inc.).

## RESULTS AND DISCUSSION

The method described by Arif *et al*. (2010) was chosen due to the hardy and fibrous nature of the date palm leaves which is quite similar to oil palm leaves. The protocol is quite simple and provides sufficient DNA yield, and appears convenient for routine extraction of DNA from oil palm samples.

With the same amount of the starting material (0.1 g) processed using the un-modified protocol of Arif *et al*. (2010), the DNA quality and yield varied and was generally inconsistent (*Figures 1* and *2*). Futhermore, the original protocol only utilises 0.1 g starting tissue with 500 μl buffer in 1.5 ml eppendorf tube. The DNA yield obtained from 0.1 g of oil palm tissue was generally less than 50 μg, which is not sufficient for some applications such as restriction fragment length polymorphism (RFLP).

**Modifications**

In order to obtain higher DNA yields, we replaced the 1.5 ml eppendorf tube with 15 ml volume Falcon tube, thus the starting material could be increased to 2 g. The lysis buffer was then increased to 7.5 ml. Liquid $N_2$ may not be necessary in the method described by Arif *et al*. (2010) because the authors used fresh tissue. However, this is not possible in cases where the leaf samples are stored frozen in -80°C freezer, prior to extracting DNA, which is the case for most samples in our laboratory. The sampling site is about 300 km away from the laboratory, so it is not possible to do extraction on the same day the samples are collected. Due to the distance, the samples are cleaned and frozen in liquid nitrogen as soon they arrive at the laboratory and kept in -80°C freezer for long-term storage. Thus, the use of liquid nitrogen could not be avoided to minimise oxidation and for maintaining the tissues at low temperature during the grinding process.

The other modification made was the addition of 2% of freshly prepared PVP-40 (w/v) into the lysis buffer. The PVP combines with polyphenols present in the samples to form a complex that is easily separated from the DNA, resulting in a cleaner final product (John, 1992). The 1% β-mercaptoethanol, 0.5 M ascorbic acid, 0.4 M DIECA were also added, as previously described (Chen *et al*., 1999; Stein *et al*., 2001; Puchooa, 2004). Pirttilä *et al*. (2001) reported that PVP, together with β-mercaptoethanol inhibits the oxidation of the secondary metabolites. Weising *et al*. (1995) suggested that different concentrations of β-mercaptoethanol are effective in preparing clean DNA of sufficient yield from various plant species. In this study, 1% β-mercaptoethanol was found optimal for *E. guineensis*. The RNAse treatment was also included in the modified protocols to remove RNA contaminants.

**DNA Yield and Purity**

The modified protocol resulted in higher quality genomic DNA (purity ratio ~ 1.8 – 1.9) (*Figure 1*) of sufficient quantity (*Figure 2*). The quality of DNA obtained was further tested by restriction enzyme digestion. Undigested DNA showed a clear and intact band of high molecular weight (*Figure 3*). The digested DNA was observed as 'smear' on the agarose gel for all of the samples tested which indicate complete digestion by the respective restriction enzymes. As expected, the smears from the four bp cutter showed a lower mobility range compared to the six bp cutter further confirming the quality of the DNA.

The DNA was also found suitable for use in the SSR assay. The DNA was readily amplified using simple SSR primer (*Figure 4a*) giving the expected amplicon size and profile. The DNA was also suitable for use in high throughput SNP analysis using the Illumina platform (*Figure 4b*). In fact, all 10 palms recorded successful genotype calls for SNP with GenTrain score >90%. An example of a successful SNP calling of marker EG20050927.69728. Contig1.571 clearly shows three well-defined groups, two homozygotes (AA and BB) and one heterozygotes (AB), with no overlap between them. These results show that the rapid DNA extraction protocol is suitable for high throughput assays that require DNA of the highest quality.

## CONCLUSION

The advantage of this procedure over the original method Arif *et al*. (2010) is that it allows larger amount of tissue to be used as starting materials.

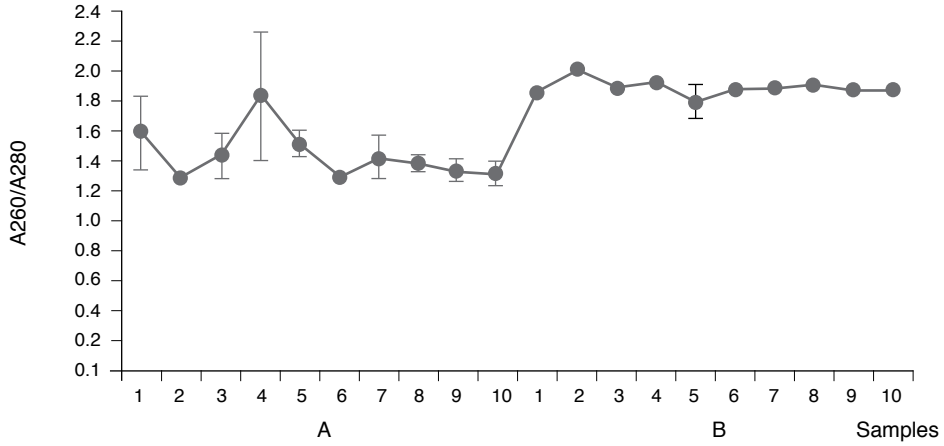


*Figure 1. The purity profile of genomic DNA. A set of 10* E. guineensis *samples isolated using A: the unmodified Arif* et al. *(2010) method and B: the modified protocol. Bar represents deviation to the mean of three replicates.*

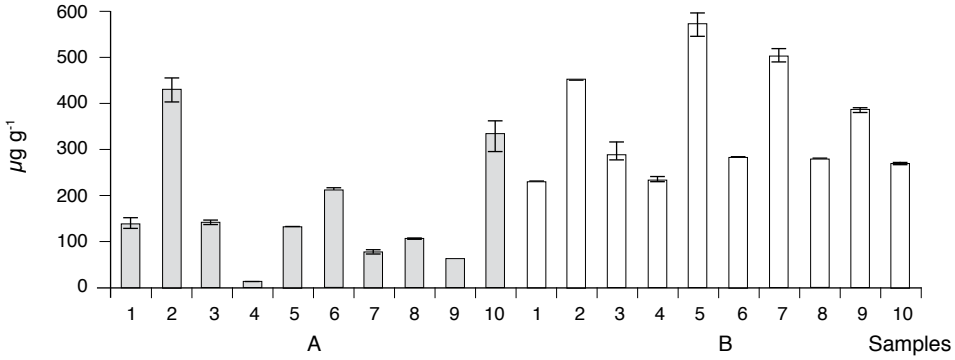


*Figure 2. DNA concentration obtained from the same set of* E. guineensis *sample. Samples 1-10 extracted using A: the unmodified Arif* et al. *(2010) and B: the modified method. Bar represents standard deviation for the mean of three replicate.*

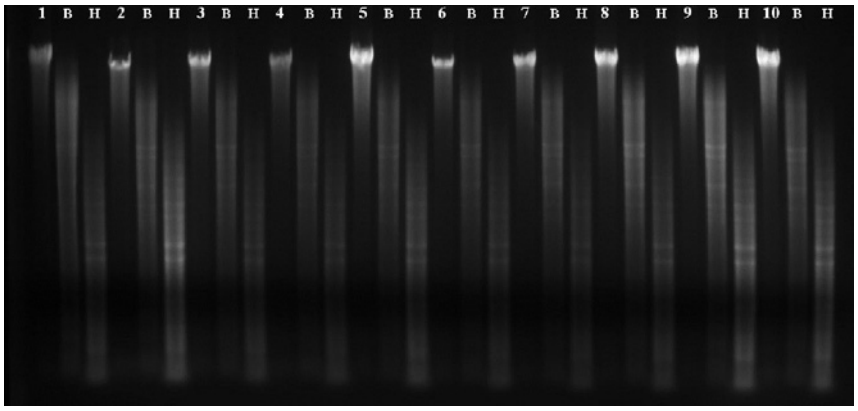


*Figure 3. Total genomic DNA isolated from spear leaves of* E. guineensis *using modified protocol. No. 1 to 10 represent undigested genomic DNA; B: BgIII-digested genomic DNA and H: HaeIII-digested genomic DNA.*

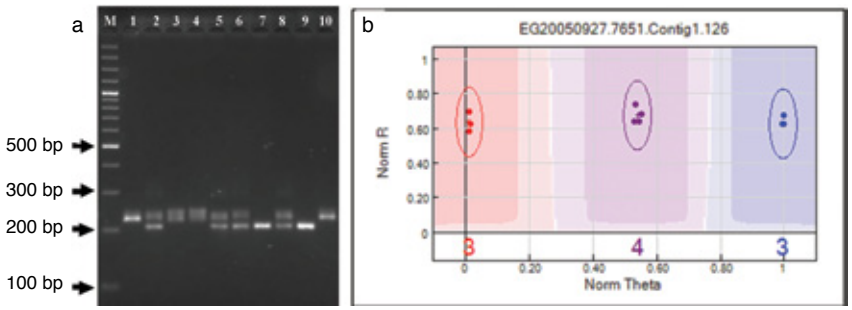


*Figure 4. (a) Simple sequence repeat (SSR) pattern obtained from the DNA extracted using the modified protocol. Ten different DNA sample (lanes 2-11) amplified using the SSR primer mEgCIR2332. Lane 1 is 100 kb DNA ladder. (b) Clustering pattern of SNP EG20050927. 69728.Contig1.571 shown for 10 samples of the African germplasma.*

Higher yield of genomic DNA can be obtained for various analysis and long-term storage. The method is simple, reliable, and can be concluded in two days. A laboratory technician can easily process up to 12 samples per day. Futhermore, the quality and yield of the DNA is similar to that of the the conventional method. Therefore, we conclude that the modified method can yield DNA for routine molecular biology studies of oil palm and perhaps also useful for other plant species.

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