Jurnal Manajemen Hutan Tropika, *30*(1), 144–154, April 2024
EISSN: 2089-2063
DOI: 10.7226/jtfm.30.1.144

Scientific Article
ISSN: 2087-0469

Optimizing DNA Extraction and Selecting Suitable Regions for Biodiversity Assessment: A Study on *Shorea leprosula*

Henti Hendalastuti Rachmat[1], Kusumadewi Sri Yulita[1], Fifi Gus Dwiyanti[2], Arida Susilowati[3*], Nawwall Arrofaha[4], Susila[5], Irsyad Kamal[6], Iskandar Zulkarnaen Siregar[2]

[1]Research Centre for Ecology and Ethnobiology, National Research and Innovation Agency, Jl. Raya Jakarta-Bogor Km. 46, Bogor, Indonesia 16911
[2]Department of Silviculture, Faculty of Forestry and Environment, IPB University, Academic Ring Road Campus IPB Dramaga, Bogor, Indonesia 16680
[3]Faculty of Forestry, Universitas Sumatera Utara, Jalan Lingkar Kampus, Kampus USU Kwala Bekala, Simalingkar A, Deli Serdang, Indonesia 20353
[4]Department of Biology, Faculty of Science and Technology, UIN Syarif Hidayatullah Jakarta. Jl. Ir. H. Juanda No. 95, Tangerang Selatan, Indonesia 15412
[5]Research Centre for Biosystematics and Evolution, National Research and Innovation Agency, Jl. Raya Jakarta-Bogor Km. 46, Bogor, Indonesia 16911
[6]Department of Biology, Faculty of Science and Technology, UIN Walisongo Semarang. Jl. Walisongo No. 35, Semarang, Indonesia 50185

Received October 6, 2023/Accepted February 21, 2024

Abstract

The extraction method plays a crucial role in obtaining high-quality DNA samples, which is indispensable for various molecular biology techniques and analyses, enabling a deeper comprehension of genetic information and biological processes. The objectives of the study were: a) to optimize the chloroplast DNA extraction protocol by comparing modified CTAB methods and GeneAid for both leaf and wood samples of Shorea leprosula, a major commercial timber species, and b) to identify a suitable cpDNA region that exhibits variability and universality across taxa. Total DNA was analyzed by gel electrophoresis followed by Sanger sequencing to determine the amplification success. The results revealed that trnL intron, trnL-trnF, and trnG yielded readable sequences of the expected length (maximum 586 bp, 480 bp, and 908 bp, respectively), while the rps16 intron failed to assemble a contig. The petL-psbE region provided long readability for reverse sequences (769 bp) but not for the forward sequence (195 bp). Higher successful DNA extraction was achieved from the leaves compared to the woods. The lower sequencing quality may be attributed to suboptimal primer design, the structural features of the regions resulting from extensive repetitive sequences, and the suboptimal condition of the extraction method in eliminating wood chemical compounds.

Keywords: cpDNA, tropical tree, genetic variation, trnL-trnF, trnG

**Correspondence author, email*: *arida.susilowati@usu.ac.id*

Introduction

Shorea leprosula is a member of the Dipterocarpaceae family. It is locally known as '*meranti tembaga*' and is widely distributed throughout the aseasonal tropical rainforests of Southeast Asia (Symington, 1943; Ashton, 1982). *S. leprosula* naturally grows in Peninsular Malaysia, Peninsular Thailand, the Indonesian islands of Java and Sumatra, and Borneo/Kalimantan (Ashton, 1982; Newman et al., 1996; Lee et al., 2000a; Rudjiman, 2002; Pooma & Newman, 2017). *S. leprosula* has a high commercial value for wood or timber production and is commonly called 'light red *meranti*' (Ashton, 1982; Kessler & Sidiyasa, 1994; Wahyudi et al., 2014). Harvested timber has been a target for illegal logging (Miranda Montero et al., 2020). With over 700 recognized species, species determination within dipterocarps can be challenging due to morphological similarities and cryptic species, and the need for molecular approaches to resolve taxonomic uncertainties. Proper identification of the species can facilitate the implementation of sustainable management practices and traceability of timber products, supporting responsible and legal trade (Tsumura et al., 2011; Ng et al., 2022).

Methodological optimization is essential when extracting DNA from tree species for various genetic studies to ensure accurate and reliable results. The choice of DNA extraction method can significantly impact the quality and quantity of DNA obtained (Gryson, 2010), thereby influencing downstream analyses, such as genetic diversity assessments, molecular marker development (Nuroniah et al., 2010; Rana et al., 2013), and evolutionary studies (Heckenhauer et al., 2018). By optimizing the extraction method, researchers can maximize DNA yield (Rohland &

Jurnal Manajemen Hutan Tropika, *30*(1), 144–154, April 2024
EISSN: 2089-2063
DOI: 10.7226/jtfm.30.1.144

ISSN: 2087-0469

Hofreiter, 2007; Särkinen et al., 2012), minimize contaminants (Kim et al., 2017), and preserve the integrity of genetic material (Zimmermann et al., 2008), ultimately enhancing the validity and robustness of their genetic studies in tree species. There are many published protocols for the extraction of DNA from various plant species and tissues (Murray & Thompson, 1980; Dellaporta et al., 1983; Rogers & Bendich, 1985; Doyle & Doyle, 1987; Wagner et al., 1987; Stewart & Via, 1993; Jobes et al., 1995; Kim et al., 1997; Tibbits et al., 2006). Many of these protocols recommend extracting DNA from needles, leaves, or buds. Extraction of DNA from fresh materials, such as leaves or shoots, is a common practice in the molecular biology of tropical forest species (Kajita et al., 1998; Cannon & Manos, 2003). However, DNA extraction from wood (sapwood and heartwood) is more difficult than that from leaves because of the higher quantity of secondary metabolites of phenolic compounds and lignin, and because the concentration of leaf DNA is higher than that of wood DNA (Liepelt et al., 2006; Swetha et al., 2014). This is because of the small amount of DNA present in woody tissues, even in living trees (Abe et al., 2011).

Some authors (Murray & Thompson, 1980; Doyle & Doyle, 1987; Wagner et al., 1987) suggest the use of extraction buffers containing cetyltrimethylammonium bromide (CTAB), while others (Stewart & Via, 1993; Kim et al., 1997) introduce PVP-40 (polyvinylpyrrolidone; mole weight 40,000) to remove contaminating components of DNA. Therefore, it is important to determine the most efficient DNA extraction protocol for wood. Wood DNA extracts are usually highly degraded, so it is important to select multiple copies of target gene sequences to increase the success of PCR amplification (Cano, 1996; Deguilloux et al., 2002).

The aims of this study were: a) to determine the appropriate extraction method for isolating the leaves and wood of *S. leprosula* by comparing two extraction methods, CTAB and GeneAid Kit, b) to determine the optimized chloroplast DNA extraction protocol through comparison between modified CTAB methods and GeneAid kit protocols, and c) to identify a suitable cpDNA region that exhibits variability and universality across taxa.

Methods

Plant materials Plant materials in the form of leaves and wood were obtained from *mature S. leprosula* trees. Fresh leaf samples were dried with silica gel, whereas wood samples were collected in sterile containers. Leaf and wood samples were stored at room temperature in the Molecular Systematics Laboratory of the National Research and Innovation Agency, Indonesia. DNA isolation and molecular analysis were carried out at the Molecular Systematics Laboratory of the National Research and Innovation Agency. This study used two samples of *S. leprosula* leaves and wood, which were extracted using CTAB (Doyle & Doyle, 1987) with modification and Genomic DNA Mini Kit (Plant) from GeneAid. Samples were processed immediately upon arrival from the field. The duration of samples storage, until they are isolated, is varied, but attempts to do so as quickly as possible, no more than a month after they are collected from the field.

Sample preparation After collecting the *S. leprosula* wood sample during the field survey, it was crucial to clean the sample from dust and dirt. Leaf samples were wiped using Kimwipes that had been moistened with 70% alcohol. This step ensured that the parts used for isolating the genetic material were free from contaminants. For the CTAB method, 60 mg of leaf and wood were weighed, while 20 mg was weighed for commercial kits. These samples were then scraped into smaller pieces and finely ground into a powder, preparing them for further analysis.

DNA extraction To ensure the integrity of DNA samples, conditions must be maintained throughout the DNA isolation process to prevent contamination from the surrounding environment. In this study, two distinct tree organs, namely wood and leaves, were extracted using two DNA extraction protocols: the modified CTAB method and the modified Genomic DNA Mini Kit (Plant) from GeneAid. The specific treatment details can be found in the sample preparation section, while the step-by-step procedures for each extraction method, tailored to the respective tree organs, are described as follows.

CTAB with modification A mixture of 60 mg of fresh leaf and wood chips with quartz sand was ground to a fine powder using a mortar and pestle. The fine powder was then inserted into a 1.5 mL microtube with an additional 700 µL of extraction buffer and 14 µL mercaptoethanol. The extraction buffer of 500 mL 2x CTAB consisted of 10 g CTAB 0.054 mol L^{-1}, 40.908 g NaCl 1.4 mol L^{-1}, 6.055 g Tris HCl 0.098 mol L^{-1}, and 3.722 g EDTA 0.016 mol L^{-1}. The samples were then homogenized in a vortex until the whole sample was mixed with buffer, followed by incubation in a water bath for at least 3 hours at 65 °C (modified from the initial protocol). In the initial protocol, the samples were incubated for 10–30 minutes at 65 ºC. We made several modifications to the subsequent lab steps, which were tailored based on our direct experience in extracting Dipterocarpaceae wood samples). The microtubes were inverted every 30 minutes to ensure an evenly homogenized content. When finished, 600 µL of chloroform-isoamyl alcohol (24:1) was added to the microtube. Next, the mixture was centrifuged for 5 minutes at 10,000 rpm. When the contents began to separate and form layers of supernatant, organic materials, and chloroform, the uppermost layer of the supernatant was transferred to a new microtube using a micropipette. This process was repeated twice. Subsequently, 500 mL of cold isopropanol was added to the supernatant, mixed, and stored in a freezer overnight. Next, the microtube was centrifuged for 5 minutes at 10,000 rpm to form precipitates. The separated fluid was discharged from the microtube and replaced with 500 mL of 70% ethanol, followed by further centrifugation for 2 minutes and another fluid was discarded. This process was performed twice. The pellets or DNA precipitates were then dried at room temperature for 30 minutes (with the tube cap opened) before 30 µL of nuclease-free water was added (modified from the initial protocol; on the initial protocol, the DNA was dissolved in 50 µL of nuclease-free water or TE buffer after the first rinsing with 70% ethanol). Finally, the microtubes were flicked, and the isolated DNA was subsequently used

Jurnal Manajemen Hutan Tropika, *30*(1), 144−154, April 2024
EISSN: 2089-2063
DOI: 10.7226/jtfm.30.1.144

ISSN: 2087-0469

for PCR amplification.

Genomic DNA mini kit (plant) GeneAid with modification The process of DNA isolation using the Genomic DNA Mini Kit (Plant) GeneAid mainly followed the manufacturer's instructions with modifications. DNA isolation was performed using a GeneAid kit as follows: The sample prepared from the sample preparation section was mixed with quartz sand and ground into a fine powder using a pestle and mortar. The fine powder was then transferred to a 1.5 mL microcentrifuge tube. A total of 400 µL GP1 Buffer or GPX1 Buffer and 5 µL RNase A were added to the sample tube and mixed with a vortex. Next, the samples were incubated at 60 °C for 30 minutes. During incubation, the tube was inverted every 5 minutes (80 µL of elution buffer was required per sample) and heated to 60 °C. A total of 100 µL of GP2 Buffer was added and mixed with a vortex and then incubated on ice for 3 minutes. Then, the samples were centrifuged for 5 min at 13,000 rpm (modified from the manufacturer's protocol; the sample was not centrifuged for 5 min at 13,000 rpm in the initial protocol). The filter column was placed into a 2 mL collection tube, and the mixture was transferred to the filter column. Samples were centrifuged again for 1 minute at 3,500 rpm, after which the filter column was discarded. The supernatant from the 2 mL collection tube was carefully transferred to a clean 1.5 mL microcentrifuge tube. A total of 1.5 volume of GP3 buffer was added to the tube and then homogenized with a vortex for 5 seconds. Next, the GD column was placed into a 2 mL collection tube and 700 µL of the mixture (and the remaining precipitate) was transferred to the GD column. Samples were centrifuged at 13,000 rpm for 2 minutes. The flow-through in the collection tube was discarded and then the GD column was put back into the 2 mL collection tube. The remaining mixture was added to the GD column and the sample was centrifuged at 13,000 rpm for 2 minutes. The flow-through in the collection tube was discarded and then the GD column was put back into the 2 mL collection tube. A total of 400 µL W1 Buffer was added to the GD column and then centrifuged at 13,000 rpm for 30 seconds. The flow-through in the collection tube was discarded and then the GD column was put back into the 2 mL collection tube. A total of 600 µL of wash buffer was added to the GD column and followed by centrifugation at 13,000 rpm for 30 seconds. The flow-through in the collection tube was discarded and then the GD column was put back into the 2 mL collection tube. Next, the samples were centrifuged for 3 minutes at 13,000 rpm to dry the column matrix. The optional step to remove pigment residue was performed; a) after adding the wash buffer, 400 µL absolute ethanol was added to the GD column; b) samples were centrifuged at 13,000 rpm for 30 seconds; c) the flow-through in the collection tube was discarded and then the GD Column was put back into the 2 mL collection tube; d) the sample was centrifuged for 3 minutes at 13,000 rpm to dry the column matrix. The dry GD column was transferred to a clean 1.5 mL microcentrifuge tube. A total of 80 µL of pre-heated elution buffer was added to the centre of the column matrix (modified from the manufacturer's protocol; on the initial protocol, the DNA was dissolved in 100 µL of pre-heated elution buffer). The tube was left for 35 minutes to ensure that the elution buffer was completely absorbed. The sample was then centrifuged at 13,000 rpm for 30 seconds to elute the purified DNA.

PCR amplification Total genomic DNA from leaves and wood was isolated using the CTAB and Genomic DNA Mini Kit (Plant) from GeneAid. Five molecular markers of non-coding chloroplast regions, namely, *trn*L intron, *trn*L-*trn*F, *trn*G intron, *rps*16 intron, and *pet*L-*psb*E were selected to perform PCR amplification and DNA sequencing in this study with details of the nucleotide sequences of each combination primers are shown in Table 1.

The PCR mix with a total volume a of 12.5 µL consisted of a PCR master mix (My taq HS Red Mix 2x 6.25 µL from Bioline), 2 µM forward and reverse primers (forward and reverse primers 0.25 µL), nuclease free water 4.75 µL, and approximately 1 µL DNA template. The reaction was carried out in Sedi G Thermo Cycler (Wealtec) with optimum conditions as follows: initial denaturation at 94 °C for 3 minutes, denaturation at 94 °C for 30 seconds, annealing for 30 seconds and extension at 72 °C for 1 minute and 30 seconds, followed by final extension at 72 °C for 4 minutes. The annealing temperature was varied for each marker used. The annealing temperature was 53 °C for the *trn*L intron, *trn*L-*trn*F, and *rps*16 intron, 51 °C for the *trn*G intron, and 50 °C for *pet*L-*psb*E. The PCR amplification process lasted

Table 1 Primer sequences for non-coding chloroplast regions used in this study

No	Marker	5′-3′ primer *sequence*	Reference
	*trn*L intron		
1	Forward	CGA AAT CGG TAG ACG CTA CG	(Taberlet et al., 1991)
	Reverse	GGG GAT AGA GGG ACT TGA AC	
	*trn*L-*trn*F		
2	Forward	GGT TCA AGT CCC TCT ATC CC	(Taberlet et al., 1991)
	Reverse	ATT TGA ACT GGT GAC ACG AG	
	*trn*G intron		
3	Forward	GCG GGT ATA GTT TAG TGG TA	Yoshimura, unpublished
	Reverse	CCT CTG TCC TAT CCA TTA GAC	
	*rps*16 intron		
4	Forward	AAA CGA TGT GGT ARA AAG CAA C	(Shaw et al., 2005)
	Reverse	AAC ATC WAT TGC AAS GAT TCG ATA	
	*pet*L-*psb*E		
5	Forward	AGT AGA AAA CCG AAA TAA CTA GTT A	(Shaw et al., 2007)
	Reverse	TAT CGA ATA CTG GTA ATA ATA TCA GC	

Jurnal Manajemen Hutan Tropika, *30*(1), 144–154, April 2024
EISSN: 2089-2063
DOI: 10.7226/jtfm.30.1.144

ISSN: 2087-0469

for 35 cycles. The PCR products were visualized on a 0.5% agarose gel with GelRed added through an electrophoretic process and lasted for 30 minutes with a voltage of 100 volts. After electrophoresis process was completed, the target band was photographed using a gel documentation system (Bioinstrument, ATTO Biosystems Inc.). The amplified PCR products were sent to the 1st Base company for Sanger sequencing.

Data analysis Data from the sequenced results in the form of forward and reverse sequences were combined to obtain a complete sequence with the help of the ATGC version 4.3.5 (Genetyx Co., Japan) program. Furthermore, MEGA X software was used to evaluate the nucleotide composition of each marker (Kumar et al., 2016).

Results and Discussion

DNA extraction quality of CTAB and GeneAid protocols
The success of DNA isolation in terms of DNA integrity, a parameter used for assessment, can be determined by visualizing the appearance of bands on agarose gel. In this study, the quality of the DNA extracted from the total genome was evaluated using electrophoresis. Figure 1 demonstrates the presence of smears in one leaf sample, specifically leaf 2, when employing both the CTAB and GeneAid KIT extraction methods. Gel electrophoresis revealed that the CTAB method yielded distinct bands of high-molecular-weight DNA, including smeared bands of contaminants, in comparison to the DNA isolated by the GeneAid method (Figure 1). According to Rahmadara et al. (2022), a clear band without smearing in the visualization results indicates good-quality DNA isolation, while the presence of smears suggests the presence of contaminants in the extraction results. Interestingly, no bands were observed when DNA was isolated from wood samples using either the CTAB extraction or the GeneAid Kit. The GeneAid kit utilizes silica-based membrane technology in the form of a spin column (Dhaliwal, 2020); however, it appears to be less effective in removing polyphenolic and protein compounds (Rahmadara et al., 2022).

PCR amplification To verify the isolated DNA from the two extraction methods, PCR amplification was conducted using non-coding chloroplast genome markers, including the *trn*L intron, *trn*L-*trn*F, *trn*G intron, *rps*16 intron, and *pet*L-*psb*E. The results presented in Figure 2 demonstrate the successful amplification of leaf and wood samples using both the DNA templates extracted by the CTAB and GeneAid extraction methods. The PCR products exhibited clear bands and single copies for all markers utilized in the study, indicating successful amplification of the leaf and wood DNA samples extracted by both methods. These findings support the efficacy of both extraction techniques in producing amplified DNA fragments based on the distinct bands observed.

Work protocol verification based on sequencing results
The isolated total genomic DNA should be further checked and verified by performing PCR and sequencing analysis. Of the five markers, the longest amplicon size was produced by the *trn*G intron for leaves by CTAB extraction (908 bp), followed by the *trn*G intron for leaves by GeneAid extraction (894 bp). In the *trn*L intron marker, only intact sequence results were obtained for *S. leprosula* leaves, both with CTAB and GeneAid extracted DNA templates with sequence lengths of 586 bp, respectively. However, for wood extracted with CTAB or GeneAid with the *trn*L intron marker, the sequenced results could not be assembled because of the messy sequence results on the forward primers. This was also found in the *trn*G intron marker, where the wood sequences extracted with CTAB and GeneAid could not be assembled due to the poor sequences in the forward primers.

In the *trn*L-*trn*F marker, it is known that the length of the sequences is relatively the same between leaves and wood extracted using both CTAB and GeneAid. The sequence length using the CTAB DNA extraction template on leaves and wood with *trn*L-*trn*F markers was 479 bp, whereas the sequence length using GeneAid DNA extraction template on leaves and wood was 480 bp. Based on the results of this study (Table 2), it can also be seen that the PCR product sequences of *trn*G intron marker leaves using CTAB DNA template extraction resulted in longer sequence lengths (908 bp) compared to the sequence length using the GeneAid kit DNA template (894 bp). Meanwhile, the *rps*16 intron and *pet*L-*psb*E markers from leaf and wood sequences could not be assembled using the DNA templates CTAB and GeneAid. The *rps*16 intron sequence is messy and is characterized by the presence of repetitive DNA (repeated nucleotide sequences). However, the *pet*L-*psb*E marker resulted from poor forward primer sequences, characterized by messy electroferogram quality with overlapping peaks (Table 2). CTAB method is known for its ability to effectively remove contaminants and impurities, such as polyphenolic compounds and proteins, that can interfere with DNA analysis (Xu et al., 2010; Turaki et al., 2017). As a result, DNA extracted using the CTAB method is generally of higher purity, which allows for longer and more reliable readable DNA sequences. The addition of PVP to the extraction buffer in the CTAB extraction method is very helpful in removing polyphenols (Karaca et al., 2005), while CTAB aims to

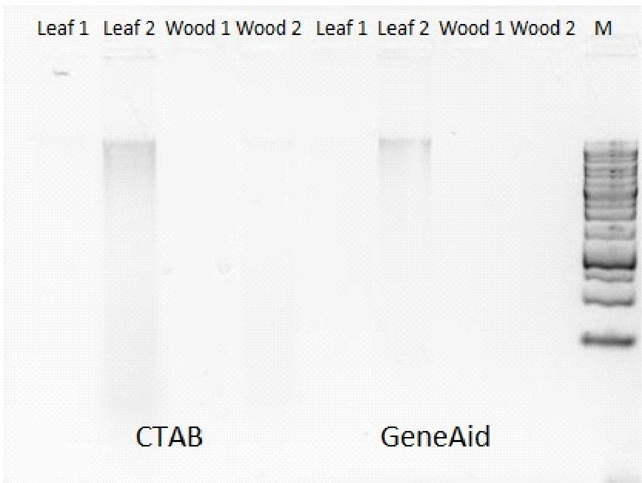
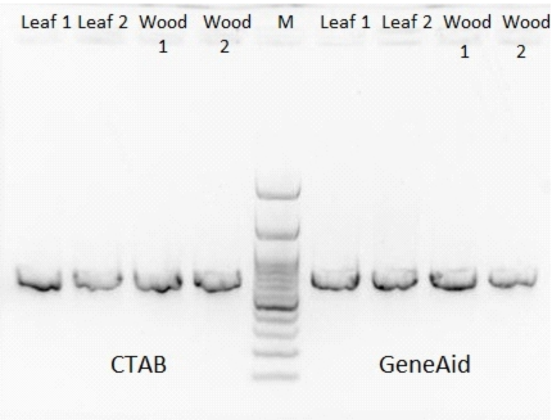
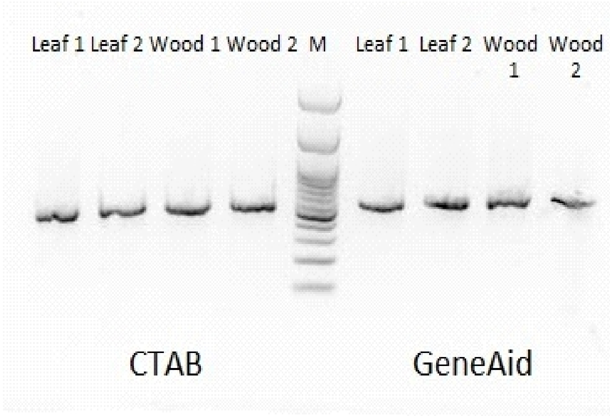


Figure 1 Representative photo of agarose gel electrophoresis containing isolated DNA using the CTAB and GeneAid protocols.

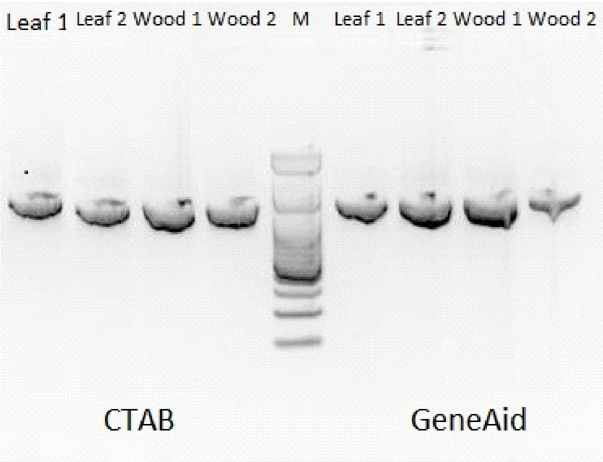
Jurnal Manajemen Hutan Tropika, *30*(1), 144–154, April 2024
EISSN: 2089-2063
DOI: 10.7226/jtfm.30.1.144
Scientific Article
ISSN: 2087-0469



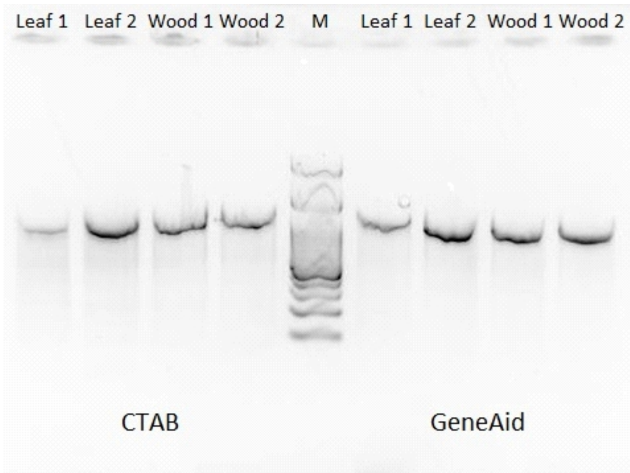
a) *trn*L intron



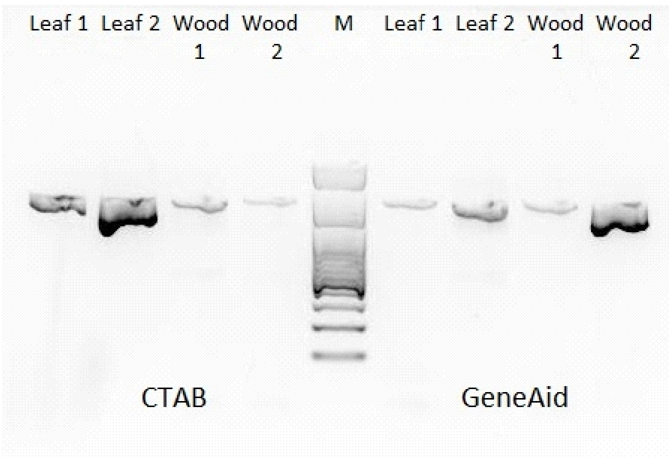
b) *trn*L-*trn*F



c) *trn*G intron



d) *rps*16 intron



e) *pet*L-*psb*E

Figure 2 Representative photos of agarose gel electrophoresis of multiple marker PCR products using template DNA extracted with CTAB and GeneAid protocols. a) *trn*L intron, b) *trn*L-trnF, c) *trn*G intron, d) *rps*16 intron, and e) *pet*L-*psb*E.

remove polysaccharides efficiently during DNA extraction (Syamkumar et al., 2005; Sahu et al., 2012). The addition of PVP to the extraction buffer can also reduce polyphenol contamination because it covalently binds to these compounds and precipitates back during chloroform extraction (Ibrahim, 2011).

On the other hand, the GeneAid plant extraction kit utilizes a different approach based on silica-based membrane

Jurnal Manajemen Hutan Tropika, *30*(1), 144–154, April 2024
EISSN: 2089-2063
DOI: 10.7226/jtfm.30.1.144
Scientific Article
ISSN: 2087-0469

technology in the form of a spin column (Rahmadara et al., 2022). Although this method is convenient and can yield satisfactory results for many applications, it may not be as effective in removing certain contaminants and impurities as the CTAB method. *S. leprosula* is known to contain polyphenols in its leaves (Abasolo et al., 2009; Risnasari et al., 2019), and its bark/wood has been reported for several shorea species (Kawamura et al., 2011; Syafriana et al., 2020). The presence of residual polyphenolic compounds and proteins in the DNA extracted using the GeneAid kit can hinder the readability and sequencing of DNA, resulting in shorter readable DNA sequences compared with the CTAB method.

The poor sequencing quality of the forward primers used in this study could be due to suboptimal primer design. The forward primer may have mismatches or suboptimal binding conditions at the target locus, resulting in inefficient amplification and poor sequencing results (Eckert & Kunkel, 1991; Francis et al., 2017). In contrast, reverse primers may have been designed more effectively, leading to successful amplification and clear sequencing outcomes (Liu & Naismith, 2008). The target locus itself can influence primer binding and amplification efficiency. It is possible that the forward primer region contains variations or structural features, such as secondary structures or repetitive sequences that impede efficient amplification (Treangen & Salzberg, 2012). These characteristics can hinder the binding and extension of the forward primer, resulting in poor sequencing results. The reverse primer targeting a different locus may not be affected by these inhibitory factors.

In contrast, the *rps*16 intron locus shows a failure when the length of the nucleotide is very short and messy in both strands of the sequence. By observing the resulting sequence structure (Figure 3), it can be seen that there is a massive repetitive section of the sequence strand that is amplified by the *rps*16 intron. From the screening of 5 markers, only the *rps*16 intron showed amplification failure in both forward and reverse sequence directions. This shows that the characteristics of the target locus of the *rps*16 intron in the *S. leprosula* in this study were not successfully amplified by the

Table 2 The nucleotide lengths of the five markers used in the study

No	Marker and samples	Length of forward sequences (bp)		Length of reverse sequences (bp)		Length of contig or assemble (bp)		Expected length
		CTAB	GeneAid	CTAB	GeneAid	CTAB	GeneAid	
1	*trnL* intron							
	Leaf	546 bp	546 bp	534 bp	534 bp	586 bp	586 bp	457–499 bp
	Wood	79 bp	79 bp	443 bp	443 bp	-	-	
2	*trn*L-*trn*F							
	Leaf	430 bp	430 bp	437 bp	437 bp	479 bp	480 bp	355–437 bp
	Wood	433 bp	433 bp	437 bp	438 bp	479 bp	480 bp	
3	*trnG* intron							
	Leaf	908 bp	894 bp	890 bp	893 bp	908 bp	894 bp	863 bp
	Wood	890 bp	890 bp	messy	messy	-	-	
4	*rps*16 intron							
	Leaf	270 bp	270 bp	192 bp	192 bp	-	-	526 bp
	Wood	270 bp	270 bp	192 bp	192 bp	-	-	
5	*pet*L-*psb*E							
	Leaf	195 bp	messy	531 bp	588 bp	-	-	956 bp
	Wood	195 bp	195 bp	769 bp	769 bp	-	-	

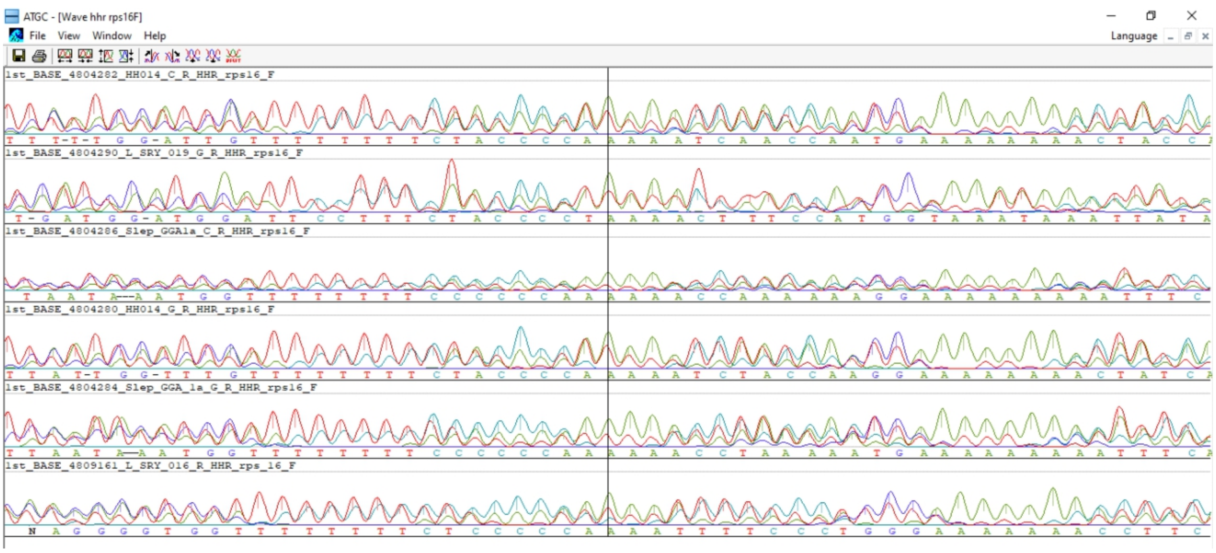


Figure 3 *Shorea leprosula* sequence electropherogram with *rps*16 intron markers.

primer pairs used. The character of the amplified target locus may also be an obstacle in other studies because until now, no other *S. leprosula* individuals that are amplified by the *rps*16 intron have been identified, which can be accessed on the NCBI global database database.

The results showed that in several primers, only leaves were amplified and resulted in a complete set of sequences, namely the *trn*L intron, *trn*L-*trn*F, and *trn*G intron markers. Leaves are composed of relatively soft and cellular tissues, which makes it easier to disrupt cell walls and release DNA (Doyle & Doyle, 1987; Murray & Thompson, 1980; Williams & Ronald, 1994). In contrast, wood is composed of harder and more lignified tissues (Kärkönen & Koutaniemi, 2010), making it more difficult to extract DNA. Leaves contain a higher density of living cells than wood, which typically consists of dead cells with thick cell walls (Haroen & Dimyati, 2006). Living cells in the leaves contain a higher concentration of intact DNA, making the extraction more efficient (Varma et al., 2007). Wood samples can contain various compounds, such as polyphenols, tannins, and lignin, which can inhibit DNA extraction and downstream applications, such as PCR. These inhibitors can interfere with the DNA isolation process and hinder the purity and yield of the extracted DNA (Porebski et al., 1997; Filippis & Magel, 1998; Jhala Vibhuti et al., 2015). The presence of polysaccharides in DNA makes it thick and resembles gum, causing difficulties in loading (Sablok et al., 2009). Polysaccharide contamination also inhibits the Taq polymerase activity (Karaca et al., 2005). Oxidized polyphenols bind to DNA and inhibit PCR amplification (Sahu et al., 2012). Chloroplasts are notably concentrated within the mesophyll cells of leaves and play a pivotal role in harnessing light energy (Kirchhoff, 2019). Conversely, in wood, chloroplasts are present in parenchyma cells, although their concentration is generally lower than that observed in leaves (Mishra et al., 2018). The process of extracting DNA from wood for chloroplast DNA (cpDNA) amplification poses increased challenges compared to leaves (Liepelt et al., 2006; Finkeldey et al., 2010); Swetha et al., 2014). This difficulty arises because of the necessity for additional steps in wood DNA extraction, including the removal of potentially interfering compounds such as lignin (Finkeldey et al., 2010). The distinct distribution of chloroplasts in leaves and wood, coupled with the complexities of wood DNA extraction, underscores the importance of tailored approaches when studying chloroplast DNA in these tissues.

The results showed that for large-scale investigations, but with limited funds, CTAB could be the best method for extracting *S. leprosula* DNA from leaves or wood. Although this method requires more time, it can produce pure DNA of good quality with successful amplification. Sequence length is one of the success factors of the quality of extracted DNA because for some molecular investigations, the amplification of nucleotide length becomes very important (Deguilloux et al., 2003; Rachmayanti et al., 2009). However, in this study, the DNA plant extraction kit method also provided good quality DNA, which was successfully amplified and could be used in molecular research for leaf and wood tissue samples. Kits are easy to work with, simple, and fast. When time is an important consideration, the utilization of a plant DNA extraction kit is the key to fast and reliable DNA isolation from plant tissue.

The results presented in Table 2 demonstrate the successful amplification of leaf and wood samples using both the CTAB and GeneAid extraction methods. Notably, *trn*L-*trn*F markers yielded complete and well-amplified sequences compared with the other four markers. These findings align with previous studies (Yulita et al., 2005; Kamiya et al., 2011, 2012; Rachmat et al., 2012) that have utilized *trn*L-*trn*F markers for molecular analysis of the Dipterocarpaceae family, particularly the *Shorea* genus. The *trn*L-*trn*F marker, derived from a non-coding region of the chloroplast genome, is widely used to infer evolutionary relationships across taxonomic levels. It has been used to study the relationships between and within genera (Bayer & Starr, 1998; Bayer et al., 2000), among species (González et al., 2002), and within populations (Okaura & Harada, 2002). Thus, *trn*L-*trn*F markers can be considered universal markers that produce high-quality sequences for both leaf and wood extractions in *S. leprosula*.

Conclusion

Higher success of DNA extraction was achieved from the leaves than from the wood parts. This is because wood samples may contain various compounds, such as polyphenols, tannins, and lignin, which can inhibit DNA extraction and downstream applications such as PCR. PCR and sequencing of the five cpDNA markers used in the study found that the *trn*L-*trn*F marker can be considered as universal markers that produce high-quality sequences for both leaf and wood extraction in *S. leprosula.* Whereas the results of The *rps*16 intron sequence is messy and characterized by repetitive DNA (repeated nucleotide sequences). In addition, *trn*L intron and *trn*G intron markers for wood extraction template DNA¸ *pet*L-*psb*E resulted from poor forward primer sequences characterized by messy electropherogram quality with overlapping peaks. CTAB and its kits are highly applicable for the extraction of *S. leprosula* DNA. For large-scale investigations with limited funding, CTAB may be the best method for extracting *S. leprosula* DNA from leaves and wood. However, when time is an important consideration, the utilization of plant DNA extraction kits is key for the fast and reliable isolation of plant tissue DNA.

Acknowledgment

This research was supported by the Rumah Program-National Research and Innovation Agency through Letter of Decree of Head of Research Organization of Life Sciences and Environmental Number 10/III.5/HK/2023 which was received by Henti Hendalastuti Rachmat, World Resources Institute's (WRI) Forest Program grant (Project Code: R4586) entitled “Indonesian-based wood identification program” awarded by Norwegian Agency for Development Cooperation (NORAD) which was received by Iskandar Z. Siregar and Talenta Research Grant Universitas Sumatera Utara Number 13388/UN5.1.R/PPM/2023, which was received by Arida Susilowati.

Disclosure Statement

The authors declare no competing interests.

References

Abasolo, M. A., Fernando, E. S., Borromeo, T. H., & Hautea, D. M. (2009). Cross-species amplification of *Shorea* microsatellite DNA markers in *Parashorea malaanonan* (Dipterocarpaceae). *Philippine Journal of Science*, *138*(1), 23–28.

Abe, H., Watanabe, U., Yoshida, K., Kuroda, K., & Zhang, C. (2011). Changes in organelle and DNA quality, quantity, and distribution in the wood of *Cryptomeria japonica* over long-term storage. *IAWA Journal*, *32*(2), 263–272. https://doi.org/10.1163/22941932-90000056

Ashton, P. S. (1982). Dipterocarpaceae. In *Flora malesiana I, Spermatophyta* (pp. 237–552). Rijksherbarium.

Bayer, R. J., Puttock, C. F., & Kelchner, S. A. (2000). Phylogeny of South African Gnaphalieae (Asteraceae) based on two noncoding chloroplast sequences. *American Journal of Botany*, *87*(2), 259–272. https://doi.org/10. 2307/2656914

Bayer, R. J., & Starr, J. R. (1998). Tribal phylogeny of the Asteraceae based on two non-coding chloroplast sequences, the *trn*L Intron and *trn*L/*trn*F Intergenic spacer. *Annals of the Missouri Botanical Garden*, *85*, 242–256. https://doi.org/10.2307/2992008

Cannon, C. H., & Manos, P. S. (2003). Phylogeography of the Southeast Asian stone oaks (*Lithocarpus*). *Journal of Biogeography*, *30*(2), 211–226. https://doi.org/ 10.1046/j.1365-2699.2003.00829.x

Cano, R. J. (1996). Analysing ancient DNA. *Endeavour*, *20*(4), 162–167. https://doi.org/10.1016/S0160-9327 (96)10031-4

Cao, C. P., Finkeldey, R., Siregar, I. Z., Siregar, U. J., & Gailing, O. (2006). Genetic diversity within and among populations of *Shorea leprosula* Miq. and *Shorea parvifolia* Dyer (Dipterocarpaceae) in Indonesia detected by AFLPs. *Tree Genetics and Genomes*, *2*(4), 225–239. https://doi.org/10.1007/s11295-006-0046-0

Deguilloux, M. F., Pemonge, M. H., Bertel, L., Kremer, A., & Petit, R. J. (2003). Checking the geographical origin of oak wood: Molecular and statistical tools. *Molecular Ecology*, *12*(6), 1629–1636. https://doi.org/10.1046/ j.1365-294X.2003.01836.x

Deguilloux, M. F., Pemonge, M. H., & Petit, R. J. (2002). Novel perspectives in wood certification and forensics: Dry wood as a source of DNA. *Proceedings of the Royal Society B: Biological Sciences*, *269*(1495), 1039–1046. https://doi.org/10.1098/rspb.2002.1982

Dellaporta, S. L., Wood, J., & Hicks, J. B. (1983). A plant DNA minipreparation: Version II. *Plant Molecular Biology Reporter*, *1*(4), 19–21. https://doi.org/10.1007/ BF02712670

Dhaliwal, A. (2020). DNA extraction and purification. *Mater Methods*, *3*, 191. https://doi.org/10.13070/mm.en.3.191

Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, *19*(1), 11–15.

Eckert, K. A., & Kunkel, T. A. (1991). DNA polymerase fidelity and the polymerase chain reaction. *Genome Research*, *1*(1), 17–24. https://doi.org/10.1101/gr.1.1.17

Filippis, L. De, & Magel, E. (1998). Differences in genomic DNA extracted from bark and from wood of different zones in Robinia trees using RAPD-PCR. *Trees*, *12*(6), 377–384. https://doi.org/10.1007/PL00009723

Finkeldey, R., Leinemann, L., & Gailing, O. (2010). Molecular genetic tools to infer the origin of forest plants and wood. *Applied Microbiology and Biotechnology*, *85*(5), 1251–1258. https://doi.org/10.1007/s00253-009-2328-6

Francis, F., Dumas, M. D., & Wisser, R. J. (2017). ThermoAlign: A genome-aware primer design tool for tiled amplicon resequencing. *Scientific Reports*, *7*, 44437. https://doi.org/10.1038/srep44437

González, D., Vovides, A., & Lammers, T. G. (2002). Low intralineage divergence in *Ceratozamia* (Zamiaceae) detected with nuclear ribosomal DNA ITS and chloroplast DNA *trn*L-F non-coding region. *Systematic Botany*, *27*(4), 654–661.

Gryson, N. (2010). Effect of food processing on plant DNA degradation and PCR-based GMO analysis: A review. *Analytical and Bioanalytical Chemistry*, *396*(6), 2003–2022. https://doi.org/10.1007/s00216-009-3343-2

Haroen, W. K., & Dimyati, F. (2006). Sifat kayu tarik, teras, dan gubal *Acacia mangium* terhadap karakteristik pulp. *Berita Selulosa*, *41*(1), 1–7.

Heckenhauer, J., Samuel, R., Ashton, P. S., Abu Salim, K., & Paun, O. (2018). Phylogenomics resolves evolutionary relationships and provides insights into floral evolution in the tribe *Shoreae* (Dipterocarpaceae). *Molecular Phylogenetics and Evolution*, *127*, 1–13. https://doi.org/ 10.1016/j.ympev.2018.05.010

Ibrahim, R. I. H. (2011). A modified CTAB protocol for DNA extraction from young flower petals of some medicinal plant species. *Geneconserve*, *10*(40), 165–182.

Jhala Vibhuti, M., Mandaliya, V. B., & Thaker, V. S. (2015). Simple and efficient protocol for RNA and DNA extraction from rice (*Oryza sativa* L.) for downstream applications. *International Research Journal of Biological Sciences*, *4*(2), 62–67.

Jobes, D. V, Hurley, D. L., & Thien, L. B. (1995). Plant DNA isolation: A method to efficiently remove polyphenolics, polysaccharides, and RNA. *Taxon*, *44*, 379–386.

Kajita, T., Kamiya, K., Nakamura, K., Tachida, H., Wickneswari, R., Tsumura, Y., Yoshimaru, H., & Yamazaki, T. (1998). Molecular phylogeny of Dipetrocarpaceae in Southeast Asia based on nucleotide sequences of matK, *trn*L intron, and *trn*L-*trn*F intergenic spacer region in chloroplast DNA. *Molecular Phylogenetics and Evolution*, *10*(2), 202–209. https://doi.org/10.1006/mpev.1998.0516

Kamiya, K., Gan, Y. Y., Lum, S. K. Y., Khoo, M. S., Chua, S. C., & Faizu, N. N. H. (2011). Morphological and molecular evidence of natural hybridization in *Shorea* (Dipterocarpaceae). *Tree Genetics and Genomes*, *7*, 297–306. https://doi.org/10.1007/s11295-010-0332-8

Kamiya, K., Nanami, S., Kenzo, T., Yoneda, R., Diway, B., Chong, L., Azani, M. A., Majid, N. M., Lum, S. K. Y., Wong, K. M., & Harada, K. (2012). Demographic history of *Shorea curtisii* (Dipterocarpaceae) inferred from chloroplast DNA sequence variations. *Biotropica*, *44*(5), 577–585. https://doi.org/10.1111/j.1744-7429.2011.00834.x

Karaca, M., İnce, A. G., Elmasulu, S. Y., Onus, A. N., & Turgut, K. (2005). Coisolation of genomic and organelle DNAs from 15 genera and 31 species of plants. *Analytical Biochemistry*, *343*(2), 353–355. https://doi.org/10.1016/j.ab.2005.03.021

Kärkönen, A., & Koutaniemi, S. (2010). Lignin biosynthesis studies in plant tissue cultures. *Journal of Integrative Plant Biology*, *52*(2), 176–185. https://doi.org/10.1111/j.1744-7909.2010.00913.x

Kawamura, F., Ramle, S. F. M., Sulaiman, O., Hashim, R., & Ohara, S. (2011). Antioxidant and antifungal activities of extracts from 15 selected hardwood species of Malaysian timber. *European Journal of Wood and Wood Products*, *69*(2), 207–212. https://doi.org/10.1007/s00107-010-0413-2

Kessler, P. J. A., & Sidiyasa, K. (1994). *Trees of the Balikpapan-Samarinda area, East Kalimantan, Indonesia: A manual to 280 selected species*. Tropenbos Foundation.

Kim, C. S., Lee, C. H., Shin, J. S., Chung, Y. S., & Hyung, N. I. (1997). A simple and rapid method for isolation of high quality genomic DNA from fruit trees and conifers using PVP. *Nucleic Acids Research*, *25*(5), 1085–1086. https://doi.org/10.1093/nar/25.5.1085

Kim, D., Hofstaedter, C. E., Zhao, C., Mattei, L., Tanes, C., Clarke, E., Lauder, A., Sherrill-mix, S., Chehoud, C., Kelsen, J., Conrad, M., Collman, R. G., Baldassano, R., Bushman, F. D., & Bittinger, K. (2017). Optimizing methods and dodging pitfalls in microbiome research. *Microbiome*, *5*, 52. https://doi.org/10.1186/s40168-017-0267-5

Kirchhoff, H. (2019). Chloroplast ultrastructure in plants. *New Phytologist*, *223*(2), 565–574. https://doi.org/10.1111/nph.15730

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, *33*(7), 1870–1874. https://doi.org/10.1093/molbev/msw054

Lee, S. L., Wickneswari, R., Mahani, M. C., & Zakri, A. H. (2000a). Inheritance of allozyme in *Shorea leprosula* (Dipterocarpaceae). *Journal of Tropical Forest Science*, *12*(1), 124–138.

Lee, S. L., Wickneswari, R., Mahani, M. C., & Zakri, A. H. (2000b). Genetic diversity of a tropical tree species, *Shorea leprosula* Miq. (Dipterocarpaceae), in Malaysia: Implications for conservation of genetic resources and tree improvement. *Biotropica*, *32*(2), 213–224. https://doi.org/10.1111/j.1744-7429.2000.tb00464.x

Liepelt, S., Sperisen, C., Deguilloux, M. F., Petit, R. J., Kissling, R., Spencer, M., De Beaulieu, J. L., Taberlet, P., Gielly, L., & Ziegenhagen, B. (2006). Authenticated DNA from ancient wood remains. *Annals of Botany*, *98*(5), 1107–1111. https://doi.org/10.1093/aob/mcl188

Liu, H., & Naismith, J. H. (2008). An efficient one-step site-directed deletion, insertion, single and multiple-site plasmid mutagenesis protocol. *BMC Biotechnology*, *8*, 91. https://doi.org/10.1186/1472-6750-8-91

Miranda Montero, J. J., Wright, E. M., & Khan, M. N. (2020). *Illegal logging, fishing, and wildlife trade: The costs and how to combat it*. World Bank Group. https://policycommons.net/artifacts/1282895/illegal-logging-fishing-and-wildlife-trade/

Mishra, G., Collings, D. A., & Altaner, C. M. (2018). Cell organelles and fluorescence of parenchyma cells in *Eucalyptus bosistoana* sapwood and heartwood investigated by microscopy. *New Zealand Journal of Forestry Science*, *48*, 13. https://doi.org/10.1186/s40490-018-0118-6

Murray, M. G., & Thompson, W. F. (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research*, *8*(19), 4321–4326. https://doi.org/10.1093/nar/8.19.4321

Newman, M. F., Burgess, P. F., & Whitmore, T. C. (1996). *Manual of dipterocarps for forester: Borneo Island light hardwoods, Anisptera, Parashorea, Shorea (red, white and yellow meranti)*. Royal Botanic Garden Edinburgh & CIFOR.

Ng, C. H., Ng, K. K. S., Lee, S. L., Zakaria, N. F., Lee, C. T., & Tnah, L. H. (2022). DNA databases of an important tropical timber tree species *Shorea leprosula* (Dipterocarpaceae) for forensic timber identification. *Scientific Reports*, *12*, 9546. https://doi.org/10.1038/s41598-022-13697-x

Nuroniah, H. S., Gailing, O., & Finkeldey, R. (2010).

Development of SCAR markers for species identification in the genus *Shorea* (Dipterocarpaceae). *Silvae Genetica*, *59*(6), 249–257. https://doi.org/ 10.1515/sg-2010-0035

Okaura, T., & Harada, K. (2002). Phylogeographical structure revealed by chloroplast DNA variation in Japanese beech (*Fagus crenata* Blume). *Heredity*, *88*(4), 322–329. https://doi.org/10.1038/sj.hdy.6800048

Pooma, R., & Newman, M. F. (2017). *Shorea leprosula*. https://doi.org/10.2305/IUCN.UK.2017-3.RLTS.T33123A2833148.en

Porebski, S., Bailey, L. G., & Baum, B. R. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Molecular Biology Reporter*, *15*(1), 8–15. https://doi.org/10.1007/BF02772108

Rachmat, H. H., Kamiya, K., & Harada, K. (2012). Genetic diversity, population structure and conservation implication of the endemic Sumatran lowland dipterocarp tree species (*Shorea javanica*). *International Journal of Biodiversity and Conservation*, *4*(14), 573–583. https://doi.org/10.5897/IJBC12.045

Rachmayanti, Y., Leinemann, L., Gailing, O., & Finkeldey, R. (2009). DNA from processed and unprocessed wood: factors influencing the isolation success. *Forensic Science International. Genetics*, *3*(3), 185–192. https://doi.org/10.1016/j.fsigen.2009.01.002

Rahmadara, G., Hanifah, N. F., Rismayanti, R., Purwoko, D., Rochandi, A., & Tajuddin, T. (2022). Comparison of DNA isolation methods that derived from leaves of a potential anti-cancer rodent tuber (*Typhonium flagelliforme*) plant. *International Journal of Agriculture System*, *10*(2), 93. https://doi.org/10.20956/ijas.v10i2.3966

Rana, R., Villarin, R., Gailling, O., Finkeldey, R., & Polle, A. (2013). Height growth, wood density and molecular markers to distinguish five tree species of Dipterocarpaceae grown at same site. *Bangladesh Journal of Scientific and Industrial Research*, *47*(4), 407–414. https://doi.org/10.3329/bjsir.v47i4.14070

Risnasari, I., Nuryawan, A., & Siallagan, N. F. (2019). Characterization of particleboard from waste tea leaves (*Camellia sinensis* L) and meranti wood (*Shorea* sp.) using urea-formaldehyde adhesive and it's formaldehyde emission. *Proceedings of the International Conference on Natural Resources and Technology*, *1*, 261–264. https://doi.org/10.5220/0008552702610264

Rogers, S. O., & Bendich, A. J. (1985). Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. *Plant Molecular Biology*, *5*(2), 69–76. https://doi.org/10.1007/BF00020088

Rohland, N., & Hofreiter, M. (2007). Comparison and optimization of ancient DNA extraction. *BioTechniques*, *42*(3), 343–352. https://doi.org/10.2144/000112383

Rudjiman, A. (2002). *Identification manual of Shorea* spp. ITTO Project-Faculty of Forestry Gadjah Mada University.

Sablok, G., Gahlot, P., Gupta, A. K., Pareek, K., & Sekhawat, N. S. (2009). Extraction of PCR-usable DNA from trees adapted to arid environment. *Plant Omics Journal*, *2*(3), 103–109.

Sahu, S. K., Thangaraj, M., & Kathiresan, K. (2012). DNA extraction protocol for plants with high levels of secondary metabolites and polysaccharides without using liquid nitrogen and phenol. *ISRN Molecular Biology*, *2012*, 205049. https://doi.org/10.5402/2012/205049

Särkinen, T., Staats, M., Richardson, J. E., Cowan, R. S., & Bakker, F. T. (2012). How to Open the Treasure Chest? Optimising DNA Extraction from Herbarium Specimens. *PLoS ONE*, *7*(8), e43808. https://doi.org/10.1371/journal.pone.0043808

Shaw, J., Lickey, E. B., Beck, J. T., Farmer, S. B., Liu, W., Miller, J., Siripun, K. C., Winder, C. T., Schilling, E. E., & Small, R. L. (2005). The tortoise and the Hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany*, *92*(1), 142–166. https://doi.org/10.3732/ajb.92.1.142

Shaw, J., Lickey, E., Schilling, E., & Small, R. (2007). Comparison of whole chloroplast genome sequence to choose noncoding regions for phylogenetic studies in Angiosperms: The tortoise and the Hare III. *American Journal of Botany*, *94*, 275–288. https://doi.org/10.3732/ajb.94.3.275

Stewart, C. N., & Via, E. L. (1993). A rapid CTAB DNA isolation technique useful for RAPD fingerprinting and other PCR applications. *Biotechniques*, *14*(5), 748–751.

Swetha, V. P., Parvathy, V. A., Sheeja, T. E., & Sasikumar, B. (2014). Isolation and amplification of genomic DNA from barks of *Cinnamomum* spp. *Turkish Journal of Biology*, *38*(1), 151–155. https://doi.org/10.3906/biy-1308-5

Syafriana, V., Rachmatiah, T., & Utama, N. W. (2020). Aktivitas antibakteri ekstrak metanol kulit batang meranti sarang punai (*Shorea parvifolia* Dyer) terhadap *Staphylococcus aureus* dan *Propionibacterium acnes*. *Jurnal Farmasi Udayana*, *2020*(*Special Issue*), 160–170. https://doi.org/10.24843/jfu.2020.v09.i03.p04

Syamkumar, S., Jose, M., & Sasikumar, B. (2005). Isolation and PCR amplification of genomic DNA from dried capsules of cardamom (*Elettaria cardamomum* M.). *Plant Molecular Biology Reporter*, *23*(4), 37–41. https://doi.org/10.1007/bf02788890

Symington, C. F. (1943). *Foresters' manual of dipterocarps*.

Malayan forest record no. 16. Kuala Lumpur: Penerbit Universiti Malaya.

Taberlet, P., Gielly, L., Pautou, G., & Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, *17*(5), 1105–1109. https://doi.org/10.1007/BF00037152

Tibbits, J. F. G., McManus, L. J., Spokevicius, A. V, & Bossinger, G. (2006). A rapid method for tissue collection and high-throughput isolation of genomic DNA from mature trees. *Plant Molecular Biology Reporter*, *24*(1), 81–91. https://doi.org/10.1007/BF02914048

Treangen, T. J., & Salzberg, S. L. (2012). Repetitive DNA and next-generation sequencing: Computational challenges and solutions. *Nature Reviews Genetics*, *13*(1), 36–46. https://doi.org/10.1038/nrg3117

Tsumura, Y., Kado, T., Yoshida, K., Abe, H., Ohtani, M., Taguchi, Y., Fukue, Y., Tani, N., Ueno, S., Yoshimura, K., Kamiya, K., Harada, K., Takeuchi, Y., Diway, B., Finkeldey, R., Na'iem, M., Indrioko, S., Ng, K. K. S., Muhammad, N., & Lee, S. L. (2011). Molecular database for classifying *Shorea* species (Dipterocarpaceae) and techniques for checking the legitimacy of timber and wood products. *Journal of Plant Research*, *124*(1), 35–48. https://doi.org/10.1007/s10265-010-0348-z

Turaki, A. A., Ahmad, B., Magaji, U. F., Abdulrazak, U. K., Yusuf, B. A., & Hamza, A. B. (2017). Optimised cetyltrimethylammonium bromide (CTAB) DNA extraction method of plant leaf with high polysaccharide and polyphenolic compounds for downstream reliable molecular analyses. *African Journal of Biotechnology*, *16*(24), 1354–1365. https://doi.org/10.5897/ajb2017.15942

Varma, A., Padh, H., & Shrivastava, N. (2007). Plant genomic DNA isolation: An art or a science. *Biotechnology Journal*, *2*(3), 386–392. https://doi.org/10.1002/biot.200600195

Wagner, D. B., Furnier, G. R., Saghai-Maroof, M. A., Williams, S. M., Dancik, B. P., & Allard, R. W. (1987). Chloroplast DNA polymorphisms in lodgepole and jack pines and their hybrids. *Proceedings of the National Academy of Sciences*, *84*(7), 2097–2100. https://doi.org/10.1073/pnas.84.7.2097

Wahyudi, A., Sari, N., Saridan, A., Cahyono, D. D. N., Rayan, Noor, M., Fernandes, A., Abdurachman, Apriani, H., Handayani, R., Hardjana, A. K., Susanty, F. H., Karmilasanti, Ngatiman, Fajri, M., Wiati, C. B., & Wahyuni, T. (2014). *Shorea leprosula Miq and Shorea johorensis* Foxw: *Ekologi, silvikultur, budaya dan pengembangan*. Balai BesarPenelitian Dipterokarpa.

Williams, C. E., & Ronald, P. C. (1994). PCR template-DNA isolated quickly from monocot and dicot leaves without tissue homogenization. *Nucleic Acids Research*, *22*(10), 1917–1918. https://doi.org/10.1093/nar/22.10.1917

Xu, J., Aileni, M., Abbagani, S., & Zhang, P. (2010). A reliable and efficient method for total rna isolation from various members of spurge family (Euphorbiaceae). *Phytochemical Analysis*, *21*(5), 395–398. https://doi.org/10.1002/pca.1205

Yulita, K. S., Bayer, R. J., & West, J. G. (2005). Molecular phylogenetic study of *Hopea* and *Shorea* (Dipterocarpaceae): Evidence from the *trn*L-*trn*F and internal transcribed spacer regions. *Plant Species Biology*, *20*(3), 167—182. https://doi.org/10.1111/j.1442-1984.2005.00136.x

Zimmermann, J., Hajibabaei, M., Blackburn, D. C., Hanken, J., Cantin, E., Posfai, J., & Evans, T. C. (2008). DNA damage in preserved specimens and tissue samples: A molecular assessment. *Frontiers in Zoology*, *5*, 18. https://doi.org/10.1186/1742-9994-5-18