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Quantitative and Qualitative Test of the Fecal Sampel From Sumatran Elephant (*Elephas maximus sumatranus*)

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Abstract *:*
Sumatran Elephants (Elephas maximus sumatranus) was one of the endemic Sumatran fauna that was protected by Indonesia Law. The Acacia Industrial Forest District Penyabungan in the landscape of Padang Sugihan was one of the elephant's habitats in South Sumatra. Habitat degradation and fragmentation, illegal hunting and conflicts become the major threats for the elephant population. Genetic study was one of the efforts to establish a conservation policy. It was necessary to test the quantitative and qualitative of DNA, at the extractions stage from fecal samples, in order to get the standard results on DNA concentration which will be analyzed later. The method used in this study was direct observation as the initial survey to determine the sampling location. Fecal samples were preserved in absolute ethanol at -20°C. The extraction process was carried out using the Quick-DNA™ Fecal/Soil Microbe Miniprep Kit. Samples were tested quantitatively using a Nanodrop Thermo Scientific, with the expectation that the concentration results were considered to be 1.6-1.8 and continued with the qualitative test of DNA through electrophoresis gel agarose and UV Trans-illuminator of the 20 samples/ There were only 12 samples that showed positive results (there were DNA bands). The results of this quantitative and qualitative test could be used to determine a viable sample to be used as a product in the genetic analysis stage of Sumatran Elephants in the Acacia Industrial Forest of South Sumatra.
Keywords: Fecal DNA, Padang sugihan landscape, South Sumatra, Sumatran Elephant

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1. Introduction

Sumatran Elephants was one of the endemic Sumatran that is protected according to Government Regulation No. 7 Year 1999 about the Preservation of Plants and Animals and and revision of the regulation by the Minister of Environment and Forestry No. P 106 Year 2018 [10];[11]. Based on the IUCN Red List of Threatened Species, Sumatran Elephant is categorized as Critically Endangered. [6] suggested that the Sumatran subspecies is monophyletic based on mitochondrial DNA patterns. This suggests that Sumatran Elephants should be managed separately from other Asian elephants [9]. Major threats that have to be faced by this charismatic subspecies were conversions of forests into human settlement and agricultural areas, causing conflicts with human. Afterward, many wild elephants have been directly killed. Elephants are also targets of illegal killing for their ivory. A systematic study on the population of Sumatran Elephants lacks from most of the elephant's distributional range, and currently Sumatran Elephant lives only in seven provinces, which are under increased pressure of habitat loss and imminent conflicts with human [2]; [9].

South Sumatra was one of Province of Sumatran Elephant distributional range. Based on the data of Sumatran Elephant distribution, the Sugihan Landscape is one of the habitat pockets with the highest number of individuals in South Sumatra. According to [14], habitat pocket of Sugihan-Simpang Heran have 15-52 individuals. This habitat pocket includes Wildlife Reserve area of Padang Sugihan, production forest in the form of lowland, swamp and peat ecosystems with acacia plantations and swamp shrubs as the dominant vegetation.

Elephants have 56 chromosomes that encode the nature of genotype and phenotype in each. Information about the genetic diversity of a population is needed to determine

conservation policies, management, and strategies [12]; [14]. Genetic analysis of elephants can use fecal samples by observing the mitochondrial D-loop area. Research on genetic studies of Sumatran Elephants through feces is considered to have a lower risk compared to blood samples, especially in wild elephants. According to [13], determining the number of populations through genetic analysis can be done with DNA D-loop Mitochondria from blood or elephant feces.

Padang Sugihan Landscape is one of the elephant habitats in South Sumatra. Habitat degradation and fragmentation, poaching and conflict pose major threats to elephant populations. Molecular studies have never been carried out on Sumatran Elephant populations in the Ogan Komering Ilir Penyabungan District, South Sumatra. Genetic analysis is now closely related to conservation, but the high cost of making this method has not been widely used. In DNA analysis, knowing the quality and quantity of samples is important. According to [1], fecal genetic sampling has not been adopted as regular practice to endangered species. The improving cost-efficiency and efficiency tools for generating a good quality of fecal DNA is needed to effectively support wildlife management.

2. Materials and Methods

Samples and study area

We have conducted field work fom December 2017 – March 2018. Fecal samples were taken from 20 different individuals from the remnant forest of acacia concession in Penyabungan District, Ogan Komering Ilir South Sumatra. The samples were taken non-invasively with fresh categories (not less than 72 hours) and reasonably fresh (up to 7 days) and clean from fungus (Figure 1). Each sample was inserted into a 1,5µL microtube which already contained the absolute ethanol. Samples were then stored at -20°C. To minimize the level of degradation and damage to DNA, samples should be handled not more than 72 hours [15].

Extraction methods

One hundred fifty gram of fecal sample was placed on ZR BashingBead™ Lysis Tube added with 750 µL of BashingBead™, homogenized in the high-speed vortex for 30 minute and centrifuged in 10.000 rpm for 1 minutes. Transferred 400 µL supernatant to Zymo-Spin™ III-F Filter in a collection tube and centrifuged in 8.000 rpm for 1 minutes. Furthermore, it was added 1.200 µL of Genomic Lysis Buffer to the filtrate in the Collection Tube and then mixed well. Then, transfer 800 µL to Zymo-Spin™ IICR Column and centrifuged in 10.000 rpm for 1 minutes, discarded the flow through from the collection tube, repeat. Add 200 µL DNA Pre-Wash Buffer to the spin tube with a new collection tube and centrifuged in 10.000 rpm for 1 minutes. Add 500 µL DNA Wash Buffer to the spin tube and centrifuged in 10.000 rpm for 1 minutes. Transfer the spin tube to a clean 1,5 ml microsentrifuge and add 100 µL DNA Elution Buffer to the binding matrix, centrifuged 10.000 rpm for 30 second to elute the DNA. Next step, place a Zymo-Spin III-HRC Filter in a clean 1,5 ml microsentrifuge add 600 Prep Solution and centrifuged in 8.000 rpm for 3 minutes. Transfer the eluet DNA to the Zymo-Spin III-HRC Filter in a new 1.5 ml microsentrifuge and centrifuged at exactly 16.000 rpm for 3 minutes. Storage the DNA at the – 20 ^{0}C. The filtered DNA was then suitable for quantitative and qualitative analysis [18].

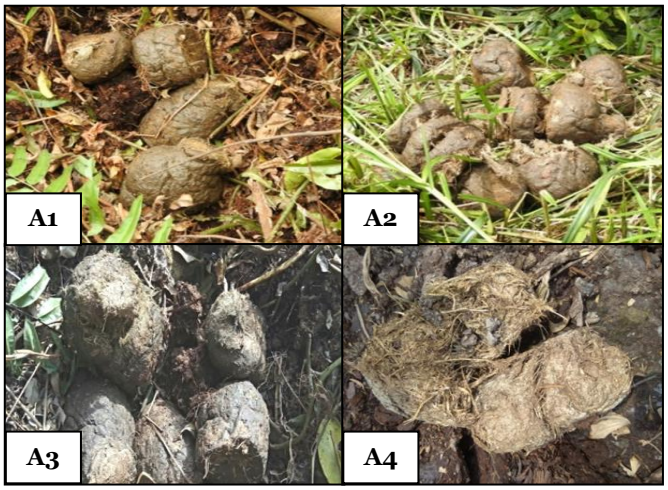


Figure 1: (A1 & A2) Fresh categories (not less than 72 hours), (A3). Reasonably fresh (up to 7 days) and clean from fungus, (A4) Estimation more than 1 month.

Quantitative and qualitative test

The quantitative test of DNA was by Nanodrop Thermo Scientific. Insert 1 µL aquadest as a control and then 2 µL of the sample in the Nanodrop, then read the DNA of UV at λ 260 nm and λ 280 nm. The measurement was performed against the concentration of purity by comparison [5].

$$\textit{Purity Concentration of the Sample} = \frac{\lambda\ 260\ \text{nm}}{\lambda\ 280\ \text{nm}}$$

The qualitative test was provided with Electroporator and visualization with Transilluminator. Previously, gel agarose had been made with 0.6 g agarose powder into an Erlenmeyer, then added 20 ml TAE 1x and homogenized by heating it for 30 seconds. Wait for the gel temperature range between 50^{o}-55^{o}C. Then add the 3 ml DNA gel stain, input into the mold, lift a comb, added TAE 1 x until the sample was submerged. Next, take 1µl loading dye Blue/Orange 6x and homogenized it with 5ml of the sample. Put on electroporator at the negative charge with 80 volt supply for 35 minutes. The results could be observed in UV Transilluminator by Camag UV Cabinet .

3. Results and Discussion

Based on field surveys in the Acacia Industrial Forest in Penyabungan District of Sugihan Landscape in Ogan Komering Ilir South Sumatra. Obtained samples varied from fresh category (72 hours) and reasonably fresh (up to 7 days), as shown in Table 2.

Table 2: Fecal Sample of Sumatran Elephant in the Acacia Industrial Forest in Penyabungan District

No	Sampel Code	Sample Type		Description/Sample Estimation (days)	Purity Concentration
		Fresh	Reasonably fresh		
1	A	√		2-3	1.73
2	B	√		2-3	1.78
3	C	√		1	1.78
4	D	√		1	1.80
5	E	√		2-3	1.76
6	F	√		2-3	1.78
7	G	√		1	1.84
8	H	√		2-3	1.78
9	I	√		2-3	1.61
10	J	√		2-3	1.78
11	K		√	5-7	1.00
12	L		√	5-7	1.00
13	M		√	4	1.58
14	N		√	4	1.60
15	O		√	5-7	1.01
16	P		√	5-7	1.14
17	Q		√	5-7	1,09
18	R		√	5-7	1,01
19	S		√	5-7	1.09
20	T		√	5-7	1.14

Extraction

Based on the extraction results, it can be seen that the DNA eluent in the fresh category sample looks clear and clean white, whereas in the reasonably fresh category sample the color of DNA eluent is white and cloudy. Fecal characteristics are still in the fresh category, a distance of 1 km smells quite strong, the fecal surface is wet and covered by a collection of mucus, morphologically has a bright green color. While the category of reasonably fresh (4-7 days) can still be considered as a sample, namely with a slightly wet condition, black with a little green, preferably still smelling, and there are no insects or fungi on the surface and inside.

Figure 2: Eluent DNA; left tube fresh categories (not less than 72 hours), and right tube reasonably fresh (up to 7 days).

Quantitative test

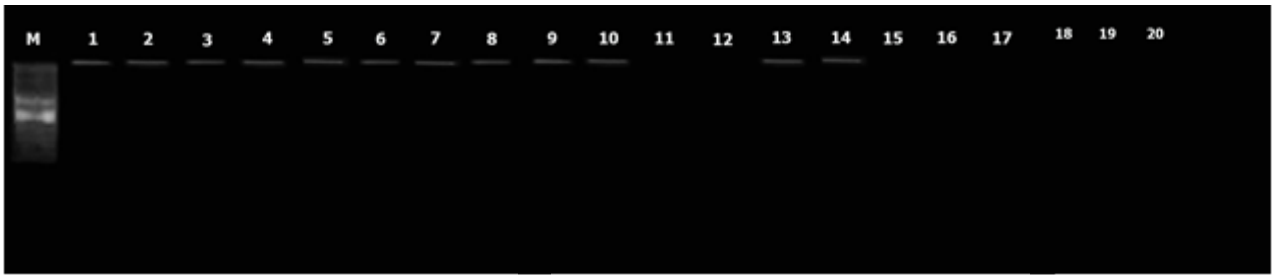
Quantitative test on the samples were carried out using Nanodrop Thermo Scientific at the absorb light of λ 260 nm and λ 260 nm. The results were shown in Table 2. The concentration of the sample purity obtained was varied, for samples with a reasonably fresh category with an estimated sample of 5-7 days; the purity values of 1.00 to 1.14 were obtained. On the other hand, the sample with the fresh category had a purity value ranging from 1.5 to 1.8 (Table 2). There was sample with code G with purity 1.84. The difference in purity value indicates that there is debris (impurities) in the sample; such samples need to be added to Nuclease-Free Water to remove debris and remaining proteins. Low purity values, can also be caused by the lack of DNA in the sample causes from conditions of the sample itself [16]. Extraction results will be used for further analysis.

The ratio of OD260/OD280 <1.8 indicates the presence of phenol or protein contamination in the extraction results [3]. The value of λ 260 / λ 280 <1.6 may contain contaminants such as proteins including RNA and other organic compounds [4]. According to [5], whereas DNA and RNA purity ranged from 1.8 to 2.0.

Qualitative test

The results of qualitative test on samples which were carried out using Bio-Rad Electrophoresis and 1st base shown in Figure 3. The standard method used to separate, identify and purify DNA fragments wass agarose gel electrophoresis. This qualitative test was carried out in three stages. The first stage is the preparation of agarose gel, electrophoresis running, and DNA visualization using a UV transilluminator.

(A)



(B)

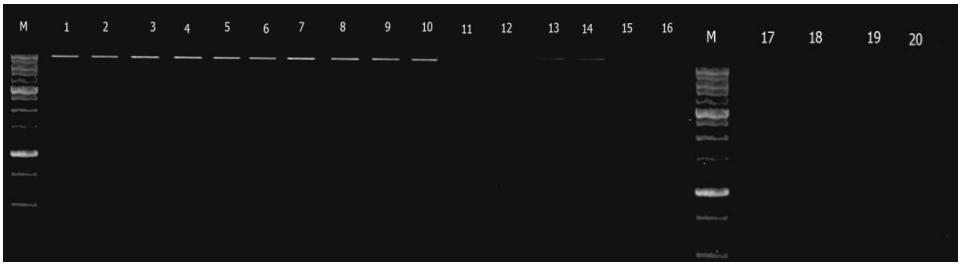


Figure 3: Result of DNA band visualization from fecal sample; (A). UV Transilluminator λ 324 nm by Camag UV Cabinet at Genetic and Biotechnology Laboratory of Biology Department, Faculty of Mathematics and Natural Sciences of Sriwijaya University, and (B) 1st Base sample quantification by gel documentation.

From the 20 samples from extraction, only 12 samples can be visualized. Samples number 11, 12, 15, 16, 17, 18, 19, and 20 have no band. The results show that no single band can be produced (Figure 3), and there is no smear indicating that the sample does not contain pure DNA eluent, and it is not recommended to proceed to the PCR and sequencing stages. Twelve of Twenty samples (A, B, C, D, E, F, G, H, I, J, M, and N) have pure DNA eluents. These samples can be used as a DNA product in the genetic analysis stage of Sumatran Elephants in South Sumatra. Reasonably fresh samples, M and N are categorized as low purity and have many debris or impurities, the DNA eluent within negative result of the electrophoresis cannot be recommended for the next stage of genetic analysis (amplification and sequencing).

Amplification Process

The Sumatran elephant fecal samples were extracted using Quick-DNATM Fecal / Soil Microbe Miniprep Kit, then amplified using a pair of mtDNA specific primers, forward MDL3 5'CCA TCT TCG TGT CCC TCT TC 3' and reverse primer MDL55' GCC ATA GCT GAA TCA CAG CA 3' with each of them having 24 bp oxytone forward (MDL3). [7]; [8]; [13].

PCR Product Electrophoresis

The PCR product was run on an evaporator using a 2% concentration gel, making a gel using 4gr agarose gel powder dissolved with 200 ml TAE 1x, the gel solution was homogeneous and hot pot was heated, the gel wass cooled at a temperature range of 50-550C then 5µL of gel staining was added. Agarosa gel which was ready to be inserted into the chamber, was added 5 µL of DNA Ladder in the first sink, then each sample of 3 µL was inserted into the next well in sequence according to the sample code.

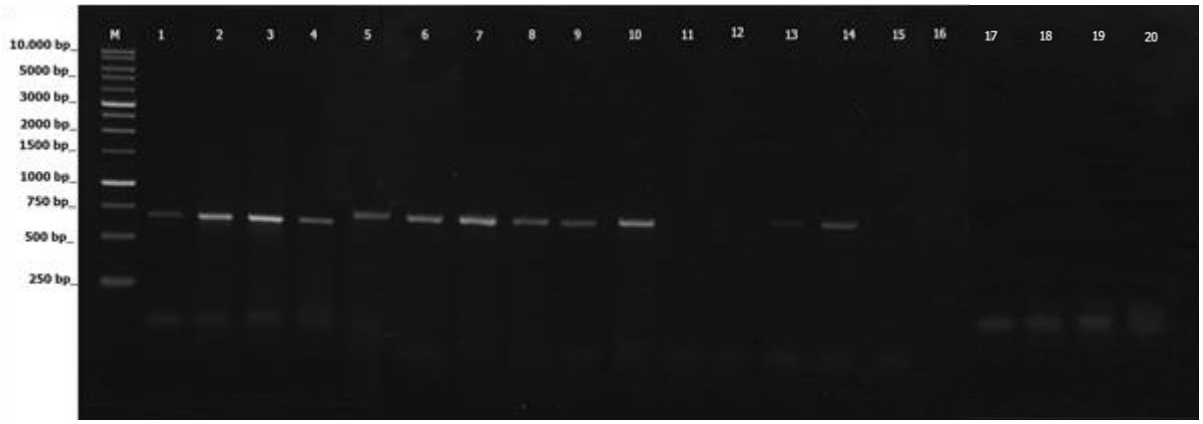


Figure 4: Result of DNA band visualization of PCR product electrophoresis from fecal sample using 1st Base sample quantification by gel documentation.

The observation results are positive and can be continued to the sequencing stage if there is a clear band marker around 630 bp (Figure 4). According to [7]; [8], the mtDNA region with MDL3 and MDL5 specific mitochondrial primers for Asian Elephants (*Elephas maximus*) generally consist of 630 bp with the first 109 bp sequence being a fragment encoding the C end and the end of the cytochrome b, sequence sequence The next 135 bp is the threonine and proline coding region of the tRNA, and the remaining sequence is the fragment of the non-coding portion of the control region, the D-loop.

The difficulty in this study is to separate pure DNA from debris (impurities), protein contaminants, and compounds contained in the remaining fiber of elephant feed such as Cellulose and Magnesium. Quantitative and qualitative tests are influenced by the limited time for encounters with wild elephants and the lack of fresh sample acquisition of less than 72 hours.

4. Conclusion

We present an effective method for taking and preparing fecal samples from Sumatran Elephant. Of the two sample categories that we used in the analysis, samples with fresh categories (not less than 72 hours) show the best quality and quantity results for DNA eluents which would be further analyzed. The reasonably fresh categories samples (up to 7 days) have low DNA quality and quantity, the samples must be immediately separated so that many repetitions will not happen during PCR amplification and make it difficult for the sequeencing process to require substantial costs. We provided several recommendations; samples that have been taken from the field must be in preparation and extraction so there will be no degradation, the laboratory work must also be done on impermeable spaces with UV radiation in sterile conditions to minimize contamination. Lastly, the study of literature and selection of suitable kits are very important to get optimal results.

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