Comparative Study on DNA Extraction's Qualitative Analysis of Captive Sumatran Elephant in Elephant Training Center, Way Kambas National Park

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Comparative Study on DNA Extraction's Qualitative Analysis of Captive Sumatran Elephant in Elephant Training Center, Way Kambas National Park

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Abstract. DNA extraction procedures may exhibit different levels of sensitivity. A preliminary effort to obtain DNA from captive Sumatran elephants (*Elephas maximus sumatranus)* in Elephant Training Center Way Kambas National Park, Lampung for building their molecular individual identification was done. DNA of 30 male and 23 female captive elephants' blood samples was extracted by QIAGEN. Qualitative analysis of DNA extraction of captive elephant was conducted using two methods, simple electrophoresis and electrophoresis based on PCR product. The aim of this project is to compare those two methods of qualitative analysis of DNA extraction. The first method used 1% agarose gel electrophoresis in the TAE buffer (Tris-Acetate-EDTA), while the second method used Polymerase Chain Reaction (PCR) technique with Glyseraldehyde-3-Phosphate Dehydrogenase (GAPDH) primer. The simple electrophoresis showed 41.5 % positive samples, while the second method showed 86.7% positive samples. The electrophoresis based on PCR product exhibited more sensitivity to detect the DNA from blood of each captive elephant.

1. Introduction

Way Kambas National Park (WKNP) with 125,621.3 ha area, is the natural habitats for five big mammals including Sumatran elephant *(Elephas maximus sumatranus)* [1]. In the last 25 years, Sumatran elephant lost its habitat and its population decreased rapidly [2]. Elephant Training Center in WKNP is one of the conservation efforts for captive Sumatran elephant due to human-elephant conflict. Genetic information is needed in elephant conservation, especially in captivity which has high inbreeding pressure. Low genetic diversity in a population will affect its survival [3]. Molecular genetic analysis approach in conservation can be applied to determine genetic diversity in Sumatran elephant. Sequencing can be done using base material, good DNA extract. Technique for DNA quality test include spectrophotometer and electrophoresis to detect gene *Glyceraldehyde-3-phosphate Dehydrogenase* (GAPDH).

2. Materials and Methods

This research was carried out between January and February 2018, in collaboration with Way Kambas National Park for sample collection and conducted in Biotechnology Veterinary Bureau Lampung.

There were 53 Sumatran elephants' blood samples, consisting of 23 female and 30 male samples. The samples were taken from Prof. Dr. Ir. H. Rubini Atmawidjaja Elephant Hospital, Elephant Training Center, WKNP [4]. The DNA extraction process were referencing to the extraction protocol of DNeasyR Blood & Tissue Kit from QIAGEN.

DNA extract quality was tested with a simple technique using agarose gel electrophoresis 1% with the addition of SYBR safe as stains and TAE buffer as mobile phase. The electrophoresis step used 100V for 17 minutes and the DNA molecules with good quality will show band luminescence that can be monitored by digi doc under UV light. DNA extract quality test in molecular technique was done with *Glyceraldehyde-3-phosphate Dehydrogenase* (GAPDH) primary that consist a pair of primers, *forward primer* and *reverse primer* (table 1).

Table 1. GAPDH primary sequence.

PriPrimary	SeSequence
FoForward	5' 5'ATCACTGCCACCCAGAAGAC3'
RReverse	5' 3'CATGCCAGTGAGCTTCCCGTT5'

DNA extract quality test with PCR GAPDH was started by mix mastering, homogenizing Platinum Blue PCR SuperMix with GAPDH primary (Reverse primer and Forward primer) and DNA. In pre denaturation phase, 95^{o}C temperature was applied for 5 minutes. Pre denaturation phase was applied to make sure the DNA genome double chains can be separated into single ones. The second step was denaturation step with 94^{o}C temperature for 20 seconds. Denaturation was the early process to separate DNA double chains into two separate chains. The third step, annealing, 57^{o}C temperature were applied for 45 seconds, as primary identifier for DNA target with unique base pairs in an organism. The fourth step is extension phase in 72^{o}C for 1 minute. Extension phase was the DNA new chain elongation phase. The fifth step was post-extension phase in 72^{o}C temperature for 5 minutes and 4^{o}C to complete the last step. The first and fifth steps were repeated once and the second, third and fourth were repeated for 35 times.

The PCR results then observed and separated with 1.5% agarose gel electrophoresis. The first well contained 100bp marker as amplicon length indicator. Electrode then connected with the power supply to induce movement from the DNA for 30 minutes with 100V. The gel moved from electrophoresis device into digi doc, visualization result was used to compare the quality test result of simple and molecular techniques.

3. Results and Discussion

Based on electrophoresis analysis on DNA quality, 13 of 23 female blood samples (figure 1) and 9 of 30 male blood samples (figure 2) showed positive results.

Figure 1. Electrophoresis results of female Sumatran elephant blood samples (n=23)

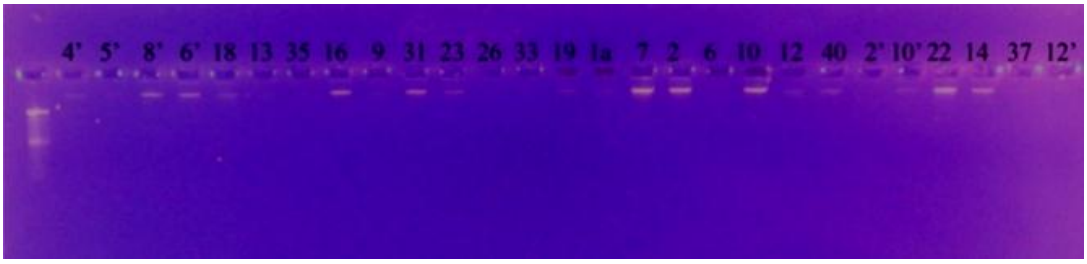

Figure 2. Electrophoresis results of male Sumatran elephant blood samples (n=30)

Positive results in electrophoresis analysis were showed by DNA band illuminescence, from SYBR *safe* bonded with the DNA. There are five factors causing the positive results, 1) high number DNA extracted, 2) suitable protocol selection, 3) good quality protocol and have been used for the same samples for better success during extraction process, 3) steril condition, 4) suitable operational standard in treating samples, 5) samples and materials condition supporting the extraction. Negative results in electrophoresis process may be affected by samples' condition, external contaminant (storage procedure, handling and shipping) and internally (changing in chemical structure in blood samples due to additive solution for preservation, the small number of DNA extracts that caused low quality of DNA band.

DNA molecular quality test using Primer of *Glyceraldehyde-3-phosphate Dehydrogenase* (GAPDH) via Polymerase Chain Reaction on certain temperature and time so that the amplification process can be proceeded and targeted DNA can be detected. Electrophoresis on its DNA molecules showed positive results on all female Sumatran elephant blood samples (figure 3), and 23 of 30 males samples (figure 4).

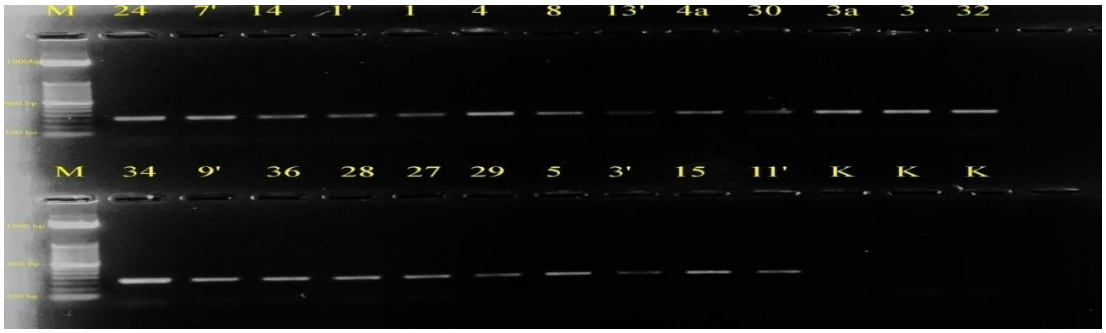

Figure 3. DNA qualitative test of female Sumatran elephants' blood samples using molecular technique

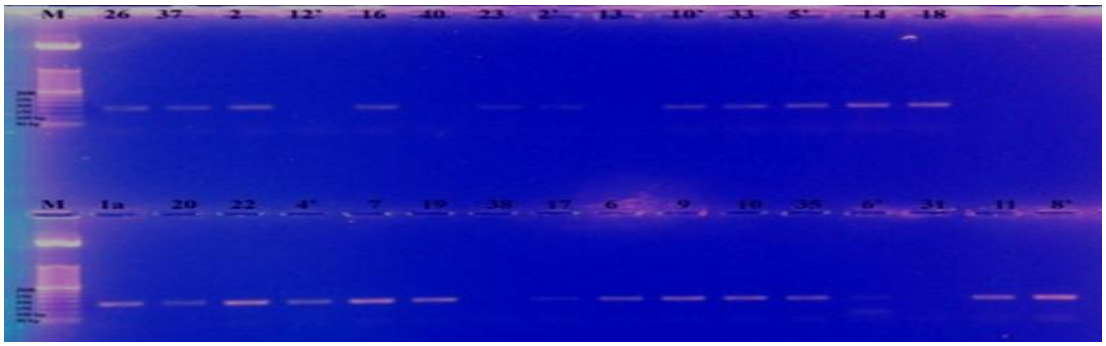

Figure 4. DNA qualitative test of male Sumatran elephants' blood samples using molecular technique

Molecular technique using GAPDH had better results than the simple technique, as primer GAPDH is gene acted coding GAPDH enzyme in organisms. Therefore, it helped to see the DNA amplicon band from the extraction that had small number DNA. The DNA molecules solved in the buffer has been multiplied through Xn PCR. Seven samples gave negative results in both simple and molecular

techniques. It may be because of the degraded samples during preservation. DNA degradation may be happened when the samples kept for a long period of time. Stabile temperature during preservation (-20 0C) is necessary to maintain the DNA quality of the samples [5]. Freezing/thawing process may affect the DNA quality, especially on DNA nitrogen base [6]. Extraction maybe also affected by the materials for homogenization and the solvent to eliminate the residue [7].

4. Conclusions

Different results were observed on DNA quality extraction when two different techniques were applied. Of 53 samples, technique using only electrophoresis showed 41.5% positive results while the second technique using PCR and GAPDH primer showed 86.7% positive results. The latter technique offer significant increase of efficiency for DNA extraction, thus reducing the time and cost associated with repeated DNA extraction.

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