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GENOMIC DNA ISOLATION OFGAJAH SUMATERA *(Elephas maximus sumatrensis)* IN ELEPHANT TRAINING CENTER, WAY KAMBAS NATIONAL PARK, EAST LAMPUNG

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ABSTRACT

Elephant Training Center, Way Kambas National Park holds 44 captive sumatran elephants*(Elephasmaximussumatrensis)*. This *critically endangered species* small population is in high risk of *inbreeding*. Its genomic DNA isolation was done to provide DNA amplification material for phylogenetic analysis. Individual blood sampels were collected from 8 different individuals based on age and sex characteristics and stored at EDTA-anticoagulated blood tubes. Qualitative test by 1% agarose gellelectrophoresis, visualized by UV transiluminator. Isolated genomic DNA was kept in -20^{0}C elution buffer solution.

Keywords: sumatran elephant, blood, DNA isolation, Way Kambas National Park.

1. INTRODUCTION

Sumatran elephant (*Elephasmaximussumatrensis*) is endemic asian elephant that lives at seven provinces in Sumatera, included Lampung (Soeharsono, 2007). Since 2011, InternationalUnion forConservationofNatureandNaturalResources(IUCN) classified the sumatran elephant as critically endangered species (Gopala*etal*.,2011). Elephant Training Center in Way Kambas National Park was established to be one of solution of this problem.

Elephant Training Center in Way Kambas National Park holds 44 captive sumatran elephants. The small size of the population of the sumatran elephant allows to increased the inbreeding probablity, then will made negative effect on gene flow in the sumatran elephant population. The bad gene flow of the population can adversely affect the viability of individual members of the population.

Research kinship patterns sumatran elephant has begun to do, especially regarding the relationship filogenik Asian elephants in Indonesia, Nepal, India and the elephants of the African continent. Fernando *et al* (2003) stated that the Sumatran elephants are related to elephants in Asia with diverse levels of phylogenetic closeness, while Sulandari and Zein (2012) states based on mitochondrial DNA genetic variation among populations of Sumatran elephants in Lampung, South Sumatra and Bengkulu is low.

Kinship patterns of captive sumatran elephants in Elephant Training Center in Way Kambas National Park has not been done. Potential high inbreeding and low genetic variation push for an immediate kinship between individual data collection of sumatran elephants in Elephant Training Center in Way Kambas National Park. Kinship patterns can be analyzed with DNA finger printing methods, for example methods Random Amplified Polymorphism DNA (RAPD) (Kumar and Gurusubramain, 2011). RAPD molecular markers are widely used in the analysis of genetic variation because it can be done without the need for data on the nucleotide sequence of the DNA template to be amplified (Yadav et al., 2012). Therefore, research on the molecular genetics of the Sumatran elephant population is an important step that must be waged. Through genetics approach, expected to conservation efforts more focused and able to rescue survival of the Sumatran elephant. RAPD molecular markers require genomic DNA as a base material for the amplification and analysis of genetic kinship.

2. MATERIALS AND METHODS

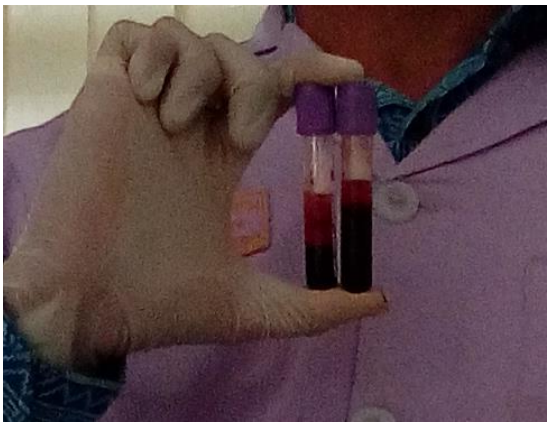
Blood sampling

Venous blood samples were obtained from 8 individual sumatran elephants in Elephant Training

Center, Way Kambas National Park, based on age and sex characteristics (Table 1). Whole bloods were collected by syringe and stored at 3 mL EDTA-anticoagulated blood tube (Pic. 1). All samples were kept at 4^{o}C before DNA isolation was performed.

Table 1. Sumatran elephants in Elephant Training Center, Way Kambas National Park

Number of Sample	Name of elephant	Sex	Age(years)
1	Agam	male	37
2	Daeng	male	29
3	Pangeran	male	2
4	Sugeng	male	6
5	Lingling	female	38
6	Suci	female	27
7	Yulia	female	3
8	Queen	female	5



Pic 1. Whole bloods were collected at 3 mL EDTA-anticoagulated blood tube

DNA Isolation

DNA was isolated from sumatran elephant's whole blood using Dneasy Blood & Tissue Protocol. Well mixed of 200 µL EDTA-anticoagulant-treated blood, 20 µL proteinase K and 200 µL buffer AL in 1,5 mL microtube was incubate in 56^{o}C for 10 minutes. Then 200 µL ethanol 96% was added followed by centrifugation at 8000 rpm for 1 minute. Washing step was done by added AW1 and AW2 with high rise centrifugation. AE buffer was added to center of the spin column for ellution step than centrifuge at 8000 rpm for 1 minute.

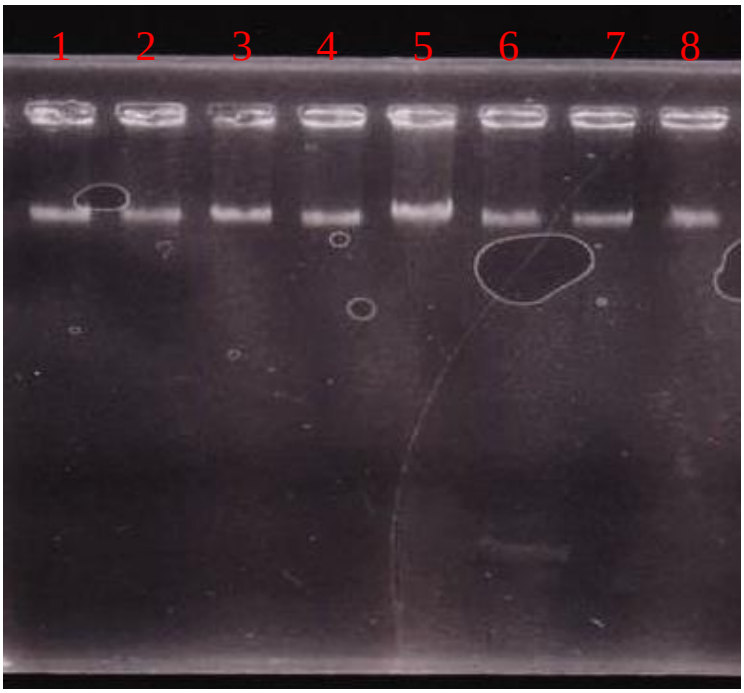
DNA Qualitative Test

Isolated DNA was test by qualitative method, loaded in 1% agarose gell electrophoresis. DNA was visualized by good view staining and photographed under ultravioled light. Isolated DNA kept at -20^{o}C before DNA amplification and another reaction performed.

RESULTS

Genomic DNA from 8 individual sumatran elephant was eluted in AE buffer sollution than kept in -20^{o}C (Pic 2). DNA was visualized by good view staining and photographed under ultravioled light as shown at Pic 3.

Pic 2. Genomic DNA from elephant eluted in buffer sollution



Pic 3. Photograph of qualitative test from genomic DNA under UV light
(1: Agam, 2: Daeng, 3: Pangeran, 4: Sugeng, 5: Lingling, 6: Suci, 7: Yulia, 8: Queen)

3. DISCUSSION

The type and condition of specimen and tissues, according to its origin, are key factors in selecting a DNA isolation method. The total genomic DNA from whole blood of sumatran elephant was isolated with Dneasy Blood & Tissue Protocol from QIAGEN®.The protocol concist of three step, lysis, washing, and elution, respectively. Lysis step using AL buffer, proteinase-K under incubating condition in 56ºC. This step will destruct the cell wall and nucleus membrane. Second step was done by twice washing step, with AW1 and AW2 buffer with centrifugation condition. This step will removed the other materials besides DNA. Third step will eluted the DNA from the sillica gell in buffer solution with AE buffer.

Using the silica-coated gell in DNA isolation step allows to reversibly bind and purify DNA away from cell debris, proteins, and another materials released upon directed cell lysis. The binding of DNA to silica gell seems to be driven by dehydration and hydrogen bond formation, which competes against weak electro-static repulsion. Hence, a high concentration of slat will help drive DNA adsorption onto silica, and low concentration will help to release the DNA from the silica gell. The total genomic DNA will kept in -20^{o}C condition before PCR analysis to construct the phylogenetic tree between sumatran elephant in Elephant Training Center, Way Kambas National Park.

4. CONCLUSION

The resulted quantity of genomic DNA is enough to conduct further PCR reactions. Using the above methode, good quality DNA samples from a sumatran elephant whole blood were isolated to study the phylogenetic analysis in sumatran elephant population.

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