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DNA Isolation on Captive Sumatran Elephant in Elephant Training Center, Way Kambas National Park: A First Step towards Its ID Card

Elly L. Rustiati[1], Priyambodo[1], Siti Asiyah[1], Dedi Candra[2], Diah E. Anggraini[2], Elizabeth D. Krismuniarti[2], Eko Agus Srihanto[3], Liza Angeliya[3], Nuning Nurcahyani[1], and Enny Saswiyanti[3]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Jl. Soemnatri Brojonegoro 1, Bandar Lampung 35145.
[2]Elephant Training Center, Way Kambas National Park, East Lampun
[3]Veterinary Bureau, Lampung Province. Bandar Lampung.
Email for correspondence: ely_jazdzyk@yahoo.com

Abstract

Elephant Training Center (ETC) Way Kambas National Park (WKNP) was built to support human-elephant mitigation conflict. The small population of captive sumatran elephant in ETC WKNP need a comprehensive strategy in order to maintain the genetic variation of each individual and avoid inbreeding drive. Currently, genetic studies have opened new field studies in ecology, included conservation ecology. Patterns in variation of population has been investigated by molecular method supporting species conservation effort. The captive sumatran elephant's ID Card is a necessary in database building, which included morphology, health status, and genetic profile. Genetic profile in each ID Card was filled by cytogenetic and molecular profile for RADP result, that initiated with DNA isolation. The DNA sources collected by blood sampling protocol described by Asiyah *et al.* (2016) from captive sumatran elephant in ETC, WKNP, and be carried to laboratory in cold condition. The DNA sources stored at 4^{o}C and isolated following commercial protocol. The result of DNA isolation stored at -20^{o}C until amplification analysis. DNA isolation was successfully done, for further individual genetic ID building.

Keywords: Conservation, DNA isolation, sumatran elephant's ID Card, WKNP

Background

Elephants belongs to the order of Proboscidea with only two living genera, *Loxodonta* (African elephant) and *Elephas* (asiatic elephant). Asian elephants are widely distributed, from South Asia to a larger part of Southeast Asia, in Malaysia and Indonesia, including Sumatra and Borneo (Fernando *et al.*, 2003; Vidya *et al.*, 2005). However, the asian elephant population has shown constant sign of decreased and in 1986 was listed as endangered species by the IUCN. One of the reason for such decrease can be found in the intensive illegal hunting of wild elephants (Stiles, 2004; Sukumar, 2006). However, the most serious threat faced by the population of elephants is habitat loss and fragmentation resulting from deforestation activities (Sukumar, 2006). These lost habitat combines with high human-population density, like in Asia, has resulted in Human-Elephant Conflict (HEC), where elephants can damage millions of dollars' worth of agricultural crops and numerous people and elephants are killed as a result. Based on those tragic consequences, HEC is considered to be one of the biggest conservation issues in Asia and presents an urgent challenge for governments and policy makers.

To mitigate such conflict, the Elephant Training Centre (ETC) in Way Kambas National Park, East Lampung, Sumatra, Indonesia was established in the 1985 by the Indonesian government to host wild sumatran elephants (*Elephas maximus sumatranus*) in conflict. As the wild elephant population decreased, inbreeding is one of population pressure that might occurs among captive elephants within the EEC and no lineage history were ever undertaken.

As a result, the risk of inbreeding in ETC is relatively high, thus presenting a serious threat for a successful and sustainable an elephant conservation program. To mitigate such risk, DNA profile for each animal was carried out. The small population of captive sumatran elephant in ETC WKNP need a comprehensive strategy in order to maintain the genetic variation of each individual and avoid inbreeding drive. Therefore; DNA bank data needs to be built (Priyambodo *et al.*, 2017) and blood sampling were carried out in collaboration with WKNP and medical teams (Rustiati *et al.*, 2017).

Nowadays, genetic studies have become one of new field studies in ecology, included conservation ecology. Patterns in variation of population has been investigated by molecular method supporting species conservation effort. The captive sumatran elephant's ID Card is a necessary in database building, which included morphology, health status, and genetic profile. Genetic profile in each ID Card was filled by cytogenetic and molecular profile for RADP result, that initiated with DNA isolation.

Materials and Methods

This project is conducted under research grant from Directorate of Research and Community Services, Higher Education Indonesia, and in collaboration with Way Kambas National Park. The DNA sources collected by whole blood sampling protocol described by Asiyah *et al.* (2016) from captive sumatran elephant in ETC, WKNP, and be carried to laboratory in cold condition. Consideration on each individual elephant was taken (Rustiati *et al.*, 2017). Twentyeight individual elephants have been whole blood sampled. DNA isolation was conducted successfully by silica gel colomn methods with commercial QIAGEN Dneasy and tissue kit for blood and tissue.

Three main steps included cell preparation/lysis, DNA isolation from contaminants and DNA precipitation. Detergent contained buffer was applied for lysis. It is done to break cell and nucleus membrane so that the chromosome can be isolated from the cell. DNA isolation using gradual wash buffer to separate DNA from RNA, protein and other contaminants with proteinase K and buffer AL and buffer AW. DNA was bound on silica gel and eluted with buffer AE. In the last step, DNA isolation is to precipitate DNA using elution buffer. DNA isolation success was qualitatively analyses by 1% agarose gel electrophoresis technique and observed under UV transiluminator. Loading dye was added to DNA to ease its movement during the electrophoresis. The DNA sources stored at 4^{o}C and isolated following commercial protocol. The result of DNA isolation stored at -20^{o}C until amplification analysis.

Results and Discussion

DNA was successfully separated from protein and others component (Figure 1) and kept in -40^{0}C. Qualitative test using agarose gel electrophoresis technique.

Figure 1. Isolated DNA genome in buffer solution

In electrophoresis process 1% agarose gel was applied as stationer phase, and Tris-acetate EDTA (TAE) as movement phase. DNA genome was separated in agarose gel based on electro mobility of its negative charge of DNA molecule in certain distance. Loading dye addition on DNA may clarify migration distance. The results were visualized under UV transiluminator (Figure 2). The yield of DNA was shown by electrophoretic migration of the undigested DNA samples. The success of DNA isolation process was shown on the existence of DNA band. Sumatran eleph-

ant's DNA genome was predicted more than 10kb. It is shown by its short migration distance from the well. Isolated DNA will be used for further molecular analysis, PCR-RAPD and DNA sequencing.

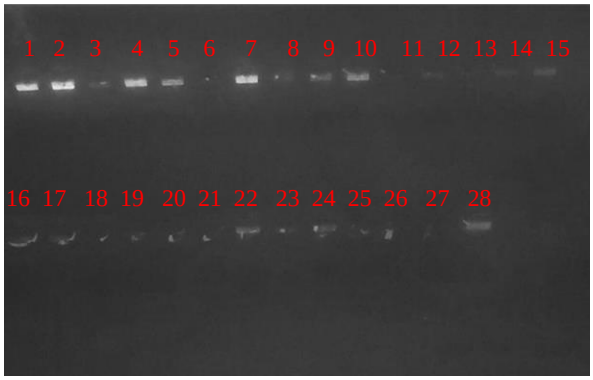

Figure 2. Visualization of qualitative test for isolation of DNA genome under UV transiluminator

Conclusion

Twentyeight whole blood samples have been successfully prepared for isolation of DNA. DNA samples were available for further individual genetic ID building and kept in -20^{o}C.

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