Ann PUBLISHER
Journal of Agricultural Sciences
E-ISSN : xxxx-xxxx
P-ISSN : xxxx-xxxx

# DNA Detection Sensitivity Test from Blood Sample Extraction of Sumatran Elephant (*Elephas Maximus Sumatranus*) Male at Elephant Training Center, Way Kambas National Park Using Simple and Molecular Methods

**Dian Neli Pratiwi[1], Priyambodo[2], Liza Angeliya[3], Eko Agus Srihanto[4], Dedi Candra[5], Elisabeth Devi Krismuniarti[6], Elly Lestari Rustitati[7]**
[1,2,7]Lampung University, Indonesia, e-mail: ely_jazdzyk@yahoo.com
[3.4]Lampung Disease Investigation Center, Indonesia, e-mail: ely_jazdzyk@yahoo.com
[5.6]Way Kambas National Park, Indonesia, e-mail: ely_jazdzyk@yahoo.com

**Abstract**. DNA detection of male sumatran elephants' (Elephas maximus sumatranus) blood samples using simple and molecular methods are ways to find out the DNA quality from extracted samples. Molecular biology analysis was done in Biotechnology Laboratory, Lampung Disease Investigation Center, under the Institution Nation Strategy Grant, Directorate General of Research Empowerment and Development, Ministri of Research, Technology and Higher Education 2018 Number 393/UN26.21/PN/2018. Sensitivity test on 12 blood samples was conducted using simple and molecular methods. Simple method is based on 1% gel agarose electrophoresis in TAE (Tris-acetate EDTA) buffer while molecular method involves Polymerase Chain Reaction (PCR) based Glyseraldehyde-3-Phosphate Dehydrogenase (GAPDH) primer. Of 12 samples extracted and tested, 10 samples using molecular method showed DNA bands. Sensitivity value (83.33%) of diagnostic calculation showed that molecular method is more sensitive in detecting DNA.

**Keywords**: DNA, Molecular Analysis, Sensitivity, Sumatran Elephant, Way Kambas National Park

## INTRODUCTION

Elephants are one of the large mammals whose existence is almost extinct and declared *critically endangered* status (IUCN, 2011). One of the subspecies of large mammals is the Sumatran elephant which lives in Lampung. The natural habitat of Sumatran elephants in Lampung is in Bukit Barisan Selatan National Park (TNBBS) and Way Kambas National Park (TNWK). The Sumatran elephant population in TNWK was 247 individuals in 2010 with an estimated 220-278 individuals (WWF, 2010). Sumatran elephant populations are declining due to the threat of habitat fragmentation, conflict with humans and poaching (Frankham et al., 2002). One of the rescue efforts carried out by TNWK is the construction of an Elephant Training Center (PLG) for the management of Sumatran elephants involved

in conflicts with the community. The population size of Sumatran elephants in PLG TNWK in 2016 was recorded at 66 heads with 36 male elephants and 30 female elephants (Rustiati et al., 2017). The number of male elephant individuals that are more than the number of female elephant individuals can also increase the chances of *inbreeding* which causes a decrease in genetic diversity and *viability* of Sumatran elephants (Frankham et al., 2002).

Information on the genetic diversity of Sumatran elephants is needed in determining policies, management and strategies for Sumatran elephant conservation efforts. Molecular genetic analysis approaches can be performed by sequencing tests to determine genetic diversity in Sumatran elephants. Sequencing tests are carried out using extracted DNA that has good quality. One of the methods used in testing the quality of DNA extraction results is using agarose gel electrophoresis (Asiah, 2016). Another DNA extraction quality test method is the molecular method with PCR (*Polymerase Chain Reaction*) using GAPDH (*Glyceraldehyde 3 phosphate dehydrogenase*) primers (Rustiati *et all.,* 2018). The PCR method is a very precise technique and is often used for molecular biology because it is easier and faster (Chen et al., 2009). DNA amplification techniques from specific DNA sources from a small number of different genes (Yusuf, 2010). GAPDH primers are sequences of nitrogenous bases that encode the enzyme GAPDH. GAPDH primers are used to determine the efficiency of mRNA synthesis (cDNA sequence) by *reverse transcription* and mRNA expression quality test.

Diagnostic testing is one way to determine the sensitivity of a method. Diagnostic testing is a test performed to determine the probabilistic of a case. Sensitivity, specificity and agreement value (Kappa test) are the results obtained in diagnostic testing. Sensitivity is the ability of a test to detect a material until the material is no longer detected (gives a result of 0) (Dohoo *et all*., 2003). Research on the sensitivity of DNA detection from the extraction of male Sumatran elephant (*Elephas maximus sumatranus*) blood samples at the Way Kambas National Park Elephant Training Center using simple and molecular methods with GAPDH was carried out with the aim of determining the sensitivity value of simple and molecular methods in detecting the presence of extracted DNA in Sumatran elephant blood samples so as to provide information on good Sumatran elephant DNA analysis methods for support efforts to trace the genetic diversity of Sumatran elephants at PLG TNWK.

## METHOD

### A. Simple test

A total of 12 DNA samples from the extraction of blood of male Sumatran elephants (Elephas maximus sumatranus) were used as material in this study. DNA samples from the extraction of male Sumatran elephant blood used came from elephant blood sample collections at PLG TNWK (Rustiati et al., 2017). A simple method is a DNA quality test extracted from male Sumatran elephant blood samples (N = 12) with 1% agarose gel electrophoresis in TAE buffer (TriacetateEDTA). Elecrophoresis is performed at a voltage of 100 V for 17 minutes in the *chamber*. The results of electrophoresis are visualized using *Digital document* (*Digidoc*).

### B. Molecular Test

A total of 12 DNA samples extracted from the blood extraction of male Sumatran elephants (*Elephas maximus sumatranus*) were used as samples in this study. DNA samples from the extraction of male Sumatran elephant blood used came from elephant blood sample collections at PLG TNWK (Rustiati et al., 2017). The molecular method used in this study was to test the quality of DNA extracted from male Sumatran elephant blood samples using the *Polymerase chain reaction* (PCR) technique with *Glyseraldehyde-3-Phosphate Dehydrogenase* (GAPDH) primers .

Test the quality of extracted DNA by molecular methods through three stages, namely the *mix master* and *template* stages, the amplification stage, and the agarose gel electrophoresis stage. The amplification stage with PCR goes through five stages, namely predenaturation with a temperature of 95 0 C in 5 minutes, denaturation with a temperature of 94 0 C in 20 seconds, *annealling* with a temperature of 57 0 C in 45 seconds, extention with a temperature of 72 0 C in 1 minute, and post-extention with a temperature of $72^0$ C in 5 minutes. The denaturation, *annealling*, and *extension* stages undergo 35 cycles of repetition. PCR products (amplicon) are elecrophoresis with 1.5% agarose gel in TAE buffer. Electrophoresis is performed at a voltage of 100 V for 30 minutes in the *chamber*. The results of electrophoresis are visualized using *Digital document* (*Digidoc*).

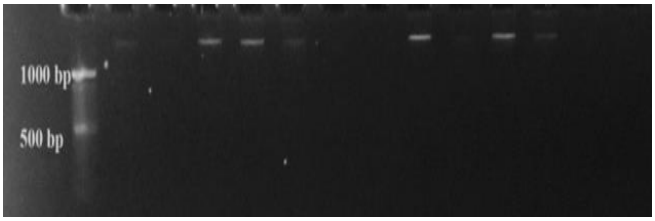
### C. Diagnostic Tests

Measurement of sensitivity values of simple and molecular methods is carried out using diagnostic testing with the following formula:

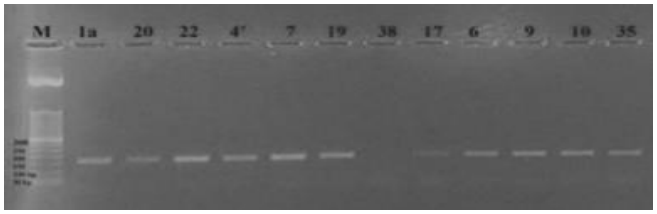
$$\text{Sensitivitas} = \frac{a}{a+c} \text{ x } 100\%$$

## DISCUSSION

The electrophoresis results of DNA samples extracted from Sumatran elephant blood extraction using simple and molecular methods were visualized using Digidoc. The results obtained from visualization of agarose gel using *Digidoc* are DNA band luminescence (Figure 1). In the simple method test, DNA luminescence was seen in 5 DNA samples, while as many as 7 other samples did not show DNA band luminescence. In the molecular test of 12 DNA samples extracted that have gone through the PCR stage with GAPDH primers and electrophoresis, DNA samples that showed the luminescence of DNA bands were seen as many as 10 samples. Manyof the other 2 samples showed no visible luminescence of DNA bands. The number of samples that showed visible luminescence of DNA bands was more than 80% of the total sample.




(a)




(b)

**Figure 1.** DNA visualization of male Sumatran elephant extraction at Way Kambas National Park Elephant Training Center using (a) simple and (b) molecular methods.

In a simple method, the absence of DNA band luminescence in 7 extracted DNA samples can be caused by DNA degradation in the sample so that the quality of the DNA produced is low and the small amount of DNA extracted also results in invisible DNA band luminescence during electrophoresis. Fachtiyah (2011) stated that the quality of DNA and the amount of DNA extracted affect the thickness of DNA band luminescence during electrophoresis. The number of samples that showed invisible luminescence of DNA bands was more than 50% of the total samples.

The molecular method using *the Polymerase chain reaction* (PCR) technique with *Glyseraldehyde-3-Phosphate Dehydrogenase* (GAPDH) primer was chosen because it has

more sensitivity and specificity to DNA molecules. Test the quality of extracted DNA by molecular methods through three stages, namely the *mix master* and *template* stages, the amplification stage, and the agarose gel electrophoresis stage. The amplification stage with PCR is regulated with a certain temperature and time so that the amplification process can run and a target DNA sample is obtained. PCR products (amplicon) are electrophoresis on 1.5% agarose gel. A higher concentration of agarose gel is expected to result in more DNA molecules being captured. Higher gel concentrations increase the agarose gel density value so that it can detect amplicon DNA that has a smaller size than genomic DNA.

The results obtained from agarose gel visualization using *Digidoc* showed that of the 12 DNA samples extracted from electrophoresis, 10 DNA samples from the extraction showed DNA band luminescence while the other 2 samples did not show visible DNA band luminescence. PCRelectronics play a role in doubling the amount of DNA contained in the sample. In the amplification stage, samples containing a small amount of DNA are multiplied through five stages, namely *predenaturation, denaturation, annealing*, extention, and *post extention.* At the amplification stage, temperature optimization is carried out to obtain an optimal temperature so that DNA with good quality is obtained. The deneturation, *annealing* and *extension* stages are repeated 35 times so that samples with a greater amount of DNA than before are produced. As Mullis and Fallona (1989) in Yuwono (2006) said that this *polymerase chain reaction* (PCR) is used to multiply DNA molecules. This shows the advantages of the PCR method, where this method can be used on samples with a small amount of DNA.

The invisibility of DNA band luminescence can be caused by two things. First is the damage to the sample due to the storage process. DNA degradation can occur in samples that are stored for too long so that the quality of the DNA produced is relatively low, and the sample storage temperature must be stable (-20 $^{0}$ C) to maintain the quality of DNA in the sample so that DNA degradation does not occur (Manela C., 2015). The second is the *freezing/thawing* process during the research process can also cause DNA damage which causes low quality DNA samples. The *freezing/thowing* process has an effect on the strength of DNA nitrogenous base strands (Davis *et all*., 2000). The presence of DNA in the sample is influenced by the stage of sample extraction. Samples that have been damaged cause DNA extraction with low quality or damaged. Factors that can affect the extraction yield are the homogenization of the material used and the type of solvent used to remove the

extracted residues (Syafaruddin et al., 2011). The extraction stage is very important because it affects the damage to the DNA used as a sample.

Comparison of test results with simple and molecular methods, namely simple tests showed positive results as many as 5 samples, while molecular tests showed positive results as many as 10 samples (Table 1). The molecular test results show samples with more positive results than simple test results. This is because the number of DNA molecules in the sample has been doubled through the PCR process, so the possibility of the sample showing positive results on visualization will be higher.

**Table 1. DNA detection results from the extraction of male Sumatran elephant blood samples at PLG TNWK using simple and molecular methods**

| No | Elephant Name | Simple Test Results | Molecular Test Results |
|---|---|---|---|
| 1 | Boy | + | + |
| 2 | Cuni | + | + |
| 3 | Rendi | + | + |
| 4 | Rendo | + | + |
| 5 | Renggo | + | + |
| 6 | Daeng | - | + |
| 7 | Edwin | - | + |
| 8 | Fatra | - | + |
| 9 | Senses | - | + |
| 10 | Joni | - | + |
| 11 | Josh | - | - |
| 12 | Karnangun | - | - |

Diagnostic calculations in tests with simple methods produce a sensitivity value of 41.67% and a sensitivity value obtained in molecular tests of 83.33% (Table 2). This shows that a simple test carried out can detect not only the presence of the GAPDH gene in the DNA sample but can detect the presence of other genes in the DNA so that it is not specific (specific). Simple tests performed are not very good at detecting the presence or absence of a gene in the sample. Themolecular test only detects the presence of the GAPDH gene in the DNA sample and does not detect other genes. Molecular tests carried out can detect the presence or absence of DNA well. Molecular tests have a higher sensitivity value when compared to simple so they are considered better to use.

**Table 2. Diagnostic test results of Sumatran elephant DNA samples**

| Test Method | Simple test | Molecular assay |
|---|---|---|
| Visible DNA samples | 5 samples | 10 samples |
| Sensitivity | **41,67%** | **83,33%** |

A sensitivity value of a test close to 0% indicates that the test method is not sensitive. The insensitivity of the method indicates that the method used is insensitive in detecting the presence of a material. A sensitivity value of a test close to 100% indicates that the test method is very sensitive. The high sensitivity value of the method shows that the method used is very sensitive in detecting the presence of a material. The sensitivity and specificity values will show the accuracy and precision of the tests that have been carried out.

## CONCLUSION

DNA detection from the extraction of blood samples of male Sumatran elephants (*Elephas maximus sumatranus*) using simple and molecular methods showed that more DNA band luminescence was found in molecular tests compared to simple tests. Molecular tests are more sensitive to the presence of DNA compared to simple tests as evidenced by the sensitivity value of molecular tests that are higher than the sensitivity value of simple tests.

## BIBLIOGRAPHY

Asiyah, S. 2016. DNA Qualitative Test of Sumatran Elephant ( *Elephas maximussumatranus)* at Elephant Training Center Way Kambas National Park (thesis). Float. Department of Biology, Mathematics and Natural Sciences, University of Lampung.

[IUCN] *International Union for Conservation of Nature* . 2011. IUCN *Red ListEndangered Species.* http://www. iucnredlist. org /search. accessed August 10, 2017

Fatchiyah, Estri, L.A., Sri, W., and Sri, R. 2011. *Molecular Biology. Basic PrinciplesAnalysis* . Erlangga. Jakarta.

Frankham, R., Ballou, J.D. &; Briscoe, D.A.2002. *Introduction to ConservationGenetics* . Cambridge University.

Rustiati E. L., Priyambodo , Nurcahyani N.2017. Phylogenetic Band Construction at Elephant Training Center, Way Kambas National Park Based on Cytological and

Molecular Analysis (Applied Product Report). University of Lampung.

Yuwono, T. 2006. Theory and Application of Polymerase Chain Reaction.Yogyakarta. Andi offset.