Comparison of two rapid DNA extraction for DNA based identification from fish fillet products

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Abstract. Efficiency and quality on the result of DNA extraction determined the success of species identification using DNA. High concentration and good purity of DNA are the basic principle for choosing the appropriate extraction method. The objective of this research is to find an effective and efficient method from two different rapid DNA extractions, KAPA Express Extract Kit (KAPA Biosystem) and Chelex® 100 (Biorad). We used each method to extract 44 fish fillet samples from Jabotabek (Jakarta, Bogor, Tangerang, Bekasi) modern retail and traditional markets. We found the KAPA Express Extract Kit (KAPA Biosystem) had fast extraction time approximately 15 minutes for PCR-ready DNA faster than 20 minutes using the Chelex® 100 (Biorad) method. All the PCR-ready DNA are visualized with gel electrophoresis and both method resulted in positive DNA bands. We propose the use of KAPA Express Extract Kit (KAPA Biosystem) than the Chelex® 100 (Biorad) method because the fast and easily to use for the next analyses such as PCR and DNA sequencing.

Keywords: DNA, extraction, identification, fish, fillet

1. Introduction

The development of molecular biology technology has had an impact in the field of biological sciences as a whole. Some branches of science such as agricultural taxonomy and forensic science have applied special DNA analysis. DNA analysis in the field of forensic science, used in criminal cases such as murder or livelihood. DNA also aims to find out the phylogeny of an organism by comparing markers from parts of an organism [1]. DNA analysis is carried out by DNA extraction which can be done on samples of microorganisms, plants and animals. The extraction method is carried out well and correctly in order to obtain a good DNA sample [2].

The principle of extraction is separation and purification which consists of three stages, cell wall destruction (lysis), separation of DNA from foreign components and purification. The KAPA Express Extract Kit (KAPA Biosystem) and Chelex® 100 (Biorad) methods are fast extraction methods that are designed in a mixture of concentration and size so they are more practical, efficient excess and higher level of purity. KAPA Express extract is a protease and a thermostable buffer system that allows PCR-ready DNA extraction in just 15 minutes. KAPA Express Extract Kit (KAPA Biosystem) Reaction is easily carried out in one tube without the need for hazardous chemicals and several washing steps, greatly reducing the risk of sample loss and contamination [3]. Chelex® 100 (Biorad) is a
divinylbenzene styrene copolymer containing paired iminodiacetate ions, which acts as a chelating group in binding to polyvalent metal ions [4]. The research aims to obtain and compare between the two extraction methods that have different speeds, the KAPA Express Extract Kit (KAPA Biosystem) and Chelex® 100 (Biorad).

2. Materials and method

2.1. Samples
The 44 Fish fillet samples were obtained by random sampling from Jabotabek (Jakarta, Bogor, Tangerang, Bekasi) retail and traditional markets were collected (table 1). All samples were stored in the refrigerator at 4 ºC based on each sampling location. The 44 samples were collected from June 14th–16th 2019 and the samples were stored on three days after collected.

2.2. Sampling
Fish fillet samples were handled sterile by placing them on sterile trays then cut into small 2 mm sizes using sterile razors and inserted into the eppendorf tube with sterile tweezers for DNA extraction.

2.3. DNA extraction
Pieces of each fish fillet are prepared to carry out the DNA extraction process with two methods of DNA extraction and see the concentration and purity of DNA using a nanophotometer (IMPLEN) at wavelength A260/A280.

2.3.1. Chelex® 100 (Biorad).
DNA extraction used 44 fish fillet samples from Jakarta, Bogor, Tangerang, Bekasi modern retail and traditional markets. The first stage of fish fillet DNA extraction is the chelex solution made by mixing 5 grams of chelex powder at 50 mL of distilled water. Then 200 μL of Chelex® 100 (Biorad) solution and a sample of approximately 2 mL or smaller is mixed into a 1.5 mL tube, then incubated at the waterbath at a temperature of 60 ºC for 20 minutes and vortexed for 5–10 seconds every 5 minutes during the 20 minute incubation. The mixture was re-incubated at 103 ºC for 25 minutes and vortexed for 5–10 seconds at 5 minutes once during the 25 minute incubation. Then, it was centrifuged for 2 minutes at a speed of 13,000 rpm. Then the 100 μL supernatant containing the DNA sample was transferred to a new tube and stored at -20 ºC.

2.3.2. KAPA express extract kit (KAPA Biosystem).
DNA extraction used 44 fish fillet samples from Jakarta, Bogor, Tangerang, Bekasi modern retail and traditional markets. The first stage of extraction of
fish fillet DNA was mixing together 88 μL PCR-grade water, 10 μL 10 x KAPA express Extract Buffer, 2 μL KAPA express Extract Buffer Enzyme and fish fillet samples 2 mm in size into 1.5 mL tubes [5].

2.3.3. The mixture was incubated at waterbath with temperature 60 ºC for 10 minutes and vortexed for 5–10 seconds in 5 minutes during the 10 minutes incubation. Then re-incubated at 95 ºC for 5 minutes and vortexed for 5–10 seconds. Then, centrifuge for 2 minutes at a speed of 13,000 rpm. Then the supernatant of 50–100 μL containing DNA samples was transferred to a new tube and stored at -20 ºC.

2.4. Polymerase chain reaction (PCR)
The reaction mixture with the fish primer was made by mixing 9 μL of nuclease free water, 0.25 μL forward primer (FishF15`TCAACCAACCACAAAGACATTGGCAC3`), 0.25 μL reverse primer (FishR15`TAGACTTCTGGGTGGCCAAAGAATCA3`), 12.5 μL GoTaq® Green Master Mix, 2X, and 3 μL template DNA as into a 0.2 mL PCR tube. The PCR tube is then inserted into the thermal cycler. The temperature and time of each PCR process, as well as the number of cycles arranged on the PCR machine, was as follows: cycle one with a temperature of 95 ºC for 2 minutes, cycle two for 35x with a temperature of 94 ºC for 30 seconds, temperature 54 ºC for 30 seconds and temperature 72 ºC for 1 minute, the third cycle with a temperature of 72 ºC for 10 minutes and the last cycle at 4 ºC.

2.5. Electrophoresis
The stages of electrophoresis are divided into four parts, making 1 % agarose gel, loading samples into well electrophoresis, running electrophoresis, and gel documentation. Preparation of 1 % agarose gel was carried out as follows: 0.50 g agarose powder and 50 mL Tris Acetic Acid (TAE) / loading buffer was mixed in a 100 mL Schott bottle. The bottle is heated in the microwave for 60–90 seconds, then 5μL of Red 1:10000 x Gel is added. The tray and comb are prepared and the gel is poured into the mold. After the gel hardens, the sample is loaded into the electrophoresis well with the gel inserted into the chamber and running buffer added. Then loading dye, DNA markers, parafilm were prepared by mixing 1 μL loading dye with 5 μL DNA above the parafilm. The mixture is inserted into the well of the electrophoresis gel using a micropipette. Electrophoresis is carried out using an electrophoresis apparatus. After the genome sample and PCR sample and 1 kb markers and positive control is loaded, the chamber is closed, and the electrophoresis apparatus is connected to the power supply (100 V). The process will last for 20 minutes until the DNA and marker samples reach the gel tip. Finally, the power is turned off and the gel is removed from the chamber.

2.6. Gel documentation system
The gel documentation process is done by using a gel documentation system that is connected to a computer. The gel is slowly moved into the Gel Doc machine, then the Gel Doc machine is closed tightly and the UV light on the Gel Doc machine is turned on. Photos of electrophoresis results are observed and stored on a computer.

3. Results and discussion
Positive DNA band results on electrophoresis gel in genome samples using two different methods (figure 1) and on PCR samples using two methods are seen through gel documentation (figure 2). DNA band results are then categorized based on whether or not they are genome samples or PCR samples. Positive DNA band results from both methods and from PCR samples and graph genomes are graphed. The largest to lowest number of samples obtained is 44 samples on the PCR samples using the KAPA express extract kit (KAPA Biosystem) extraction method, 31 samples on the genome samples using the extraction method Chelex® 100 (Biorad), the 25 genome samples using the Chelex® 100 (Biorad) extraction method and 17 PCR samples using the KAPA express extract kit extraction method (figure 3).
Figure 1. (a), (b), (c) Genomic DNA samples extracted with the Chelex 100® (Biorad); and (d), (e), (f) Samples extracted with the KAPA Express Extract Kit (KAPA Biosystem). M = 1K/1000bp DNA marker (SMOBIO); K+ = positive control.

Figure 2. (a), (b), (c) Amplification from genomic DNA samples extracted with the Chelex® 100 (Biorad); and (d), (e), (f) Samples extracted with the KAPA Express Extract Kit (KAPA Biosystem). M = 1K/1000bp DNA marker (SMOBIO); K+ = positive control.
The genome DNA concentration using the KAPA express extract kit (KAPA Biosystem) extraction method averaged 476.43 ng/μL, the lowest concentration was 60 ng/μL in the JS1 sample and the highest was 3400 ng/μL in the JU2 sample. Whereas the extraction method of Chelex 100® (Biorad) averaged 325.4 ng/μL, the lowest concentration was 26.5 ng/μL in the JB2 sample and the highest concentration was 695 ng/μL in the sample SJ6 (figure 4) The results of genome DNA purity using the KAPA express extract kit (KAPA Biosystem) extraction method averaged 1.122, the lowest purity was 0.841 on the BK2 sample and the highest was 1.580 on the JU2 sample. Whereas the extraction method of Chelex 100® (Biorad) averaged 1.394, the lowest purity was 1.065 in the JT4 sample and the highest purity was 2.028 in the JP3 sample (figure 5).

Figure 3. Amount of DNA samples between genomic DNA and Amplification from genomic DNA. Comparison of Chelex® 100 (Biorad) method with KAPA Express Extract Kit (KAPA Biosystem) method

Figure 4. Yield and quality of DNA using Chelex® 100 (Biorad) method and KAPA Express Extract Kit (KAPA Biosystem) method. Stock graphs representing min: minimum, max: maximum and average values showing the concentration ratio.
The successful amplification of a target sequence by PCR depends on the amount of extracted nucleic acids from the samples using rapid DNA extraction method [6]. The advantages of the Chelex® 100 (Biorad) and KAPA Express extraction Kit (KAPA Biosystem) methods are similar in the time of rapid DNA extraction. PCR samples and genome samples from electrophoresis using Chelex® 100 (Biorad) are low and not many DNA bands are formed, this is because there are contaminants that are carried along during extraction. The literature states that Chelex® 100 (Biorad) method is not capable of removing all cellular components and other contaminants that may be present in the sample [6]. It could be that the Chelex® 100 (Biorad) is a PCR inhibitor. The chelator for polyvalent ions, the magnesium requirements for the polymerase to work, would be captured, therefore any resin present would affect the efficiency of PCR amplification and inhibitory primers attached to the template so the sample could not be amplified [7]. The results of the PCR samples from the KAPA Express Extract Kit (KAPA Biosystem) have a high value as seen by the DNA band at the time of electrophoresis This is because KAPA Express Extract Kit (KAPA Biosystem) contains a thermostable protease and an efficient lysis buffer so that it quickly and reliably extracted DNA from a variety of fish tissue products [8]. The value of concentration and purity of the two methods of DNA extraction is not significant because the differences may be due to the basic principles of these methods. The KAPA Express Extract Kit (KAPA Biosystem) and the Chelex® 100 (Biorad) method are not affinity methods and require several steps, which may result in low concentrations and purity. The amplification of the sequence not only depends on the extraction method used. There are other factors, such as the number of gene copies, the size of the region to be amplified, and the PCR conditions.

4. Conclusion

The two methods designed to produce PCR-ready DNA have the advantages and disadvantages, the results of experiment fitting that KAPA Express extracts Kit (KAPA Biosystem) the method is better than Chelex® 100 (Biorad) method in the efficiency of extracting DNA and PCR analysis.

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References
[1] Hubert N and Hanner R 2015 DNA Barcodes 3 44-58
[2] Nicholl D S T 2008 An Introduction to Genetic Engineering (Cambridge: Cambridge University Press)
[3] Smorenburg P, Siebritz M, Geldart A and Kasinskas R W 2015 Rapid qPCR analysis from highly inhibited tissue and blood sample extractions with KAPA PROBE FORCE qPCR Kits and KAPA Express Extract available at https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=2ahUKEwjnjuzgt47lAhUNVH0KHcMkCl0QFjAAegQIAbAC&url=https%3A%2F%2Fwww.n-genetics.com%2Fproducts%2F1104%2F1033%2F13642.pdf&usg=AOvVaw3CiyY1bue5Tu7ApjilXf_c
[4] Ip S C, Lin S W and Lai K M 2015 Sci. Justice 55 200-8
[5] KAPA Biosystems 2016 KAPA Library Preparation Kit available at https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=2ahUKEwiK-4zl47lAhVLfysKHWGJHDUQFjAAegQIAXAC&url=https%3A%2F%2Fwww.kapabiosystems.com%2Fdocument%2Fkapa-library-preparation_kr0410%2F3Fl%3D1&usg=AOvVaw06kuBeBqxblD3PVs1aHuX7
[6] Ruiz-Fuentes J L et al. 2015 Int. J. Mycobacteriol 4 284-9
[7] Hu Q, Liu Y, Yi S and Huang D 2015 Forensic. Sci. Int. Genet. 16 94-7
[8] Rehbein H and Schiefenhöve K 2012 J. Aquat. Food Prod. Technol. 21 86-96