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DNA Isolation of Chicken Feathers from the Base of the Young Feathers, the Base of the Old Feathers, and the Tip of the Feathers

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Background: The goal was to provide information on DNA templates in chicken samples so that the molecular research sampling process may employ feathers instead of hurting the test animals. The sample used consisted of 10 Bangkok chickens which were sampled for young feathers and old feathers and the tips of the feathers. **Method:** Quantitative techniques by comparing the results of DNA isolation which were analyzed using a nano photometer and then confirmed using real-time PCR with the SYBR green method. **Result:** The analysis of purity and concentration showed that at the base of young chicken feathers, the average value of purity was at 1,790, with an average value of the concentration of 4,210. At the base of the old feather, the average purity value was 0.638, with an average concentration value that was not detected. Likewise, at the tip of the feather, the average purity value is 0.894, and the concentration value is not detected. Confirmation tests performed on all samples using the real-time PCR melt curve method showed that all samples were detected with a Tm value of 78.5 for young feathers, 78.5 for old feathers, 79.0 for positive controls and 78.7 for positive controls, while negative controls were not detected. **Conclusion:** DNA isolation can be carried out at the base of the young feathers, the base of the old feathers, and the feathers' tips.

Analisis Hasil Isolasi DNA dari Bulu Ayam yang Dicuplik dari Pangkal Bulu Muda, Pangkal Bulu Tua dan Ujung Bulu

Kata kunci:
Bulu ayam;
Pangkal Bulu;
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A B S T R A K

Backgrond: Dengan tujuan memberi informasi tentang penggunanan DNA template pada sampel ayam sehingga proses sampling untuk penelitian molekuler bisa menggunakan bulu sehingga tidak menyakiti hewan uji. Sampel yang digunakan terdiri dari 10 ekor ayam bangkok yang disampling bulu muda dan bulu tua serta ujung bulu. **Metode:** Menggunakan metode kuantitaif dengan membandingkan hasil isolasi DNA yang dianalisis menggunakan nano photometer kenudian dikonfirmasi menggunakan real time PCR dengan metode SYBR green. **Hasil:** Analisis kemurnian dan konsentrasi menunjukan bahwa pada pangkal bulu ayam muda, rata-rata nilai kemurnian berada pada 1.790 dengan nilai rata-rata konsentrasi 4.210. Pada pangkal bulu tua rata-rata nilai kemurnian berada pada 0.638 dengan nilai rata-rata konsentrasi yang tidak terdeteksi. Begitupun pada ujung bulu nilai kemurnian rata-rata 0.894 dan nilai konsentrasi tidak terdeteksi. Uji konfirmasi yang dilakukan terhadap seluruh sampel menggunakan metosde melt curve real-time PCR menunjukan semua sampel terdetksi dengan nilai Tm pangkal bulu muda 78.5, pangkal bulu tua 78.5, ujung bulu 79.0 dan kontrol positif 78.7, sedangkan untuk kontrol negatif tidak terdeteksi. **Kesimpulan:** isolasi DNA dapat dilakukan pada pangkal bulu muda, pangkal bulu tua dan ujung bulu.

Introduction

In principle, DNA isolation through a red chloroform extraction process, the sample will go through three main processes, namely lysis (destruction of cell walls), separation of DNA from protein/cellulose and purification. Each of these stages plays an essential role in the success of the DNA isolation process to get good results, namely

results whose purity is in the range (1.7-2) and concentrations above 20 ng / µl (Qiagen, 2014). In a DNA analysis using animal samples, the most important thing to note is the ethical issue of the object of research.

This problem is fundamental to pay attention to because it concerns how the test animals used as the object of research do not experience pain or pain when the sample is taken. Therefore, other references are needed in taking DNA samples. Generally, in chicken samples, samples were taken from the blood (Abinawanto et al., 2019) and lower abdominal incisions (Kamagi, 2017). These two types of samples certainly have different ways and risks when compared to the sampling method in the form of chicken feathers. The selection of chicken samples is due to the large number of studies using chickens with limited reference information regarding sampling as the object of research. Besides that, this research is also expected to be another solution.

Because the kit used does not have specifications for use on chicken feathers, the preliminary test stage through optimization is essential. The data obtained can be used as a source of information for similar research in using similar extraction kits so that knowledge of information about the characteristics of the kit and its functions can be enriched from the results of this study. This study aims to become a source of information in taking samples for research purposes on poultry in general or chickens in particular. The novelty aspect of this research lies in the initial idea of selecting a sample that will be the reference source for similar research.

Methods

The materials in this study were chicken feathers, RNA free water, Dneasy mericon Food Kit (50) paint extraction kit. 69514 (Qiagen). QuantiNova SYBR Green RT-PCR Kit Real-Time PCR Kit (100) paint. 208152 (Qiagen).

Sample Preparation

The sample was weighed as much as 1 g, added 1 mL of Food Lysis Buffer, and 25 µl of proteinase K then vortex for 15 seconds until homogeneous. Incubate (temperature 65°; 60 minutes; 1100 rpm), let the sample stand at room temperature for 30 seconds, and then put it in an ice block/freezer for 10 minutes. Then, centrifugation at 2500xg for 10 minutes. Pipette 500 µL of Chloroform into a new 2 mL tube Carefully remove the 700 µL transparent layer in step 6, without touching the precipitation at the bottom of the tube. Put it in a 500 µl tube containing Chloroform (step 1-6). Vortex for 15 seconds, then centrifuges at 14000xg for 15 minutes. Take 350 µL of the transparent layer. Please put it in Qiacube, use the standard method with 60 ul of E.B. buffer elution. The eluted DNA can be used directly for real-time PCR processing or stored at -20o or -80o for long storage.

Data analysis

Data analysis was carried out by looking at the average values of purity and concentration by a nanodrop microphotometer at a wavelength of A260-A280 and comparing them. Good results are shown with a purity value of 1.7 - 2.1 and a concentration of > 20 ng / µl.

Data analysis confirmation test with real-time PCR was carried out based on two main criteria: (i) Ct (Cycle threshold) analysis which looked at the value of sample Ct and compared it with controls. (ii) Analysis of melting temperature (Tm), which is the melting point at the temperature at which melting occurs and comparing the melting point to the positive control (Sophian et al., 2020).

Results

DNA Isolation

Data analysis of template DNA using the Mericon Food kit can be seen below (Table 1). The data below shows DNA isolation results for samples from the base of young hair, the base of old hair, and the tip of the hair.

Table 1. DNA Isolation Results Data

Sample	A260-A280 purity	Concentration
Young Feather Base	1.630	4.400
Young Feather Base	1.577	4.100
Young Feather Base	1.950	3.900
Young Feather Base	1.804	4.150
Young Feather Base	1.473	4.050
Young Feather Base	1.886	4.150
Young Feather Base	1.483	4.300
Young Feather Base	2.150	4.300
Young Feather Base	2.073	4.250
Young Feather Base	1.875	4.500
The base of Old Fur	0.071	Not detected
The base of Old Fur	0.433	Not detected
The base of Old Fur	0.324	Not detected
The base of Old Fur	0.237	Not detected
The base of Old Fur	0.364	Not detected
The base of Old Fur	0.119	Not detected
The base of Old Fur	0.297	Not detected
The base of Old Fur	0.361	Not detected
The base of Old Fur	0.333	Not detected
The base of Old Fur	0.289	Not detected
Feather Tip	0.869	Not detected
Feather Tip	0.906	Not detected
Feather Tip	0.927	Not detected
Feather Tip	0.979	Not detected
Feather Tip	0.935	Not detected
Feather Tip	0.935	Not detected
Feather Tip	0.717	Not detected
Feather Tip	0.872	Not detected
Feather Tip	0.833	Not detected
Feather Tip	0.974	Not detected

From the data above, if an average meal will be presented as (Table 2). The analysis of purity, meanwhile the concentration analysis, showed that the concentration value was not detected at the base of the old hair and the tip of the bladder. At the base of young hair, the purity values are 1,473 - 2,150 with a concentration of 3,900 -

4,500. This result can be said to be better when compared to the purity and concentration like at the base of the old hair and the tips of the hair. According to O'Neill et al., (2011), a purity value above 2 indicates that the extracted sample is contaminated with RNA, while a purity value below 1.8 indicates the sample is contaminated with a protein.

Table 2. Data Mean Value of DNA Isolation

Sample	Purity	Concentration
Young Feather Base	1.790	4.210
The base of Old Fur	0.638	Not detected
Feather Tip	0.894	Not detected

The method that is often used to analyze isolated DNA is the reading method using a nano photometer. According to Held. P.G., (2001), the analysis of concentration and purity was carried out using a nano photometer, which reads DNA absorption at wavelength A260 / A280. Matlock B., (2015) expressed another opinion. Which states that the results of the purity analysis and good concentration on the sample do not guarantee a successful sample is amplified. Another equally important factor is the sample itself. However, the purity and concentration analysis method to see the quality and quality of isolated nucleic acids using a nano photometer is a reasonably effective method to determine the quality of the extraction results in a shorter time when compared to the horizontal electrophoresis method, which requires agarose gel (Sambrook et al., 1989; Kirby, 1990; Artama, 1991; Eppendorf, 2016).

Confirmation of Isolation Results Using Real-Time PCR

The PCR analysis carried out was the melt temperature (Tm) analysis using a qualitative method with the SYBR green quantinova kit. In this kit, DNA polymerase is inhibited by an enzyme which will only be active if the inhibiting enzyme is denatured by PCR at a temperature of 95o. When viewed in (Figure 1), the peak points of the observed curve vary slightly, this is due to the composition and size of the nucleotides. In the SYBR green analysis, the annealing stage plays an important role here at this stage the fluorescent signal will pick up when the DNA bands begin to separate (Dwight et al., 2011). The melting process in molecular analysis and can be used to see the specificity level of the analysis, where the single peak can be used as a reference for specificity, while the peaks that appear other than the target peak can be used as initial information to predict mutation processes (Bruzzone et al., 2013).

In (Figure 1), all samples experience amplification, even though the concentration data in (Tables 1 and 2) for old feather traps and feather tips are not detected. For the purity data, the two samples have numbers that detect the presence of nucleic acid.

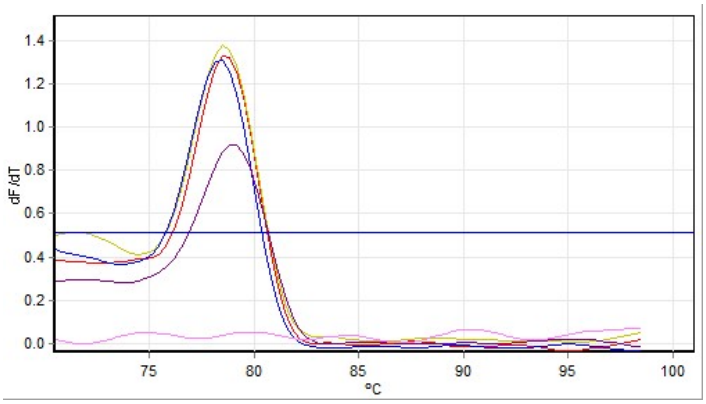

Figure 1: Results of melt cure (Tm) analysis using real-time PCR (red = positive control; yellow = light hair tips; blue = dark hair tips; purple = feather tips; light purple = negative control).

Table 3. Real-Time PCR Analysis Results

Sample	Tm value
Young Feather Base	78.5
The base of Old Fur	78.5
Feather Tip	79.0
Positive control	78.7
Negative control	Not detected

Discussion

Tissue sampling for molecular/genetic analysis in chickens is an integral part of carrying out molecular analysis. The body part used as the object for sampling must also be considered to avoid harming the test animal. Therefore, selecting the fur section as a sampling site for DNA research to avoid the sampling process that hurts the test animals.

Somebody parts that have been used as sampling sites for chickens include blood Abinawanto et al., (2019) and chest muscle tissue incisions (Kamagi, 2017).

According to Sophian, (2021), the purpose of carrying out the DNA lysis process is to remove the genetic material contained in cells so that it can be used to carry out molecular analysis. This solution can be done with the help of chemical and enzymatic processes. The chemicals and the remaining enzyme residues that are produced from the lysis process will be purified in the extraction process to produce inhibitor-free DNA. The low purity of extracted DNA could be caused by residual phenol or other reagents used in the extraction process (Matlock B., 2015).

Of the five nucleotide acid constituents that make up DNA or RNA, if you read the absorbance at wavelength A260 / A280, it will show different values for each nucleotide component, namely: guanine (1.15), adenine (4.50), cytosine (1.51), uracil (4.00) and thymine (1.47). The results of the purity analysis resulting from the absorbance readings are then averaged and will be the absorbance value. This is the basis for determining the

purity values generally for DNA analysis in the range (1.8-2.0) (Leninger, 1975).

Each type of real-time PCR has a different level of detection capability. To measure the sensitivity of the tool can use LOD analysis. The tool's smallest value that can be detected ranges from 0.01-2 p / µL (Perandin et al., 2004; L et al., 2011; Kamau et al., 2013; Xu et al., 2015; Srisutham et al., 2017).

After adding proteinase K, the samples were incubated at 70 oCwhile shaking in the DNA extraction process. This heating aims to activate the proteinase K enzyme to actively work to carry out lysis (Renshaw et al., 2015). In the centrifugation stage, macromolecules such as proteins and polysaccharides will settle at the bottom of the tube, while DNA and water are in the top layer (Sophian, 2021).

In the discussion, use references with a consistent format as in the introduction paragraph, namely the system "*name and year*". The discussion must lead to the results of research, and the language used is standard and not convoluted. Discussions should not repeat or display research results. Use references to support or compare the results of the research.

Conclusion

This study concludes that samples derived from the base of young feathers have better purity and concentration values than samples from the base of the old hair and the tips of the hairs.

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Declaration statement

The authors reported no potential conflict of interest.

References

Abinawanto, Sophian, A., Lestari, R., Bowolaksono, A., Efendi, P. S., & Afnan, R. (2019). Analysis of IGF-1 gene in ayam ketawa (Gallus gallus domesticus) with dangdut and slow type vocal characteristics. *Biodiversitas Journal of Biological Diversity*, *20*(7), 2004–2010. https://doi.org/10.13057/BIODIV/D200729

Artama, W. T. (1991). *Rekayasa Genetika*. Pusat Antar Universitas-Bioteknologi, Universitas Gadjah Mada.

Bruzzone, C. M., Tawadros, P. S., Boardman, B. A., & Steer, C. J. (2013). Enhanced primer selection and synthetic amplicon templates optimize high-resolution melting analysis of single-nucleotide polymorphisms in a large population. *Genetic Testing and Molecular Biomarkers*, *17*(9), 675–680. https://doi.org/10.1089/GTMB.2013.0113

Cnops, L., Jacobs, J., & Esbroeck, M. Van. (2011). Validation of a four-primer real-time PCR as a diagnostic tool for single and mixed Plasmodium infections. *Clinical Microbiology and Infection : The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, *17*(7), 1101–1107. https://doi.org/10.1111/J.1469-0691.2010.03344.X

Dwight, Z., Palais, R., & Wittwer, C. T. (2011). uMELT: prediction of high-resolution melting curves and dynamic melting profiles of PCR products in a rich web application. *Bioinformatics (Oxford, England)*, *27*(7), 1019–1020. https://doi.org/10.1093/BIOINFORMATICS/BTR065

Eppendorf. (2016). *Nucleic Acid Photometry. Check of critical parameters*. Eppendorf AG. www.eppendorf.com

Held. P.G. (2001). *Nucleic Acid Purity Assessment using A260/A280 Ratios. Application note*. Senior Scientist & Applications Lab Manager. Biotek.

Kamagi, D. D. W. (2017). Isolasi dan Amplifikasi mtDNA Ayam Hutan Merah dan Ayam Kampung (Gallus gallus) Sulawesi Utara. *Jurnal Sains, Matematika Dan Edukasi* , *5*(2), 162–167.

Kamau, E., Alemayehu, S., Feghali, K. C., Saunders, D., & Ockenhouse, C. F. (2013). Multiplex qPCR for Detection and Absolute Quantification of Malaria. *PLOS ONE*, *8*(8), e71539. https://doi.org/10.1371/JOURNAL.PONE.0071539

Kirby, L. T. (1990). *DNA Fingerprinting: An Introduction*. M. Stockton Press.

Leninger, A. L. (1975). Biochemistry. In *Ed. 2*. Worth Publishers.

Matlock B. (2015). *Assessment of Nucleic Acid Purity, Technical Note 52646*. Thermo Fisher Scientific.

O'Neill, M., McPartlin, J., Arthure, K., Riedel, S., & McMillan, N. (2011). Comparison of the TLDA with the Nanodrop and the reference Qubit system. *Journal of Physics: Conference Series*, *307*(1), 012047. https://doi.org/10.1088/1742-6596/307/1/012047

Perandin, F., Manca, N., Calderaro, A., Piccolo, G., Galati, L., Ricci, L., Medici, M. C., Arcangeletti, M. C., Snounou, G., Dettori, G., & Chezzi, C. (2004). Development of a real-time PCR assay for detection of Plasmodium falciparum, Plasmodium vivax, and Plasmodium ovale for routine clinical diagnosis. *Journal of Clinical Microbiology*, *42*(3), 1214–1219. https://doi.org/10.1128/JCM.42.3.1214-1219.2004

Qiagen. (2014). For Extraction of Total Nucleic Acid From a Range of Food Sampel Types. In *Dneasy Mericon Food Hand Book.* Sample & Technology Assay.

Renshaw, M. A., Olds, B. P., Jerde, C. L., McVeigh, M. M., & Lodge, D. M. (2015). The room temperature preservation of filtered environmental DNA samples

and assimilation into a phenol–chloroform–isoamyl alcohol DNA extraction. *Molecular Ecology Resources*, *15*(1), 168–176. https://doi.org/10.1111/1755-0998.12281

Sambrook, J., Fritschi, E. F., & Maniatis, T. (1989). Molecular Cloning: a Laboratory Manual. In *Ed. 2*. Cold Spring Harbor Laboratory Pr.

Sophian, A. (2021). Short Communication: Analysis of purity and concentration of extracted DNA on salted fish processed food products. *Asian Journal of Natural Product Biochemistry*, *19*(1). https://doi.org/10.13057/BIOFAR/F190104

Sophian, A., Purwaningsih, R., Lukita, B. L., & Ningsih, E. C. (2020). Detection of Salmonella typhimurium ATCC 14028 in supplement health product liquid preparation using Real-Time PCR (qPCR). *Asian Journal of Natural Product Biochemistry*, *18*(2). https://doi.org/10.13057/BIOFAR/F180202

Srisutham, S., Saralamba, N., Malleret, B., Rénia, L., Dondorp, A. M., & Imwong, M. (2017). Four human Plasmodium species quantification using droplet digital PCR. *PLOS ONE*, *12*(4), e0175771. https://doi.org/10.1371/JOURNAL.PONE.0175771

Xu, W., Morris, U., Aydin-Schmidt, B., Msellem, M. I., Shakely, D., Petzold, M., Björkman, A., & Mårtensson, A. (2015). SYBR Green Real-Time PCR-RFLP Assay Targeting the Plasmodium Cytochrome B Gene – A Highly Sensitive Molecular Tool for Malaria Parasite Detection and Species Determination. *PLOS ONE*, *10*(3), e0120210. https://doi.org/10.1371/JOURNAL.PONE.0120210