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IDENTIFICATION OF HUMAN HAIR USING DIRECT PCR TARGETING THE CYTOCHROME B GENE

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Abstract

Background*: Hair is one of the human parts that is easily scattered and easy to obtain for testing. Direct PCR which is PCR without going through the DNA extraction process has been widely used in various fields. The cytochrome b gene is one of the genes located in mitochondrial DNA. The gene is unique in that it has a small variation within one species organism but has a large variation between species.* ***Aims****: c.* ***Methods****: Hair was heated at 60^{0}C for 10 minutes then the sample was used for the PCR template. PCR was performed using the cytochrome b gene.* ***The results****: The analysis showed that human hair could still be detected on day 30 using direct PCR using the cytochrome b gene.* ***Conclusions****: This result will simplify and save time in analysis in various fields related to hair shafts.*
Keywords*: Polymerase Chain Reaction, Human hair, Cytochrome b gene*

1. Introduction

Hair is one of the body parts that humans and higher animals have. If there is a mixture of human hair and animal hair, such as cats, dogs, and monkeys, it will be difficult to distinguish because the naked eye is very similar (1). Microscope examination is a method that is often used to analyze it, but microscope examination has several disadvantages, including requiring a long time. Microscope examination also tends to be subjective, the results of observations are highly dependent on the interpretation of the observer (2,3).

Detection of hair of human origin can be done more accurately using Polymerase

Chain Reaction (PCR) analysis (4). PCR is an in vitro amplification of DNA. PCR has been utilized in various fields and has undergone various innovations/ developments. One of the developments is direct PCR. Direct PCR is a PCR analysis using the sample as a template so that it does not pass the DNA extraction process. Direct PCR has several advantages, such as speeding up analysis and saving costs. (5,6). The cytochrome b gene is a gene located in the mitochondria. The cytochrome b gene is unique in that its variation is very limited within one species and very large between one species and another (7). The location of the cytochrome b gene target in the mitochondria causes the analysis results to be more sensitive than targets located in nuclear DNA. (8).

In this study, direct PCR testing was carried out using cytochrome b in human hair. The results of the study are expected to provide convenience and speed up the analysis of hair.

2. Research Methods

A. Preparation of Direct PCR Using Human Blood

Human blood from volunteers was taken as much as 5 uL added with 45 uL of distilled water then heated at 95^0C for 10 minutes. After that, it was waited until room temperature to be used as a PCR template.

B. Preparation of Direct PCR Using Human Hair

The hair was removed from the volunteer's head. It was then placed in the sample box for 0 days and 30 days. After that, the tip of the hair shaft was put into 50 uL of distilled water and heated at 95^0C for 10 minutes. (9). After that, it was waited until room temperature to be used as a PCR template.

C. Direct PCR

This study used cytochrome b gene primers with forward sequence 5'-TAGCAATAATCCCCATCCTCCATATA T-3', and reverse sequence 5'-ACTTGTCCAATGATGGTAAAAGG-3'.

A total of 2 uL of the previously prepared template was mixed with 12.5 uL of master mix, 2 uL of forward primer, 2 uL of reverse primer, and 6.5 uL of distilled water and then homogenized. The mixture was then PCR with predenaturation 95^0 C for 1 minute, denaturation at 95^0C for 30 seconds, annealing at 55^0 C for 30 seconds, elongation at 72^0C for 1 minute post elongation at 72^0C for 5 minutes (10). PCR results were electrophoresis and observed with UV illuminator.

3. Results and Discussions

The analysis showed that human blood could be amplified by direct PCR using the cytochrome b gene (Figure 1). In this study, human blood was used as a positive control.

The cytochrome b gene primers were able to detect human blood in a mixture of human and chicken blood. (11). The

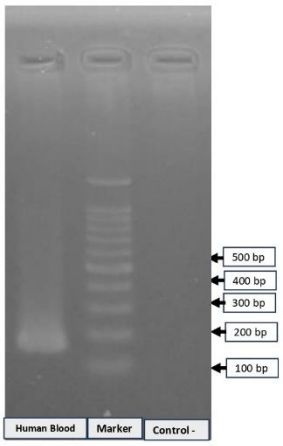
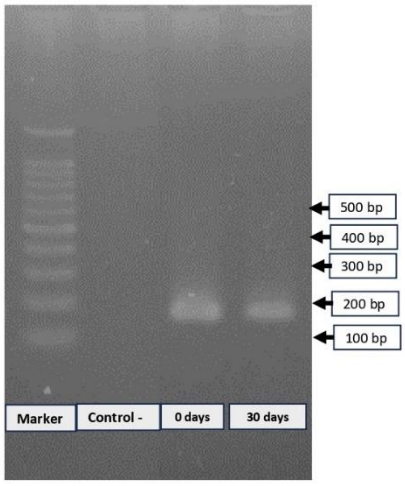


Figure 1. Direct PCR electrophoresis results using blood samples.



The results of PCR analysis showed a DNA band with a size of 157 bp appeared in both treatments (Figure 2). From these results it can be concluded that on the 30th day, human cytochrome b gene primers have also been used for identification of specimens of human origin by various researchers (12). The analysis was continued using hair as a sample. PCR analysis was conducted at 0 days and 30 days after the hair was removed from the head.

hair shaft samples can still be detected by direct PCR using the cytochrome b gene.

Figure 2. Direct PCR electrophoresis results using human hair samples

This result is particularly useful when finding a hair sample and the sample is found several days after detachment from the body. By using the direct PCR method and cytochrome b gene primers, hair samples that have fallen out for 30 days can still be analyzed and it can be confirmed whether the sample is of human or non-human origin. Direct PCR analysis also saves analysis time because it does not have to isolate DNA first.

4. Conclusions

Human blood can be detected using direct PCR with the cytochrome b gene. Hair that has been removed within 30 days can still be identified whether it comes from humans or animals. The use of direct PCR cuts the analysis time because there is no need to extract DNA.

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