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The Identification of Human and Animal Blood Mixtures Using Human Cytochrome b Gene

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***Abstract*— Human and animal blood has a similar composition so it is difficult to distinguish between the two. The aim of this study was to identify human blood from human and animal blood mixtures using the cytochrome b gene that has a species-specific DNA sequence. The DNA extracted from varied human and chicken blood was amplified using a human-specific cytochrome b gene primer. The result showed that 10 % human blood from the mixtures could be identified and the DNA extracted from the human blood could be amplified. The concentration of 0.01 ng still showed the appropriate DNA band. The primer was very sensitive and specific, so it useful for forensic purposes to verify human blood in a blood mixture.**

Keywords—Polimerase Chain Reaction (PCR), DNA, human and Animal Blood Mixtures, human cytochrome b

I. INTRODUCTION

The origins of forensic casework samples must be determined to be human or animal (non-human). Human and animal bloods especially blood has a similar composition (red blood cells, white blood cells and platelets) so it is very difficult to distinguish between the two. The classical methods for identification based on protein analysis [1,2] were are not applicable to the forensic casework.

New methods have emerged based on genetic differences. Cytochrome b markers have shown good feasibility in detecting species of origin from the sample mixtures. The nucleotide sequence of the cytochrome b gene contains species-specific information and has been used in food safety as well as in forensic investigations in a number of studies [3,4,5,6].

Furthermore, the cytochrome b gene is located in the mitochondrial genome. The mitochondrial genome of vertebrates is made up of small, circular double-stranded DNA molecules that present up to several thousand copies per cell [7]. This gene location takes advantage of the sensitivity of PCR-based DNA analysis in the forensic context.

In the present study, the authors applied PCR-based analysis using cytochrome b to identify human blood from human and chicken blood mixtures and reveal how it level in the mixtures can be detected.

II. MATERIAL AND METHODS

A. Primer ordering

This study used a human cytochrome b gene primer which was designed by Matsuda [8] and ordered from PT. Genetika Science, Indonesia. Forward primer 5'-TAGCAATAATCCCC ATCCTCCATATAT-3', reverse primer 5'-ACTTGTCCAAT GATGGTAAAAGG-3'

B. Bloods preparation

Human peripheral blood samples were obtained from the Indonesian Red Cross (PMI) while the animal blood samples were obtained from a chicken farm.

C. DNA extraction

DNA from human blood, chicken blood and human and chicken blood mixtures were extracted using the Wizard Genomic DNA Purification Kit (Promega) according to the respective manufacturer's instructions. The concentrations of the extracted DNA were determined by UV absorbance using a spectrophotometric reading at 260 nm.

D. Primer specificity test

The specificity of the primer was confirmed by the amplification of 100 ng DNA from human blood and chicken blood. The amplification was conducted in a 50 ul volume; containing 2 ul template DNA, 2X GoTaq Green Master Mix (reaction buffer (pH 8.5), 400µM of all four dNTP and 3mM MgCl2), 0,3 uM forward primer, 0.3 uM reverse primer. PCR was performed in a thermal cycler for 30 cycles of 30 s at 95^0 C, 40 s at 55^0 C, and 1 min at 72^0 C. The products of the PCR amplification were electrophoretically determined in a 2% agarose gel containing ethidium bromide in a 0.5X TBE (Tris–Borate–EDTA) buffer and visualised by UV transillumination.

E. Minimal DNA template concentration test

DNA from the human blood was diluted using aqudest (100 ng, 10 ng, 0.1 ng, 0.01 ng). Each dilution was used as a template in PCR amplification according to the primer specificity test. Minimal DNA template concentration was determined by PCR amplification of the DNA extracted from humans at levels of 0.01, 0.1, 10, 100 ng of DNA in water.

F. Minimal percentage of human blood test

Human blood and chicken blood was mixed and extracted in ratios of 0:100, 50:50, 25:75, 10:90, 5:95, 100:0. DNA from this extraction was used as a template in PCR amplification according to the previous analysis.

III. RESULTS AND DISCUSSION

A. Specificity of the primers

The specificity for human DNA was evaluated using DNA extracted from human and chicken blood as template DNA in amplifications with the human cytochrome b gene primer. In the agarose gel electrophoresis, the DNA of animals produced no visible bands whereas human DNA showed a single band of the expected size of approximately 157 bp (Fig.1)

B. Minimal DNA template

The minimal DNA template was determined by PCR amplification of the DNA extracted from human blood at levels from 0.01 to 100 ng of DNA in water. The DNA templates were still successfully amplified at 0.01 ng (Fig.2).

C. Minimal percentage of human blood

The results of the PCR reactions for the DNA extracted from human and animal blood mixtures showed that detection of the human blood was successful in mixtures from 10% to 50% level. Mixtures containing 5% human blood were not successfully amplified (Fig.3).

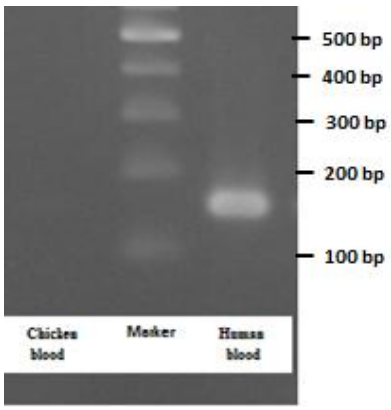

Fig. 1. Agarose gel electrophoresis (2%) of PCR product amplified from human blood and chicken blood; Marker : 100bp DNA Ladder

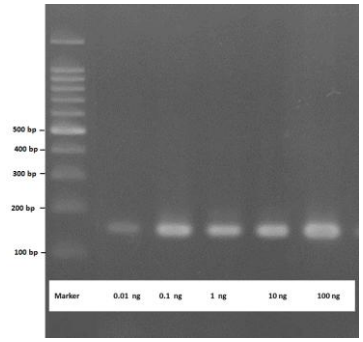

Fig. 2. The result of minimal DNA template concentration test on 2% agarose gel; 0.01, 0.1, 10, 100 ng : DNA template concentration; Marker : 100bp DNA Ladder

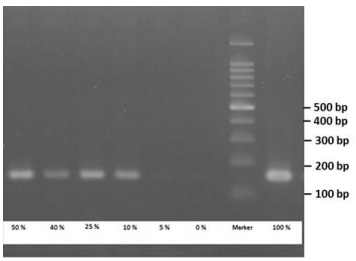

Fig. 3. The result of minimal percentage of human blood on 2% agarose gel; 50%, 40 %, 25 %, 10 %, 5%, 0%, 10% : percentage of human blood in mixtures; Marker : 100bp DNA Ladder

D. Discussion

In this study, species-specific PCR was applied for the identification of human and animal blood mixtures using a cytochrome b gene that had a species-specific sequence. According to the alignment analysis, the chicken cytochrome b gene has 13 differences in the regions targeted by the human cytochrome b gene primer. Similarly, monkey, gorilla and other primates who are closely-related with humans also have 10-13 differences in the regions targeted by the human cytochrome b gene primer. Chicken blood was therefore used as a representative of animal blood. DNA extracted from the human blood was successfully amplified while the DNA extracted from human blood was not. The DNA bands obtained were 157 bp according to Masuda [8]. It was designed to amplify the human cytochrome b gene sequence from the 902th base to 1058th base. The results also showed that the human cytochrome b gene was specifically targeted so that only the human DNA would be amplified.

The minimal DNA template of the primers depends on it sequences. The primer detected the presence of 0.01 ng of the DNA template from human blood while the other cytochrome b gene primer showed a positive result of 0.25 ng [8], 0.0001 ng [9,10], 0.01 ng [11] in the DNA template. The cytochrome b gene has many copies and is located in the mitochondrial DNA which causes it to be very sensitive and easily detected.

The 10% level of human blood from the human and chicken blood mixtures could be detected but the 5% level was not. The identification of the sample mixtures using the cytochrome b gene could be performed until a 0.1% level [3, 12].

In mammalian blood including humans, mitochondrial DNA is found in white blood cells which make up only 1% of whole human blood. Mitochondrial DNA in the 5% level mixtures did not show up in as many of the other human sample mixtures. It was one of reasons that the 5% level mixtures showed a negative result.

IV. CONCLUSION

PCR-based DNA analysis using the human cytochrome b gene primer was very specific, sensitive and simple so it was a suitable method to detect blood in the sample mixtures for forensic purposes.

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