The use of touch DNA analysis in forensic identification focusing on Short Tandem Repeat-Combined DNA Index System loci THO1, CSF1PO and TPOX

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Abstract

Forensic identification through DNA analysis is an accurate diagnostic tool. Deoxyribonucleic Acid (DNA) analysis is via DNA repetitive regions with less than 1 kb base size is called 'microsatellite' or Short Tandem Repeat (STR). At the crime scene, the perpetrator's skin may accidentally be in contact with surrounding objects, thereby transferring trace evidence to the objects. In this study DNA was obtained using "touch DNA" from two buccal smears and two smear from watches and cellphones from volunteers who had signed the consent form. Samples were isolated using DNAzol. The quantity of DNA obtained will be measured using a UV spectrophotometer. For DNA amplification using 3 STR CODIS loci namely TH01, CSF1PO, and TPOX. The last step is visualization using acrylamide gel and silver staining. Mean levels of DNA (UV-Visible Spectrophotometer) were 167.89±85.71 µg/mL for the buccal swab, 59.19±5.58 µg/mL for the watch swab, and 38.09±2.12 µg/mL for the mobile swab; the purity of the buccal swab DNA was 1.79±0.71, of the watch swab 1.69±0.76, and of the mobile swab 1.53±0.56. Visualization of PCR products on Polyacrylamide Agarose Composite Gel Electrophoresis stained with Silver and amplified using the standard primers THOI, TPOX and CSF1PO for STR Combined DNA Index System (CODIS) showed a 100% detection of amplicons. Both the buccal swab, watch swab and handphone swabs had trace amount of DNA that was sufficient to be isolated and amplified by using Polymerase Chain Reaction on the STR CODIS loci THO1, CSF1PO and TPOX.

Introduction

Deoxyribonucleic Acid (DNA) identification is a way of identifying individuals through characteristics and features that distinguish them from others. Currently the identification method has evolved towards molecular forensic DNA. DNA is the smallest unit and is present in all living things from microorganisms to higher organisms such as humans, animals and plants.[1]

Identification through DNA analysis is an accurate diagnostic tool. DNA analysis includes analysis of Variable Number of Tandem Repeat (VNTR) and Restriction Fragment Length Polymorphisms (RFLP). DNA analysis through VNTR is a DNA examination method that is based on certain repeated base sequences (also called core sequences). DNA repeated sequence areas with base size less than 1 kb (kilo base pair), are known as 'microsatellite' or Short Tandem Repeat (STR).[2]

The Federal Bureau of Investigation (FBI) designed 13 STR locus as a synergistic forensic identification system with the Combined DNA Index System (CODIS) database. The STR locus used by FBI includes TH01, TP0X, CSF1PO, vWA, FGA, D3S1358, D5S818, D7S820, D13S317, D16S539, D8S1179, D18S51, and D21S11, plus amelogenin markers used to determine the sex of the individual.[3,4]

At the crime scene the perpetrator's skin surface or part of his body is often accidentally exposed to the surrounding objects, resulting in the transfer of trace evidence to such objects. In this case one of the technologies, namely touch DNA/contact trace DNA, can be used, through the DNA that is transferred in the form of skin cells when objects are held or touched.[5] Research conducted by Yudianto *et al* concluded that there are external factors that connect the environment and the duration of exposure to the quantity and quality of DNA from earphone swabs, but can still be an alternative material in forensic identification. PCR samples showed that 126 bp (nt 34-159) was detected on mtDNA HVS II and the results were well-sequenced to earphones placed in an open space for one day.[6]

The DNA study of sweat and grease substance left by donor men on the skin of both men and women in half of cases reveals allelic combinations inherent in both the donor and the recipient. The results obtained indicate equal chances of detecting the DNA of contacting individuals.[7] Therefore, research on identification using touch DNA must be developed focusing on objects that are often used daily.

Materials and Methods

DNA was obtained using "touch DNA" from two buccal smears and two smears from watches and cellphones. DNA isolation in this study using DNAzol and DNA pellets were added with 50ul distilled water.[8-10] DNA was amplified by using STR-PCR (PowerPlex® 21 Systems, Promega, USA) targeting 3 STR autosomal loci (TH01, TPOX, and CSF1PO) with sequences:[11]

TH01:	5'-(FL)-GTGATTCCCATTGGCCTGTTC-3' 5'-ATTCCTGTGGGCTGAAAAGCTC-3'
TP0X:	5'-ACTGGCACAGAACAGGCACTTAGG-3' 5'-(TMR)-GGAGGAACTGGGAACCACAC AGGTTA-3'
CSF1PO:	5'-AACCTGAGTCTGCCAAGGACTAGC-3' 5'-(**JOE**)-CCGGAGGTAAAGGTGTCT TAAAGT-3'

The amplification process was done using a Bio Rad T100TM PCR machine for a total duration of 2 hours and 7 minutes. The temperature settings for PCR were set as follows: 96°C for 2 minutes, then 94°C

for 1 minute, 64°C for 1 minute, 70°C for 1.5 minutes, for 10 cycles, then 90°C for 1 minute, 64°C for 1 minute, 70°C for 1.5 minutes, for 30 cycles. The amplicons were stored at 4°C (12–14). Amplicons were visualised on 6% Silver Nitrate stained polyacrylamide gel electrophoresis (Bio-Rad Mini-PROTEAN®).[15]

Results and Discussion

The results were obtained by measuring the average amount of DNA using a UV-Visible Spectrophotometer. The average DNA level of all samples was at a minimum amount of 0.25 ng with a purity of 1.8-2 (Table 1).[16-18]

PCR products were visualised using Polyacrylamide Agarose Composite Gel Electrophoresis (PAGE) and the gel was stained with silver. To amplify the STR CODIS locus, the standard primers (THO1, TPOX and CSF1PO) were used and all buccal swab and property (watch and cell-phone) swab samples showed positive results signifying a 100% detection. Allele profiles in all samples were a match with the positive control K562. Figure 1 shows the visualization results from several samples from locus THO1 (amplicon product 156–195 bp) showing that all samples were detected as compared to the positive control (K562) and repeat allele 9,3:9,3. Figure 2 shows the visualization of PCR products with PAGE stained with Silver. The samples were amplified using the primer CSF1PO (amplicon product 321–357 bp) and all the results were positive as shown by the matching bands with the positive control (K562) and repeat allele 9:10. The last is Figure 3, that shows the visualization of PCR products with PAGE stained with silver and the amplicons were amplified using the CODIS STR TPOX locus (amplicon product 262-290 bp) and all samples were detected as positive (detection +) when compared with the positive control (K562) and repeat allele 8:9.

For adequate visualization of the results, sufficient levels and purity of the DNA is needed in order for the DNA to be used as an examination material, including in this case of identification and paternity test.[19-21] Failed PCR amplification is characterized by the absence of bands on agarose gel. Incomplete PCR cycles were minimized by PCR optimization for all the respective primers used.[22]

In this study, 3 STR CODIS loci were used, showing that all the samples for buccal swab and property swab (watch and cellphone) were detected positive, as well as the allele profile of each locus in each

sample showing matched results. Matched results have the understanding that the allele profile on the buccal swab is identical to the allele profile on the swab property. Only one sample showed a different allele, the sample A[2] (cellphone, allele 8:9,3; see Table 2). This could be due to contamination during DNA analysis checking process, starting from the process of sample collection. As a positive control, that is K562, which is a positive control in the examination of DNA analysis with STR CODIS and 100bp ladder markers. DNA analysis tests have a 100% accurate value, when done correctly. This DNA analysis test gave a results probability of 100%. The following is a profile table of STR CODIS allele detection results (THO1, CSF1PO, TPOX) on DNA samples (watch swabs, cellphone swabs and buccal swabs).

Research on STR CODIS as a whole has not yet been reported, with the exception of a few primers. Some studies have been conducted from several STR CODIS focus areas. The accuracy of research at the loci THO1, TPOX, CSF1PO, and has been reported in several studies including: chromosome populations and allele sequences at the THO1 locus[23], population in Thailand with 15 STR loci included THO1, TPOX, CSF1PO and vWA,[24,25] research on Chinese population in Taiwan with STR,[26] research on genetic variation in Caucasia,[27] and researched on genetic variation in the population of Filipinos and Thais living in Taiwan using 9 STR loci. In Indonesia, Novita (2005) did a research on the THO1 and TPOX allele pattern in the Bali population and a research in Madura for sibling's DNA using 13 STR locus.[28] STR loci typing method especially the THO1 locus is a reasonable, strong and efficient method making it a useful method in forensic cases.[23]

Table 1. Average amount and purity of DNA samples.

Sample	Average amount of DNA (x±SD)(μg/mL)	Average purity of DNA (λ260 nm/ λ280 nm)
Buccal swab	167.89±85.71	1.79±0.71
Watch swab	59.19±5.58	1.69±0.76
Handphone swab	38.09±2.12	1.53±0.56

x, mean; SD, standard deviation.

Table 2. Allele STR CODIS's profile.

LOCI	SAMPLE A	A[1]	A[2]	B	B[1]	B[2]
TH01	9;9.3	9;9.3	8;9.3	9;9.3	9;9.3	9;9.3
CSF1PO	9;10	9;10	9;10	9;10	9;10	9;10
TPOX	8;9	8;9	8;9	8;9	8;9	8;9

A, buccal swab sample 1; A[1], Watch swab 1; A[2],Cellphone swab 1; B , buccal swab sample 2; B[1], Watch swab 2; B[2],Cellphone swab 2.
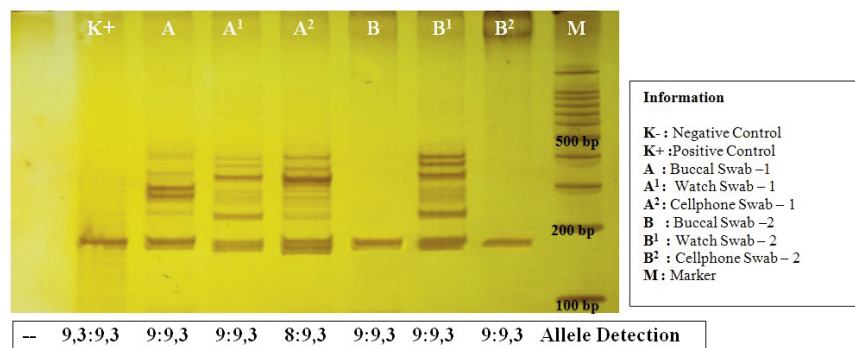

Figure 1. Visualization of STR CODIS PCR product using at locus THO1 (156–195 bp).
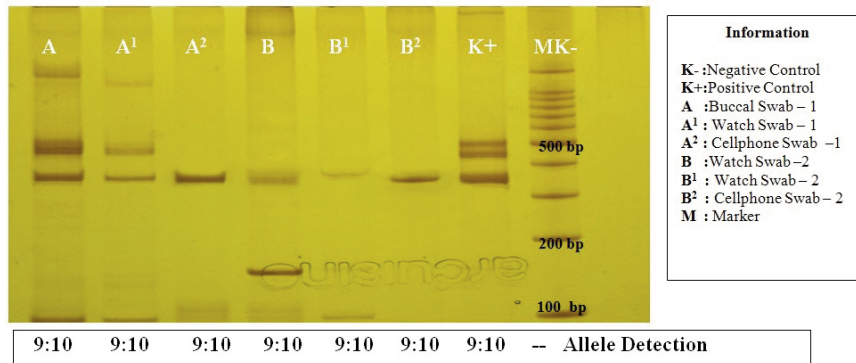
Figure 2. Visualization of STR CODIS PCR product using at locus CSF1PO (32–357 bp).
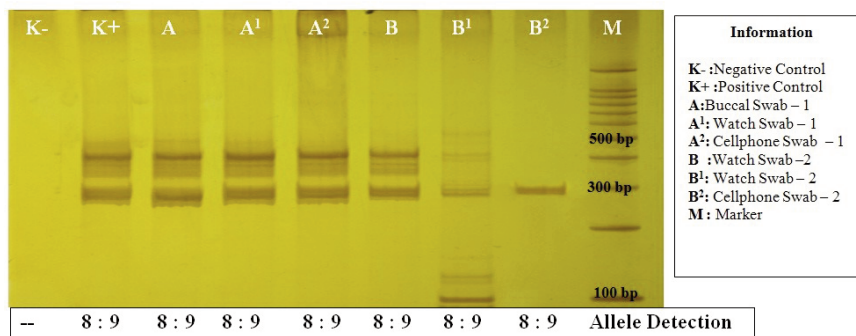

Figure 3. Visualization of STR CODIS PCR product using at locus TPOX (262–90 bp).

Conclusions

Property (cellphone and watch) swabs can be used as alternative materials in forensic identification using Touch DNA analysis with an average DNA yield of: 59.19±5.58□g/mL and: 38.09±2.12□g/mL for watch and cellphone swabs respectively. Both the buccal swab, watch swab and cellphone swabs had trace amount of DNA that was able to be isolated and amplified by using Polymerase Chain Reaction on the STR-CODIS loci THO1, CSF1PO and TPOX.

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