Fluorescent Triplex-PCR for profiling of DNA from fingerprint evidence

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Abstract

Typing of DNA from low quality forensic biological samples recovered from crime scene, *e.g.* fingerprint, can be difficult and not cost effective. In this study, a Triplex-PCR for human DNA typing was developed in order to screen DNA extracted from fingerprint samples. Three primer pairs, consisting of THO1, D8S1179, and CSF1PO, are also presented in commercial STR typing kits. Forward primers of each pair were labeled with one of the two fluorescent dyes, *i.e.* 6-carboxyfluorescein (6FAM™) and VIC™ dyes. PCR products generated from each locus are in the range of 152-196 bp, 203-255 bp, and 317-361 bp, respectively. DNA from buccal swab was used for Triplex-PCR optimization. Triplex-PCR, optimized in a total volume of 12.5 µL containing 2.5 pmol of THO1 primers and 20 pmol of each D8S1179 and CSF1PO primers. Optimal thermal cycling condition was as following; initial denaturation step at 95°C for 11 min; 28 cycles of denaturation at 94°C for 1 min, annealing at 59°C for 1 min; and extension at 72°C for 1 min, final extension at 60°C for 60 min, and held at 25°C. Full DNA profile was obtained when using as low as 250 pg DNA template. The fluorescent Triplex-PCR was then tested with DNA extracts from 1, 4 latent and 4 fluorescent powder dusted fingerprint(s). No DNA profile was obtained when the optimal condition was applied for PCR, but partial DNA profiles were generated when the number of PCR cycle was increased to 34. Highest signals were generated from samples collected from four latent fingerprints, then four dusted fingerprints and one latent fingerprint samples, respectively. Similar results were demonstrated when DNA samples were typed with AmpF*ℓ*STR® Identifiler® STR amplification kit. Partial DNA profiles were generated when samples were amplified for 34 cycles. Results from the developed Triplex-PCR was consistent to the commercial kit, however the cost was 6 times lower. This fluorescent Triplex-PCR would benefit screening a large number of crime scene samples prior to 16-loci STR analysis.

Keywords: Fingerprint, DNA Typing, STR

Introduction

Various types of biological evidence can be found in crime scene and one of them is fingerprint which is known to be a source of DNA [1-3]. Partial DNA profiles were often obtained from fingerprint evidence when analyzing DNA extracted from fingerprints using available commercial short tandem repeat (STR) amplification kits, consisting of 15 STR loci and the sex-determining locus, amelogenin gene.

The nature of DNA from fingerprint evidence is often present in minute amount or degraded. The degradation of skin cells is a result of apoptosis, also known as programmed cell death. It normally occurs in aged cells and epithelial cells, then, sloughed off from a finger. In addition, apoptosis also causes degradation of chromosomal DNA into small fragments [4, 5]. The quantity of DNA recovered may due to two main factors; 1) the amount of biological material left on touched objects, 2) the suitability of recovery and extraction methods. Previous studies demonstrated that amount of DNA left on touched surface varies among the individual [6, 7]. Moreover, loss of DNA yield can occur during steps of collection and extraction methods can lead to the loss of DNA [3, 8]. As a consequence, the extracted DNA would then be very low in both quantity and quality. Therefore, it is difficult to obtain good quality DNA profile when typing DNA from fingerprints using available commercial amplification kits, and also not being cost effective.

In this study, we developed a simple triplex-PCR which can amplify DNA extracts from fingerprints and gave consistent results with an available commercial STR amplification kit commonly used in forensic laboratories. The triplex-PCR was used to generate DNA profiles from undusted fingerprints and fingerprints dusted with fluorescent powder, and results were compared to a commercial amplification kit.

Materials and Methods

Sample preparation and DNA extraction

There were 3 groups of fingerprints used in our study: 1) one latent fingerprint, 2) four latent fingerprints and 3) four fluorescent powder dusted fingerprints. Fingerprints were generated by pressing the fingers against clean glass slides for 30 s. Fluorescent powder used was red fluorescent powders (Lynn Peavey Company, KS, USA). Each group of samples were then collected by using moisten sterile swabs and left to air-dry. Buccal swabs were also collected for reference DNA profiles.

DNA was extracted from swabs using QIAamp® DNA Mini Kit (QIAGEN, CA, USA) following the manufacturer's instructions. Quantification was carried out using UV-VIS spectrophotometry, NanoDrop™ 1000 Spcetrophotometer (Thermo Fisher Scientific, DE, USA).

DNA Amplification, Separation and Detection

The triplex-PCR consisted of three primer pairs, i.e., THO1, D8S1179, and CSF1PO. These three loci are members of the core STR loci and were selected as representatives of small, medium and large size STR loci presented in commercial STR typing kits. Primer sequences were obtained from STR-base [11], details are shown in Table 1. Modification was made by labeling forward primers of each locus with fluorescent dyes; *i.e.* 6-carboxyfluorescein (6FAM™) for D8S1179, and VIC™ for THO1 and CSF1PO.

PCR was carried out in a total reaction volume of 12.5 μL, containing 1X PCR buffer, 0.2 mM of deoxyribonucleoside triphosphates (dNTPs), 1.5 mM of $MgCl_2$, 1 unit of AmpliTaq Gold® DNA poloymerase, primers, and DNA extracts. Amplification was carried out in GeneAmp® 9700 thermocycler (Applied Biosystems, Foster City, CA) using the following condition; initial denaturation step at 95°C for 11 min; 28 cycles of denaturation at 94°C for 1 min, annealing step at 64°C for 1 min, extension at 72°C for 1 min; final extension at 60°C for 30 min, then held at 25°C, unless otherwise stated.

Amplification of STR loci using the AmpFℓSTR® Identifiler® PCR Amplification Kit (Applied Biosystems, CA, USA) was carried out according to the manufacturer's instructions. For low copy number (LCN) DNA analysis, PCR cycle was increased to 34 cycles.

Amplification products were then separated in the ABI Prism 310 Genetic Analyzer by co-injecting with the internal size standard (GeneScan™-500 LIZ®). DNA fragments size were analyzed and genotyped using GeneMapper™ *ID* Software Version 3.2 (Applied Biosystems, Foster City, CA).

The three reference DNA profiles used in this experiment were as following; *profile1* THO1 (7,9), D8S1179 (10,10), and CSF1PO (11,12); *profile2* THO1 (7, 9), D8S1179 (11,16), and CSF1PO (12,14); and *profile3* THO1 (8,10), D8S1179 (11,16), and CSF1PO (12,12).

Table 1 Primer sequences used in this study. "F" refers to the forward primer and "R" to the reverse primer for each locus. "6FAM" and "VIC" are commercially available fluorescent dyes.

Locus		Primer Sequence (5' to 3')	T_m (°C)	Size (bp)
THO1	F	VIC-GTGATTCCCATTGGCCTGTTC	64	152-196
THO1	R	ATTCCTGTGGGCTGAAAAGCTC	66	
D8S1179	F	6FAM-ATTGCAACTTATATGTATTTTTGTATTTCATG	78	203-255
D8S1179	R	ACCAAATTGTGTTCATGAGTATAGTTTC	74	
CSF1PO	F	VIC-CCGGAGGTAAAGGTGTCTTAAAGT	69	317-361
CSF1PO	R	ATTTCCTGTGTCAGACCCTGTT	64	

[a] Predicted primer melting temperatures (T_m) were calculated from the equation:

$Tm = 4(G+C) + 2(A+T)$

Results and Discussion

Singleplex PCR Optimization

Singleplex amplification was first carried out in order to test all primers using two annealing temperatures, 57°C (figure 1a) and 59°C (figure 1b), using 10 pmol of each primer. At the annealing temperature of 57°C, only PCR products of THO1 were presented. Electropherograms showed that peak heights were also lower than those carried out at 59°C. When the annealing temperature was increased to 59°C, PCR products of correct sizes, colors, and genotypes were present in all three loci; THO1 and CSF1PO peaks were green and D8S1179 peaks were blue. However, there were large differences in peak heights of products from three loci; peak heights of THO1 were highest, then CSF1PO, and D8S1179 was lowest.

To increase peak heights (or PCR products), primer concentrations were increased to 20 pmol of each primer/reaction. Singleplex amplification of three primer pairs were then carried out at 62°C. As shown in Figure 2, PCR products of all three loci increased as higher peaks were showed in all three electropherograms.

Triplex PCR Optimization

Triplex amplification was first carried out by combining all three primers in equimolar concentration (20 pmol each), at the annealing temperature of 62°C (figure 3a). PCR products of correct sizes were amplified in the multiplex PCR, however, peaks in the electropherogram were imbalanced. PCR products of the smallest locus (THO1) were the most preferentially amplified, then CSF1PO, and D8S1179, respectively. Concentration of THO1 primer pair was then reduced from 20 pmol to 10, 5, and 2.5 pmol in order to balance the peak height. As shown in figure 3b – 3d, peak heights of THO1 reduced as according to the amount of primer used. Peaks presented in the electropherogram shown in figure 3d were balanced most; ratio of THO1:D8S1179:CSF1PO primer pairs was 2.5:20:20 pmol. PCR product ratio of THO1:D8S1179:CSF1PO was approximately 3:1:2. This product ratio was also obtained when using two different DNA samples.

Annealing temperatures were also increased to 64°C and 66°C to increase amount of PCR products from D8S1179, but decrease products generated from THO1 locus. Electropherogram in Figure 4b showed that peak heights of D8S1179 increased when the annealing temperature was 64°C. In addition, peak heights of CSF1PO slightly increased as well. No allele was called (no PCR product was obtained) when annealing temperature was increased to 66°C (see figure 4c).

From these results, triplex PCR was suggested to be carried out by using 2.5 pmol of THO1, 20 pmol of D8S1179, and 20 pmol of CSF1PO primers; annealing temperature of 64°C for the thermocycling condition (details are shown in Table 2 and Figure 5).

Triplex-PCR sensitivity test

A series of 2-fold dilution of DNA template extracted from buccal swab starting from 2 ng – 62.5 pg was used in this experiment to test the sensitivity of the triplex PCR. The minimum limit for peak calling was set at 50 rfu. As shown in figure 6, PCR products from all three loci were detected in the electropherograms when 2 ng – 250 pg of DNA template were used in the triplex PCR (see figure 6a – 6d). Only THO1 peaks were presented in the electropherogram when lower amounts of DNA template were added to the reaction (see figure 6e and 6f). No PCR product was detected in the negative control (no DNA) reaction (see figure 6g).

Profiling of DNA from fingerprint

Full DNA profile was generated from only buccal swab sample using the triplex primer set. No profile was obtained from the fingerprint samples. LCN typing condition was then applied for the triplex PCR analysis, in which the PCR cycle number was increased from 28 to 34 according to Gill *et al.* [9, 10]. Full DNA profile was generated from the buccal swab sample and partial profiles were generated from 3 groups of fingerprint samples (see figure 7); however, the quality of DNA profile differed. Partial DNA profile generated from 1 undusted fingerprint gave the lowest signal as peak heights were less than 2,000 rfu (see figure 7a). In addition, alleles of D8S1179 locus, which was the most poorly amplified locus in the triplex PCR, was dropped out. This may be due to the nature of biological material (epithelial cells) available in fingerprint for the analysis was small in quantity and low in quality. Quality of partial DNA profile obtained from 4 undusted fingerprints was better than the 4 fluorescent powder dusted fingerprint (see figure 7b and 7c). This may be due to the dusting step in fingerprint enhancement that may brush away epithelial cells deposited on the surface, thus

resulting in smaller amount of cells being left. Comparing peak height and peak balance, peaks in the electropherogram of 4 undusted fingerprints were higher and in balance when compared to the fluorescent powder dusted sample. In addition, one allele of D8S1179 locus was called in the 4 undusted fingerprints DNA profile.

Same samples were amplified using Identifiler STR amplification kit and the results turned out the same way as obtained from the fluorescent Triplex-PCR. Results showed that, full 16-loci STR profile was obtained from buccal swab DNA when using the 16-STR loci AmpF*ℓ*STR® Identifiler® PCR Amplification Kit. No DNA profile was generated when fingerprint samples were DNA typed. As shown in figure 8, partial DNA profiles were obtained from LCN typing of fingerprint samples and sex-determining Amelogenin gene was generated in all samples. The highest signal was obtained from 4 undusted fingerprints (figure 8b), then 4 fluorescent powder dusted fingerprints (figure 8c), and one undusted fingerprint (figure 8a) sample, respectively. Partial DNA profiles generated from 4 fingerprints were better in quality when compared to the DNA profile generated from 1 fingerprint. Smaller loci than 200 bp (*e.g.* D8S1179, THO1, D3S1358, and D19S433) was better generated than larger ones which, in some samples, were dropout. The buccal swab sample gave a full DNA profile (figure 8d).

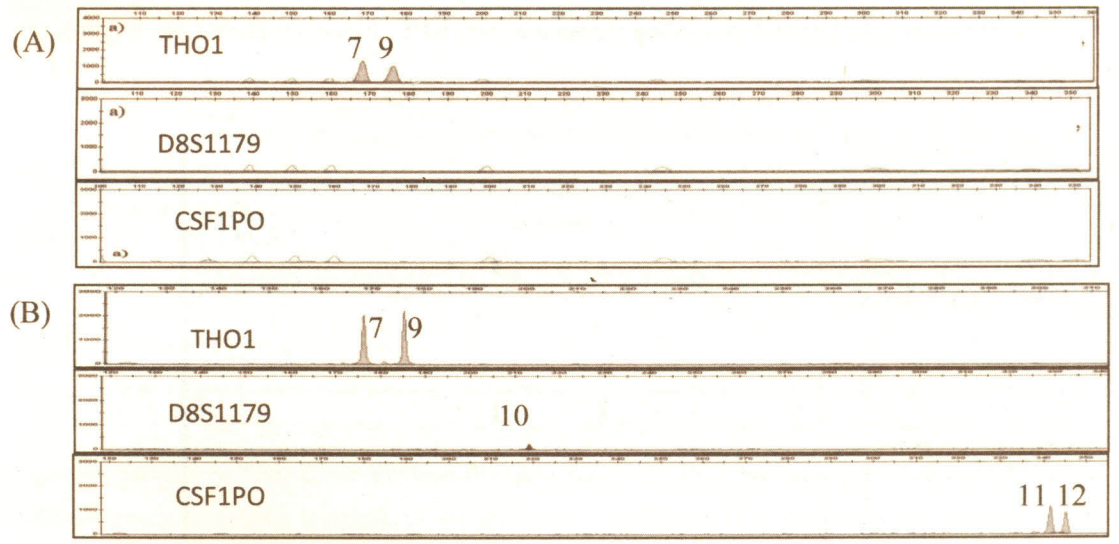
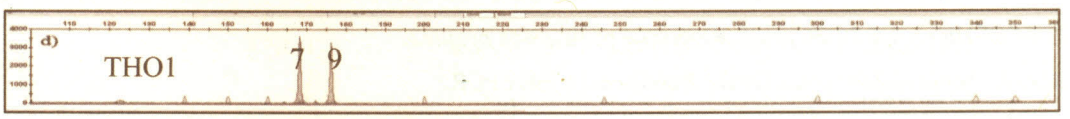


Figure 1 Electropherograms showing singleplex PCR products amplified from buccal swab DNA using 10 pmol of each primer.



Annealing temperatures were 57°C (figure 1B), and 59°C (figure 1A). Reference profile was *profile1*.

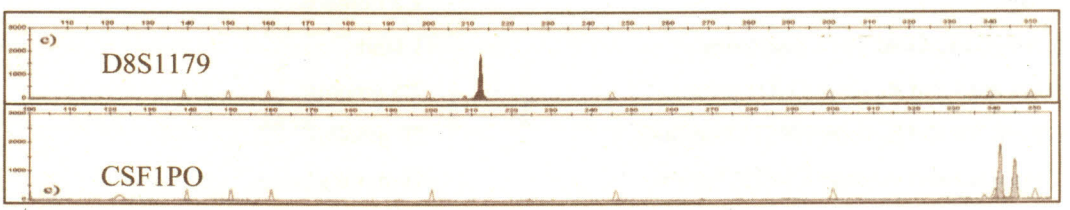


Figure 2 Electropherograms showing singleplex PCR products amplified from buccal swab DNA using 20 pmol of each primer. Annealing temperature was 62°C. Reference profile was *profile1*.

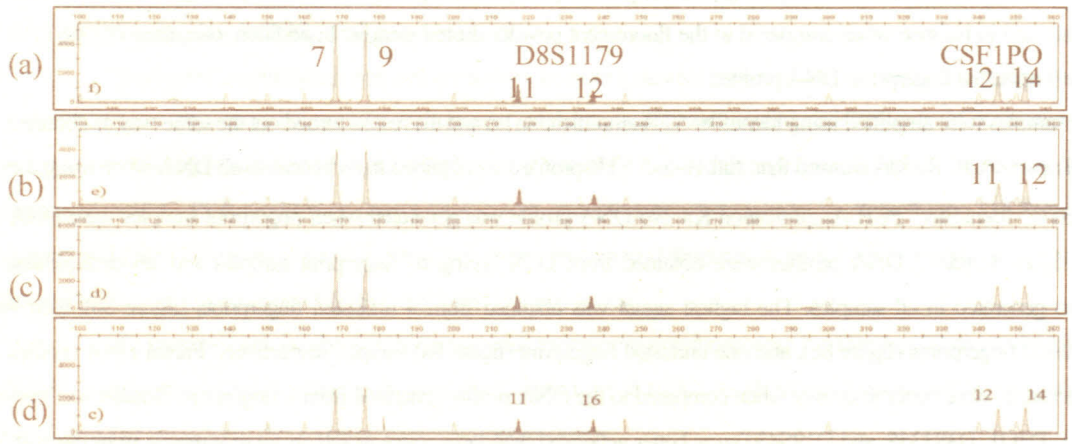


Figure 3 Electropherograms showing triplex PCR products amplified from buccal swab DNA using different amount of THO1 primers; 20 pmol (figure 3a), 10 pmol (figure 3b), 5 pmol (figure 3c), and 2.5 pmol (figure 3d). Concentrations of D8S1179 and CSF1PO primers were 20 pmol. Annealing temperature was 62°C. Reference profile was *profile2*.

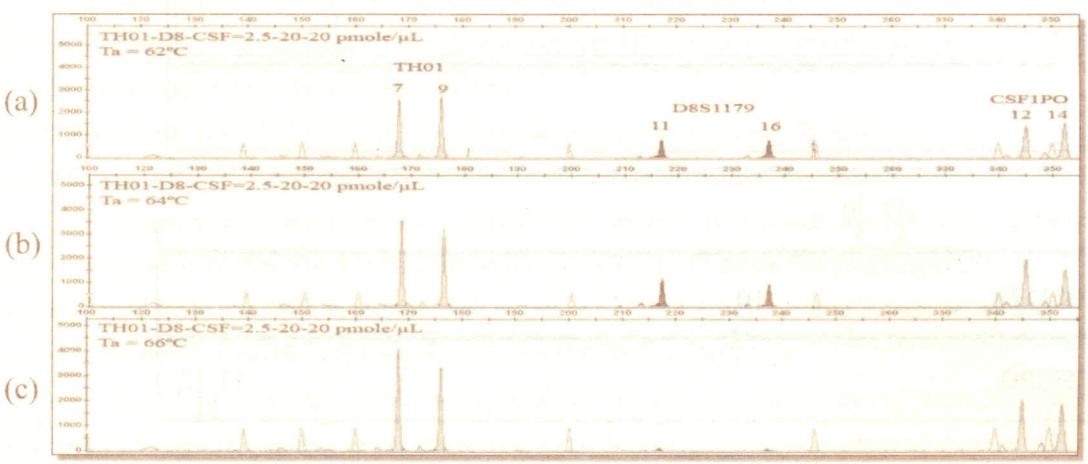


Figure 4 Electropherograms showing triplex PCR products amplified from buccal swab DNA at different annealing temperatures; 62°C (figure 4a), 64°C (figure 4b), and 66°C (figure 4c). Concentrations of primers were in the ratio of THO1:D8S1179:CSF1PO = 2.5:20:20 pmol. Reference profile was *profile2*.

Table 2 Optimized reaction components for the fluorescent triplex PCR..

PCR Reagents	**Concentration**
PCR buffer	1X
dNTPs	0.2 mmol/L
$MgCl_2$	1.5 mmol/L
AmpliTaq Gold DNA polymerase	1 Unit
Forward and Reverse THO1 primer	2.5 pmol/µL
Forward and Reverse D8S1179 primer	20 pmol/µL
Forward and Reverse CSF1PO primer	20 pmol/µL
DNA template	2 ng
Total volume reaction = 12.5 µL	

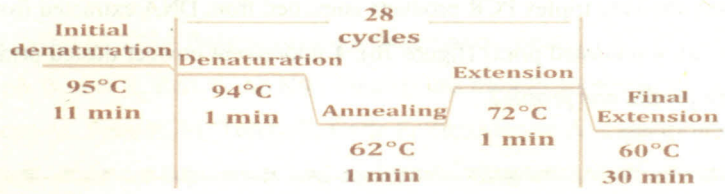


Figure 5 Optimized thermal cycling condition for the fluorescent triplex-PCR.

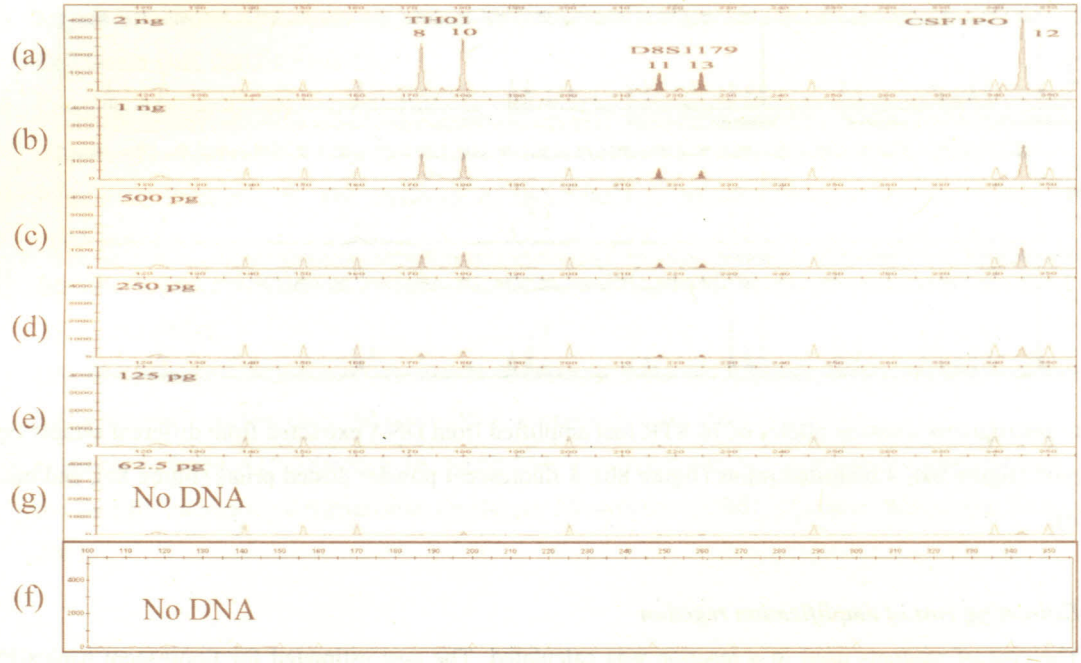


Figure 6 Sensitivity Test. Electropherograms showing triplex PCR products amplified from buccal swab DNA using different amounts of DNA template; 2 ng (figure 6a), 1 ng (figure 6b); 500 pg (figure 6c), 250 pg (figure 6d); 125 pg (figure 6e), 62.5 pg (figure 6f), and no template DNA (figure 6g). No PCR products were obtained when less than 250 pg of DNA was present in the reaction. Reference profile was *profile3*.

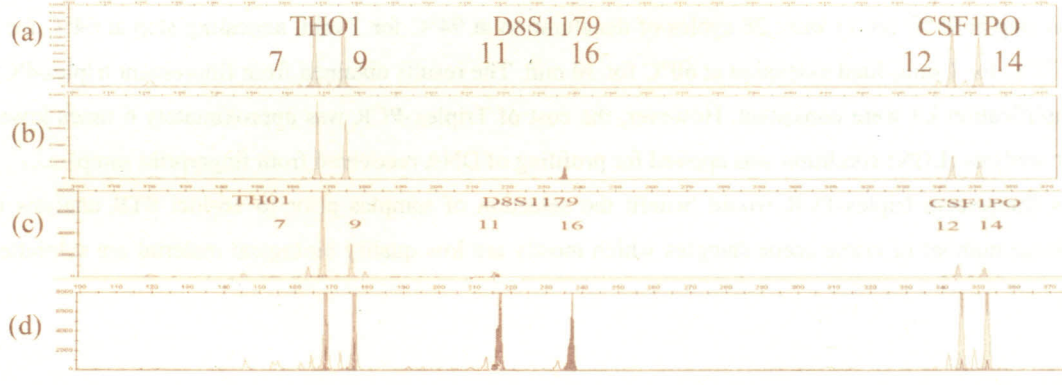


Figure 7 Electropherograms showing triplex PCR products amplified from DNA extracted from different sample types; one undusted print (figure 7a), 4 undusted prints (figure 7b), 4 fluorescent powder dusted prints (figure 7c), and buccal swab (figure 7d). Reference profile was *profile2*.

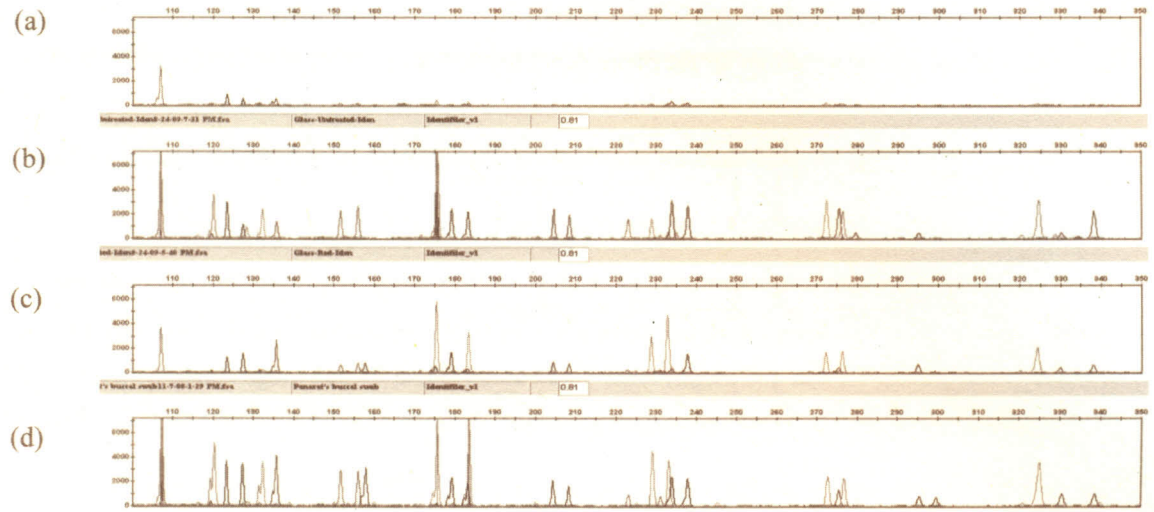


Figure 8 Electropherograms showing alleles of 16 STR loci amplified from DNA extracted from different sample types; one undusted print (figure 8a), 4 undusted prints (figure 8b), 4 fluorescent powder dusted prints (figure 8c), and buccal swab (figure 8d).

Estimating cost of amplification reaction

The cost of reagents used in a reaction was calculated. The cost estimated for fluorescent triplex-PCR reagents was 91.50 baht per reaction, while for Identifiler amplification kit was 607.23 baht. Therefore, cost of reagents for triplex PCR was approximately 6 times lower than the commercial kit.

Conclusion

The fluorescent triplex-PCR developed was proved to amplify DNA extract as low as 250 pg. The ratio of primer concentration of THO1:D8S1179:CSF1PO is 2.5:20:20 pmol, using thermocycling condition as following; initial denaturation step at 95°C for 11 min; 28 cycles of denaturation at 94°C for 1 min, annealing step at 64°C for 1 min, extension at 72°C for 1 min; final extension at 60°C for 30 min. The results obtained from fluorescent triplex-PCR and Identifiler amplification kit were consistent. However, the cost of Triplex-PCR was approximately 6 times lower. Low copy number analysis (LCN) condition was applied for profiling of DNA recovered from fingerprint samples.

This fluorescent triplex-PCR would benefit the selection of samples prior to 16-loci STR analysis in practice, when a large number of crime scene samples which mostly are low quality biological material are submitted for DNA analysis.

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