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"Only Cigarette Butt is Left, DNA Fingerprinting Traps the Theft"

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Abstract

The examination of saliva traces left on cigarette butts as evidences are complicated due to the availability of biological material in trace amounts and its rapid degradation due to extreme effects of environmental factors. This study is aimed to assess the DNA purity and quantify the amount of DNA preserved in saliva found on cigarette butts subjected to various temperatures and humidity. Isolation of cell material from biological traces on forensic evidence is often a serious challenge to solving forensic cases. Successful isolation of high-grade DNA from cell material even in critically low quantity could be achieved in examination of traces. The proper collecting and storage of the material is very important for successful DNA typing from saliva traces and epithelial cells from the lips and oral cavity. Meeting these conditions would increase the chances for successful DNA profiling of biological traces on evidence of an earlier date. In the presented forensic cases the opportunity for solving the crime was given by the vices of the suspects. In this case one cigarette butt found at scene of crime was the only evidence to detect the accused. DNA is extracted from salivary epithelial cells adheres to the cigarette butt and profiled successfully. DNA profiles of suspected accused and the cigarette butt are matched in the source.

Keywords: Cigarette Butt; Salivary Epithelial Cells; PCR; STR Profiling

Introduction

Cigarette butts are a common trace sample at crime scenes and obtaining DNA profiles from this evidence is an important capability in the forensic science repertoire. DNA extraction is a critical step in forensic analysis because quality of DNA directly affects the ability to obtain a good quality forensic profile. Cigarette butts are notably difficult samples in that they produce DNA that is contaminated with inhibitors of the polymerase chain reaction (PCR) [1]. The range of additives may lead to different levels of inhibition from different the brands, and to complicate matters, the brand is not always easily determined. Most methods in current usage solve the problem by adding a series of post-extraction steps to selectively remove inhibitors. These steps make the method complex, non-automatable, susceptible to contamination, and the many steps reduce DNA yields – a critical factor with trace samples [1,2].

Cigarette butts are one of the common carriers of saliva traces in forensic practice. Today, approximately 1.2 billion people worldwide smoke tobacco [3] and they smoke when they are nervous (*i.e.* when involved in a crime) [4,5]. The male and female smokers both are showing furious, annoyed rebellious impatient behavior [6]. Also in Finland about 10 town study was done by Marianna Virtanen.et.al. Where they studied the statistical of crime and the smoking behavior.Where they found that where there is strong smoking intensity and low average household income the the high local srime reate is high [7]. Cigarette butts found at crime scene may contain traces of saliva and attached mucosal epithelium cells from the lips of the smoker, which allows for DNA identification by profiling [8,9].

Saliva is evidence which can be encountered in forensic casework. Most probably the exhibits like toothpick, cigarette butt, bottle or postage stamp, all of which could contain saliva are most susceptible to damage by environmental factors like "Heat, Sunlight, Moisture, Bacteria, Mold. Direct sunlight and warmer conditions may also damage DNA [10].It has a high evidentiary value in identifying victims and suspects as well as exonerates an innocent individual [8]. A healthy adult produces 500 - 1500 mL of saliva in a day, at a rate of 0.5 mL/min. The quantity and quality of saliva produced, however, is influenced by several pathological and physiological conditions which includes, taste and smell stimulation, hereditary factors, hormonal status, and oral hygiene [11].

DNA samples recovered from a crime scene are frequently exposed to damaging environmental conditions such as light, heat and bacterial decomposition before they are collected for analysis. Hence, generating an evidentially valuable profile from these quality-compromised samples is a great Malaysian Journal of Forensic Sciences (2016) 7(1):10-16 11 challenge to the forensic scientist [12-13].

Materials and Methods

Materaials:

Reagents	Parameters
Forensic Buffer	1 ml Tris HCL-100ml 0.5ml EDTA Buffer -10ml 5M Nacl-10ml Make the volume up to 1000ml
Proteinase K	Appearance- Colourless solution in 50% glycerol, cont.20mM Tris.,1mM Cacl2,PH ca.7.4 Concentration 20mg solid/ml
Investigator kit	Buffer G2,Prot.K,Carrier RNA,
AmpFSTR Identifiler® PCR amplification Kit	Allelic Ladder,Ampli Taq Gold® DNA polymerase, Primers,
Hi-Di™ Formamide	CAS 75-12-7,CAS 60-00-4
Size Standard	GeneScan™-500,LIZ ™

Table 1

Instrument	Operarting Paramerers
Kits designed for this instrument	QIAGEN EZ1 Kits
Pipetting range	50-1000 µl
Protocols/main application on this instrument	Purification of DNA, mRNA, total RNA, and viral RNA and DNA
Samples per run; throughput	6 samples per run
Technical data of the instrument	Weight 48 kg, 100–240 V AC, 50–60 Hz
Technology	Magnetic-particle technology

Table 2: EZ1 Automate DNA Extraction System Parameters

Instrument	Operarting Paramerers
Capacity	96 wellx0.2ml PCR tubes/one 96 well plate
Heating/cooling	Peltier based
Capable of testing temperatures	Denaturation, Annealing & Extension steps
Block ramp rate	5.0 °C/Sec.
Sample ramp rate	4.4°C/S
Temperature range	4-99°C/S
Temperature accuracy	±0.2 °C
Customized programming	Allows a maximum of 20 steps and 99 cycles
Display	LCD touch screen, about 8.5 in

Table 3: PCR Thermal Cycler Machine

Instrument	Operarting Paramerers
Fragment Size(bp)	500bp
No. of Markers	16
Polymer	POP4
Detector	CCD
Oven Temp	60 °C
Column Size	36cm
Software	GeneMapper®

Table 4: Genetic Analyser-3130

Method: 1) Extraction of DNA from Cigarette Butt

Carefully clean the platform of workstation of laminar flow with ethyl alcohol

Take approx 0.5mm sample piece and cut respective sample into small pieces and take into 2ml micro-centrifuge sample tube

To this Add 400µl Forensic Buffer +25 µl Protease K+40 µl 1mM DTT

Vertex and spin

Incubate at 56 ˚C overnight on Thermo-Shaker

To the next day, transfer the sample into the EZ1 micro-centrifuge sample tube

Set the micro-centrifuge sample tube in the EZ1 Advanced (Quiagen) magnetic bead based liquid handling system for automate DNA Isolation

Store the extracted DNA at -20 ˚C

Extraction of DNA from Blood:-

Take 5 µl blood sample+ 97.5 µl ATL buffer+100µl AL buffer+10 µl protease K in micro-centrifuge Tube

Vortex and spin for 1 min's

Incubate at 56 ºC for 10 min's on Thermo-shaker

To this add 50 µl 99% ethanol then vortex and spin

Wait for 5 min's and transfer the supernatant into the micro-kit column

Centrifuge at 80000rpm for 1 min's and discard the filtrate

Wash the column by adding 500 µl washing solution

Centrifuge the column at 8000rpm for 2min's and discard the filtrate

Repeat the procedure

Spin the empty column at 10000rpm for 3min's

Transfer the column in new Elution Tube

Finally add 100 µl Elution Buffer to the column, centrifuge at 14000rpm for 2 min's

Remove the column and store the DNA for at -20˚C

Polymerase Chain Reaction:- (Refer Figure 1)

REAGENTS VOLUME

PCR reaction Mix -10.5 ul
Primer set- 5.25 ul
Sample input -10 ul

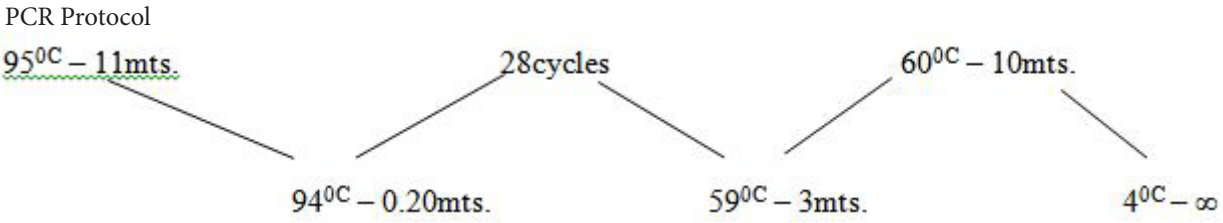

Figure 1

Genotyping: -

STR genotyping is detected and analysed on 3130 Genetic Analyser (Applied Biosystems) instrument by capillary electrophoresis of single stranded amplified DNA fragments includes following steps.

Sample Preparation for Injection
Standard Mix :
1µL PCR product
0.5 µL Size standard (for GeneScan500-LIZ®)
10-20 µLHi-Di™ formamide (PN 4311320)

Denaturation of PCR product. (90 –95 °C, 2 –5 min)

⇩

Immediately on ice or cool to 4 °C in thermal cycler Load the mixture in auto sampler on instrument for injection.

⇩

Electrophoresis is done through fine glass capillary filled with polymeric gel.
(During capillary electrophoresis, the extension products of the PCR reaction (and any other negatively charged molecules such as salt or unincorporated primers and nucleotides) enter the capillary as a result of electro kinetic injection. The extension products are separated by size based on their total charge)

⇩

DNA fragments travel through capillary according to their size & reach the window which coincides with the Laser device in the instrument.
(Shortly before reaching the positive electrode, the fluorescently labeled DNA fragments, separated by size, move across the path of a laser beam. The laser beam causes the dyes attached to the fragments to fluoresce.)

⇩

Laser excites the fluorescently labelled DNA fragments. (The laser beam causes the dyes attached to the fragments to fluoresce.)

⇩

CCD Camera behind the window records the excitation peaks. (The dye signals are separated by a diffraction system, and a CCD camera detects the fluorescence.)

⇩

Excitation peaks for 16 different loci are obtained.(Because each dye emits light at a different wavelength when excited by the laser, all colors, and therefore loci, can be detected and distinguished in one capillary injection.)

⇩

For each set of sample standard allelic ladder is run.

DATA COLLECTION software collects the data of these excitation peaks. (The fluorescence signal is converted into digital data, and then the data is stored in a file format compatible with an analysis software application.)

Results

The DNA extracted from saliva detected on cigarette butt found at scene of crime and blood sample of suspect was typed at 15 STR LOCI and gender specific Amelogenin locus using PCR Amplification technique.

Table No.5 shows that the DNA profile obtained from saliva detected on cigarette butt found at scene of crime and DNA profile obtained from blood sample of suspect is identical and from one and same source of male origin. The DNA profile of saliva detected on cigarette butt found at scene of crime and DNA profile of blood sample of suspect are matched with the maternal and paternal alleles in the source.

STR LOCUS	GENOTYPE	
	DNA Blood of Suspect	DNA Cigarette butt found scene of crime
D8S1179	10,10	10,10
D21S11	31,31.2	31,31.2
D7S820	9,10	9,10
CSF1PO	11,13	11,13
D3S1358	19,20	19,20
THO1	6,8	6,8
D13S317	8,11	8,11
D16S539	11,12	11,12
D2S1338	23,25	23,25
D19S433	15,15.2	15,15.2
vWA	17,18	17,18
TPOX	9,11	9,11
D18S51	13,16	13,16
AMELOGENIN	X,Y	X,Y
D5S818	12,13	12,13
FGA	21,24	21,24

Table 5: The results of DNA typing

Discussion

In this case a complainant, who worked as senior clerk in election commission office, filed a complaint. According to this complaint, material records and memory chips of voting machines of 2012 election were sealed in small boxes and all these boxes were kept in locked room. It was very well confirmed that all this election related material was kept in completely locked room, but it was found that this locked room was break open by pushing the door very strongly by unknown person. This unknown person broke the sealed boxes and succeeds in taking very important election material. He also removed paper seal of main hall door where election boxes were kept. By doing all this, the unknown person pretended it is a scenario of robbery to fool the people and police. During investigation, police found only cigarette butts at scene of crime. So police send these cigarette butts along with the blood sample of suspect for DNA fingerprinting.

DNA present in body fluids such as blood, semen, saliva, sweat, nasal secretion etc. get preserved after drying on any object at 37 ^{0}C forever. Due to stability and sensitivity of DNA it is possible to extract DNA from various materials which are found at scene of crime like cigarette butts, chewing gum , glass bottles and any unclaimed articles used by the used by accused . This is a DNA analysis from only cigarette butts as evidence collected from scene of crime. DNA profile of salivary epithelial cells adheres to the cigarette butt matched with the DNA profile of suspect control blood sample. So we can say that, small evidence collected from scene of crime is also very much useful to trap the criminal, only because of the sensitivity and accuracy of DNA fingerprinting technique.

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References

1. Gunther S, Herold J, Patzelt D (1995) Extraction of high quality DNA from bloodstains using diatoms. Int J Legal Med 108: 154-6.

2. Watanabe Y, Takayama T, Hirata K, Yamada S, Nagai A, et al. (2003) DNA typing from cigarettebutts. Leg Med (Tokyo) 5: S177-9

3. Oleski K, Campbell C, Patel R, Welker J (2010) DNA Extraction for obtaining DNA from Cigarette Filter Paper Collected in Paris and East Lansing for PCR Amplification Detecting p53 Mutations. Retrieved on 12th February 2016 from Ace Learning Company. Inc website: 35.9.122.184/p53mutations.pdf

4. Axelrod A, Antinozzi G, (2007) The Complete Idiot's Guide to Forensics. New York: Alpha Books.

5. Dettmeyer R (2013) Forensic Medicine. Berlin: Springer-Verlag Berlin and Heidelberg GmbH & Co KG.

6. Journal Pharmacology Biochemistry: Dinnis et al in 2002.

7. Virtanen M, Kivimäki M, Kouvonen A, Elovainio M, Linna A, et al. (2007) Average household income, crime, and smoking behaviour in a local area: The Finnish 10-Town study. Soc Sci Med 64: 1904-13.

8. Yudianto A (2009) Effectiveness of Cigarette Butts as an Alternative Material for Forensic DNA Identification with Polymerase Chain Reaction (PCR) in Short Tandem Repeat (STR) Loci. Folia Medica Indonesiana 45: 112-4.

9. Linch CA, Prahlow JA (2008) Microscopical Examination of Particles on Smoked Cigarette Filters. Forensic Sci Med Pathol 4: 228-33.

10. Giese AC (1976) How Ultraviolet Radiation Affects the Cell. Living with Our Sun's Ultraviolet Rays 1976: 43-66.

11. Roberts KA,Johnson DJ (2012) Investigations on the Use of Sample Matrix to Stabilize Crime Scene Biological Samples for Optimized Analysis and Room Temperature Storage. Unpublished note, National Criminal Justice Reference Service, U.S.

12. Thacker CR, Oguzturun C, Ball KM, Court DS (2006) An Investigation into Methods to Produce Artificially Degraded DNA. International Congress Series 1288: 592-4.

13. Scientific working Group on DNA Analysis Method (SWGDAM) (200) short tandem repeats (STR) interpretation guidelines. Forensic Science Communication 2(3) Available on line at: http://www.fbl.gov/hq/lab/fsc/backissu/july2000/string.htm