Molecular Analysis of Touched DNA Samples Collected From Pistol Surfaces
التحليل الجزيئي لمسحةعينات الحامض النوويDNA المعزول من سطوح المسدس

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Abstract

Molecular analysis of touch DNA samples from pistol surfaces is important for crime scene investigation. In this study touched samples were collected from different parts of pistol. DNA extracted and quantified using different methods, then analyzed by mtDNA sequencing and STR profiling. The results showed that pistol surfaces can be analyzed by mtDNA sequencing but not full STR profiled. These results indicated the need for improving DNA extraction and STR profile kits for analysis low DNA amount.

Key words: Touch DNA,pistol , Short Tandem Repeat [STR], sequencing[mtDNA], forensic

الملخص

التحليل الجزيئي لمسحة عينات الحامض النووي DNA لسطوح المسدس مهمة في تشخيص مسرح الجريمة. في هذه الدراسة تم جمع مسحات من اجزاء مختلفة من سطح المسدس .كذلك تم استخلاص الحامض النووي كميا"بطرق مختلفة, وتحليل الحامض النووي بتقنية التسلسل المايتوكوندريا الحامض النووي mtDNA وكذلك تحليله بتقنية تتابع متسلسل قصير STR نمط. اظهرت النتائج امكانية تحليل سطوح المسدس بتقنية المايتوكوندريا الحامض النووي mtDNA ولكن بدون وجود نمط بتقنية تتابع متسلسل قصيرSTR. هذه النتائج تشير الى الحاجة لتحسين استخلاص الحامض النووي والكتات المستخدمة لتقنية التتابع المتسلسل قصيرSTR لتحليل الكميات القليلة من الحامض النووي.

الكلمات الدالة: الحامض النووي التلامسي، المسدس، تتابع متسلسل قصير، التسلسل المايتوكونريا، العدلي

Introduction

Touch DNA is the DNA transferred from people hands to touched surfaces [1]. Thousands of cells were separated from human skin daily [2], in addition an amount of DNA were found in sweat glands [3]. Analysis of touched surfaces for evidence is so important in fatal crimes.Many studies conducted to analyze STR profiles of pistol surfaces with variable results range from no to partial profiles [4,5,6]. The standard method for analysis of crime scene is STR profiling, but with low DNA amount mtDNA sequencing usually used [7]. In this study different parts of pistol were studies for the ability to get mtDNA sequence or STR profile using different DNA extraction methods.

Materials and methods

Sample collection

Four different regions bullet, grip, magazine, cushion of Pistol surfaces were used remenant DNA was cleaned with 10% bleach, followed by ethanol 70% and finally left it for drying to remove any component bleach and ethanol that may be left from the previous cleaning process.

Volunteers did not wash their hands for at least 2hours and were asked to touch different part of the pistol [bullet, handgrip,cushion,magazine] for 1,5,10, minute [8], touched samples were collected and extracted with four different DNA extraction methods organic methods [9], Chelex [10], direct method [11], and silica kit method [12]. Isolated DNA was quantified by three methods [Nanodrop spectrophotometer, Fluorometer and Real time PCR]. Twenty samples were selected form mtDNA HV2 [heperveriable region in mitochondrial DNA human] and subjected for sequencing, seven samples for STR profiling using powerplex 18D kit, and 3130 Genetic analyzer.

Results and discussion

In this study four different methods were used for DNA extraction, organic, Chelex, direct and silica kit method.

Three different methods were used for isolated DNA quantification, Nonodrop spectrophotometer, fluorometer, and real time PCR. Real time PCR method is more accurate and sensitive but more expensive than other two methods. Nanodrop spectrophotometer is less accurate than fluorometer but is cheaper.

The recommended DNA extraction method that yields better concentration and purity was organic method, and this may be due to the powerful cell lysis solution and long incubation time. Grip and cushion surfaces are rough and expected to contain more epithelial cells after touch and yield more DNA in comparison with bullet and magazine which have smooth surface and yield low DNA content. Table (1) shows the range of DNA amount collected from pistol touched surfaces.

Table (1): DNA concentration range extracted by different methods using spectrophotometer and fluorometer.

Extraction method	Nanodrop Spectrophotometer ng/µl	Fluorometerng/µl	Purity
Organic	21.6 - 270.7	0 - 0.85	1.3 - 1.9
Silica	3.4 - 8.1	0 - 0.79	1.2 – 1.8
Direct	2 – 88	0 - 0.8	1.1 – 1.4
Chelex	5 - 67.3	0 - 1.22	1 – 1.4

The concentration of DNA isolated from bullet before shooting was more than after shooting this is due to the high temperature resulted from explosion during firing which may destroy DNA from the weapon.

DNA concentration collected from touched surfaces [bullet, grip, magazine, cushion and extracted by organic method was ranged from 21.6 to 270.7ng/µl as determined by nanodrop spectrophotometer, while ranged from [0 to 0.85ng/ µl] by fluorometer. The results show that there is a difference between the two methods in quantification of touched DNA. Although spectrophotometer method is commonly used, it can be unreliable and inaccurate [13,14]. Ultra violet (UV) absorbance measurements are not selective and cannot distinguish DNA, RNA, or protein. Values are easily affected by other contaminants e.g., free nucleotides, salts, and organic compounds and variations in base composition [15,16]. In addition, the sensitivity of spectrophotometry is often inadequate, prohibiting quantitation of DNA and RNA at low concentrations .In light of these drawbacks, the use of fluorescent dyes to quantify nucleic acids became a common alternative [15,16]. Fluorescence-based quantitation is more sensitive and is often specific for the nucleic acid of interest.

In this work, HV2 region was amplified and sequenced using direct sequencing Sanger method. The PCR product was 422bp] as determined by agarose gel electrophoresis Figure (1).

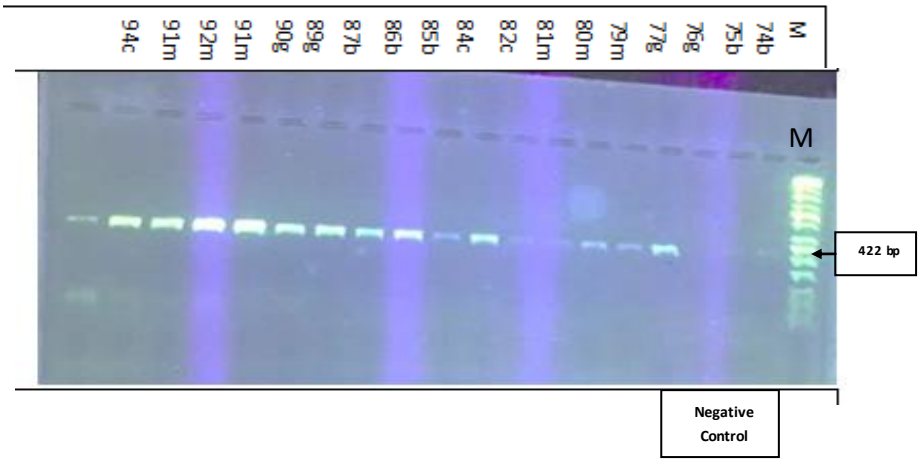

Fig. (1): Agarose gel electrophoresis of human mtDNA [HV2] PCR products. M: the DNA molecular weight marker 100bp Negative lane [0] Positive amplification of [422] for mtDNA HV2, 2٪ agarose concentration TBE buffer 1X, [60 volt/ cm for 2 hours].

Twenty mtDNA (HV2) samples extracted by the four DNA extraction methods were sequenced successfully.This is because human cells contain about 50 to several hundred mitochondrial DNA molecules [17]. The relative abundance of mitochondrial DNA has made it a powerful tool for forensic scientists [17,18].

The results showed that forward primers showed typical sequencing resultswith all extraction methods and surfaces Figure [2].

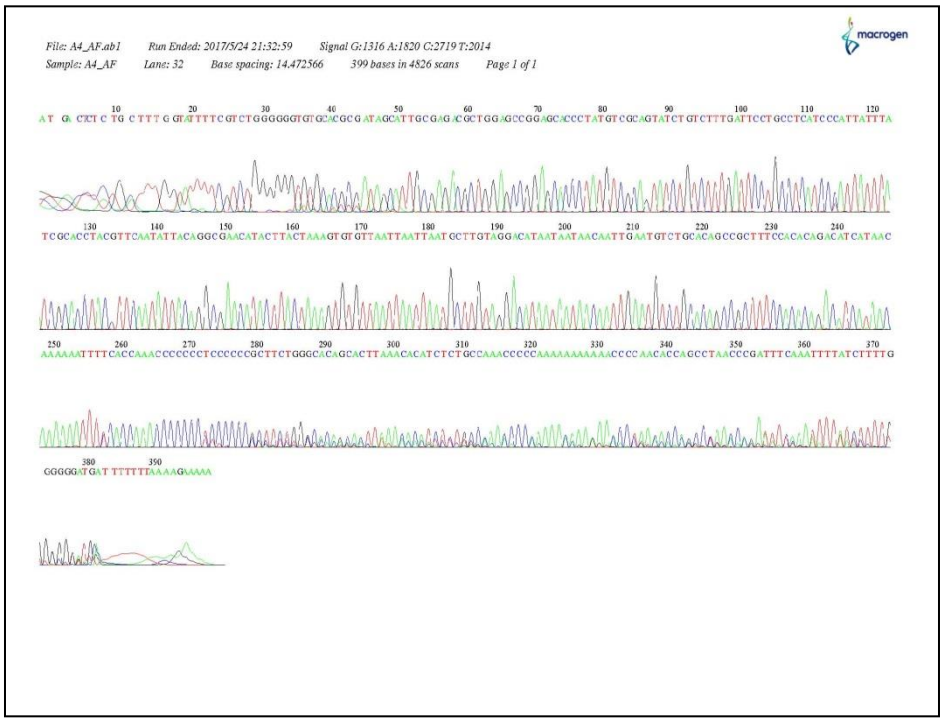

Fig. (2): Electropherogram of HV2 sequencing PCR product using forward primer. For all extraction methods used sequenceing HV2 PCR product using forward primer and reverse primer.

while reverse primers produced partial sequence for all except one sample with not optimal peaks. That mean the reverse primer should replaced with other optimised reverse primer.

These results indicate the suitability of mtDNA for analysis touched pistol surfaces in spite of the lower power of discrimination for mtDNA.

STR markers are used most frequently in current DNA testing because their length is short enough to allow amplification by PCR [19].

Figure (3) shows partial low profile peaks for the sample (83 cushion) extracted by silica method [female] due to low DNA concentration.

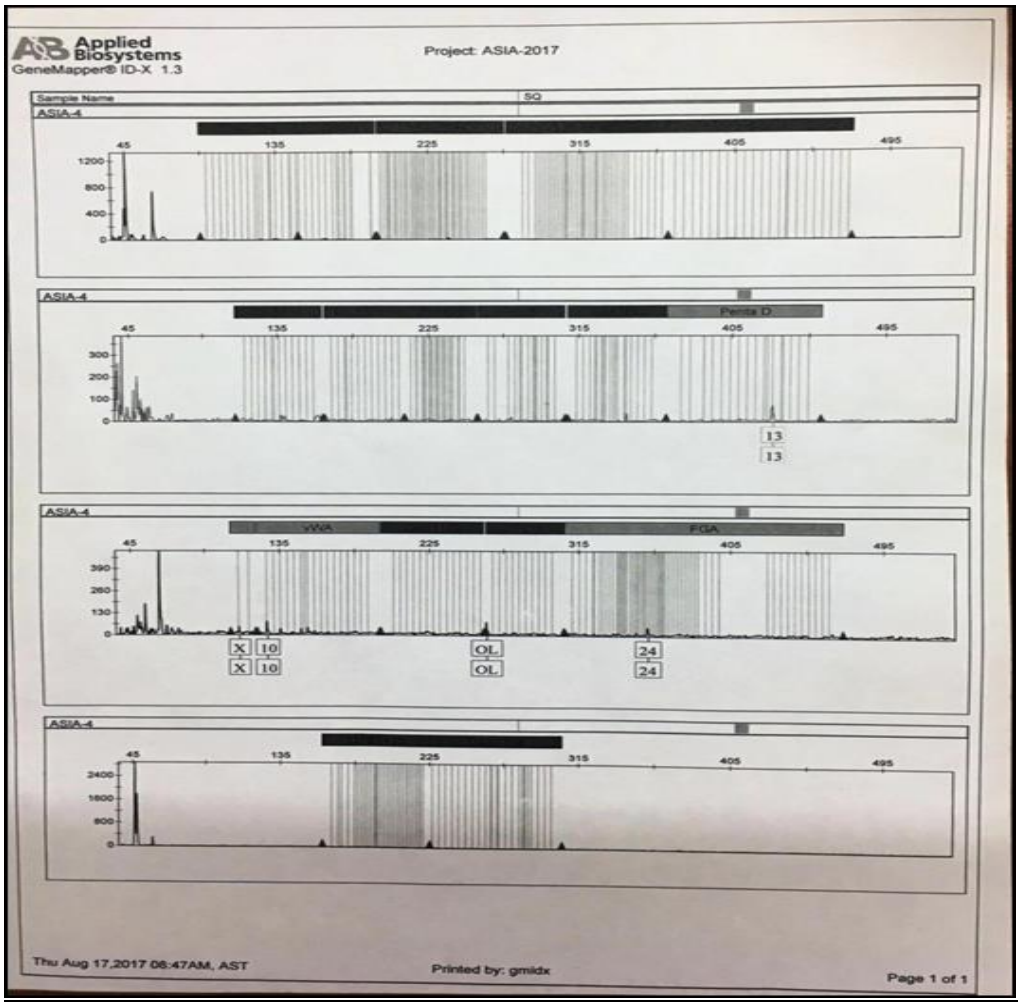

Fig. (3) Chromatogram of STR alleles using powerplex [18D] kit.

The results show after bullets after shooting the STR profile is either partial or no profile but before shooting the profile is partial. However, full mtDNA sequence was obtained after bullet shooting. This study indicate the importance of mtDNA sequencing for analysis low DNA samples and the need for improving STR kits for analysis such samples.

The importance of this study that most perpetrators use guns through their crimes. Many other studies used several techniques to improve the analysis of low amount DNA samples [20]. Methods can include making changes to the current STR practices such as: use of laser microdissection (LMD) to isolate single cells [21], increasing the quantity of the sample before PCR using whole genome amplification [WGA] [22,23].

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