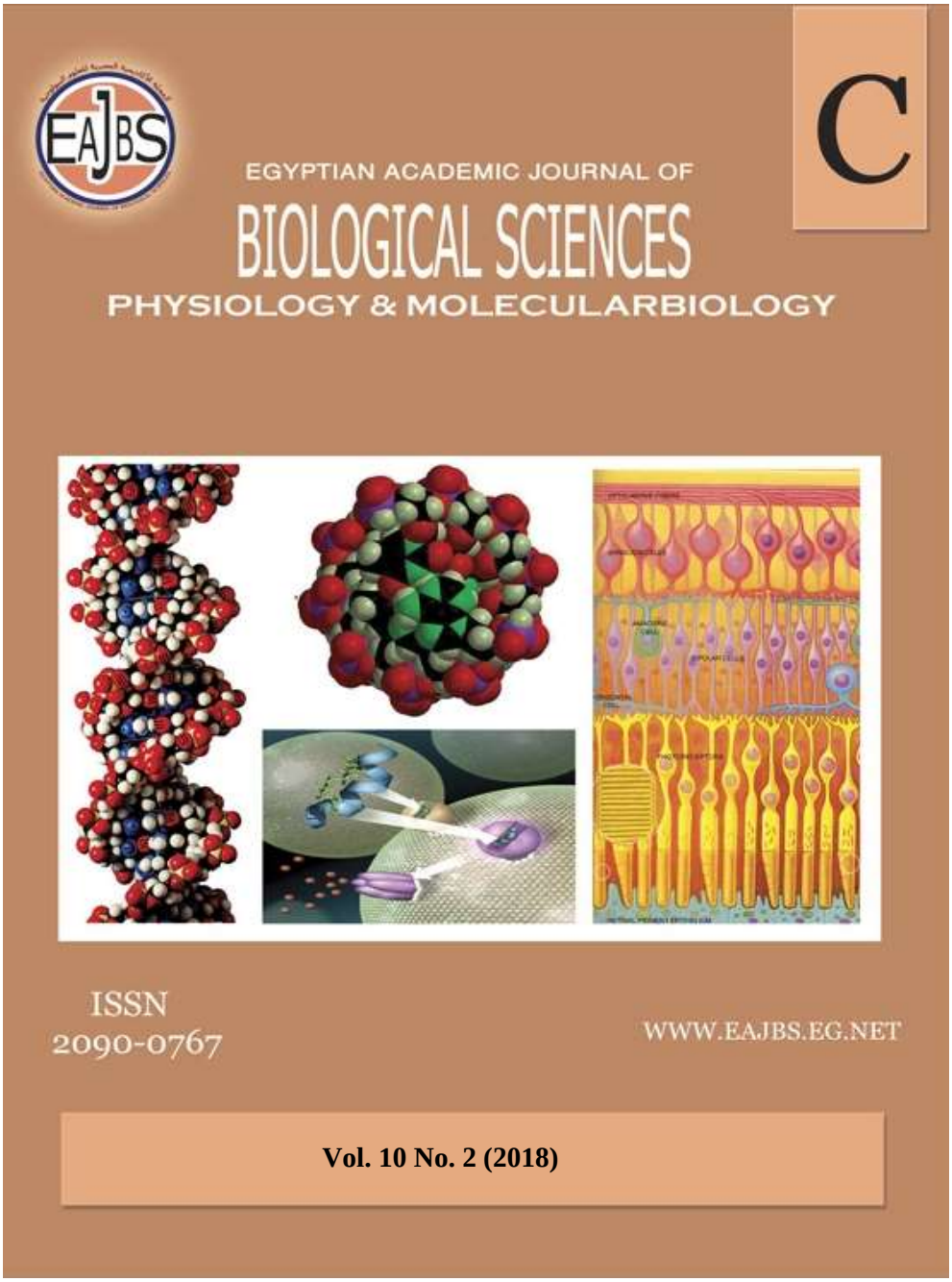

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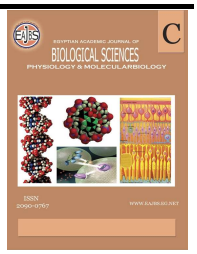

Direct PCR-Based Detection of Y Chromosome Strs Loci from Human Hair Samples

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Direct PCR, DNA profiles, hair waxes, Y-STRs.

ABSTRACT

Direct-PCR is not common for Y chromosome in forensic laboratories in Egypt. The current investigation was carried out to evaluate the priority of direct PCR technique in Y-STR test over routine extraction method. Results showed that direct PCR over hair samples obtained from five persons who do not use any Hair waxes recorded higher scale of peak heights than those subjected to a prior-PCR DNA extraction. In addition, much better results for direct PCR were obtained when one hair was subjected to direct PCR amplification than using three hairs. This technique may solve the problems of rapid and cost-reducing genotyping of forensic samples, as well as may improve the fluorescence detection of some genetic loci.

INTRODUCTION

DNA profiling and fingerprint analysis both have considerable distinctive potential that are utilized as a mean of human identification and as an integral part of a forensic laboratory's workflow (Templeton *et al*., 2017). DNA extraction is the first step in forensic analyses in order to obtain adequate amounts of DNA (Sambrook and Russell, 2001). DNA extraction purifies nucleic acid from cellular debris, endogenous proteins, and exogenous inhibitors that may interfere with enzyme activity during PCR amplification of STR loci. However, DNA extraction can result in an approximate loss of 20% to 90% of the initial template amount, dependent upon the method of extraction and accuracy of the quantification method (Van Oorschot *et al*., 2003; Balogh *et al*., 2003; and Ottens *et al*., 2013b). Factors that affect DNA extraction efficiency include the number of tube changes, the number of washing steps, and the capacity of DNA to absorb/irreversibly bind to plastic consumables (Gaillard and Strauss, 1998) and extraction matrices (Schiffner *et al*., 2005). The evolved DNA is then subjected to PCR and electrophoresis. Unfortunately, this process causes a significant loss in sample DNA ranged from 20-70% (Van Oorschot *et al*., 2003) and has the potential to introduce extraneous DNA

into the reaction (Butler, 2005). The necessity of finding improved methods to generate high-quality DNA profiles from samples that yield low amounts of DNA is of considerable interest to forensic DNA laboratories. DNA Direct polymerase chain reaction (PCR) amplification, a sample processing method in which an evidence or substrate punch is added directly to an amplification reaction without prior extraction or quantification, may improve the generation of genotyping data from such samples. Direct PCR has been widely used in microbiology since the early 1990s, where it is more commonly known as Colony PCR. Colonies of bacteria or yeast are directly amplified using specific primers as a rapid test to determine the success of cloning, or for the rapid detection of infections (Saris *et al*., 1990; Aufauvre-Brown *et al*., 1993; and Tjhie *et al*., 1994). Direct PCR reduces loss of DNA because it does not involve sample transfer or sample purification (Swaran and Welch, 2012). Since direct PCR involves less handling, introduction of errors and contamination can be reduced. Due to the nature of direct PCR, a sample is unable to be quantified prior to amplification. The extraction step is bypassed and therefore quantification is not possible. This increases the sensitivity of PCR, highlighting its benefit for latent DNA samples (Ottens *et al*., 2013a). For all mentioned above, the present study was conducted to detect the most potent amplification methodology; with or without extraction step. Results compared on the basis of difference in peak heights for the generated PCR profiles, between samples applied to the routine DNA extraction method and the same ones directly subjected to PCR.

MATERIALS AND METHODS

Sample collection and processing:

The current study was performed on five volunteers who donated their hairs. One and three hairs were taken from left and right side of the head of each person who doesn't use any hair waxes. Collected samples were exposed to two types of tests; routine extraction method and direct PCR method.

DNA extraction via Biorobot EZ1 equipment:

Extraction step was carried out by using Biorobot EZ1 equipment and Qiagen kits EZ1 DNA Investigator Kit (cat. no. 952034) according to Biorobot EZ1technical manual to obtain highly purified genomic DNA.

Amplification of certain regions on Y chromosome via PCR:

PCR amplification was carried out using PowerPlex®Y23 System (Promega) in a final volume of 13 µL for all samples according to (Promega Corporation Technical Manual # DC2305 and DC2320). PowerPlex®Y23 System depends on amplification of 23 Y chromosome loci with high diversity (Jain *et al*., 2016). Gene Amp PCR System 9700 thermal cycler (AB/LT/Thermo) was used for amplification, as described in the PPY23 Technical Manual (PP Y23 System Technical Manual, 7/12, (available at: http:// www.promega.com/resources/ protocols/technicalmanuals/101/ powerplex-y23-system-protocol/), with consideration of using half the reaction volume of the recommended protocol. Each amplification reaction contained 2.5 µl of PP Y23 Master Mix and 1.25 µl of PP Y23 Primer Pair Mix, 0.5 µl of DNA with up to 7.75 µl of distilled water.

Samples were initialized for loading in 3500 genetic analyzer through adding 1 µl of amplified sample or allelic ladder to 10 µl of Hi-Di Formamide (AB/LT/Thermo) and 0.5 µl of ILS provided with PPY23 system. GeneMapper ID (forensic data analysis and expert data software) v1.5 software (Applied Biosystems, UK) was used to analyze the fragment size for all markers

with a 50 relative fluorescence units (RFU) analytical threshold (Jain *et al*., 2016). Negative control was amplified with each batch of samples. All samples were stored at 4 ºC until use.

Statistical data analysis:

Data analysis showed as electropherograms where each locus has one peak except two loci that may have one or two peaks that termed alleles. The means of all loci were tested for significance by the multiple way analysis of variance (MANOVA) using SPSS statistics 15.0 release 15.0.0 software. Results were considered significant at $P \leq 0.05$.

All procedures of sampling followed the Ethics Regulations issued by Resolution of the Egyptian Minister of Health Population No. 238/2003, Articles 52-61 and guidelines from the 4th Meeting of EC International Dialogue on Bioethics (European group on Ethics on Science and New Technologies to the European Commission, Copenhagen, Denmark 19 June 2012). All the collected hairs contained the full hair structure (including the hair root).

RESULTS

Results presented in table (1) and illustrated in figures (1- 4) showing the data of Y chromosome profiles of both one and three hairs samples for five males who exposed to two technical methods; routine extracted method prior to entering samples to PCR, and Direct PCR technique. All amplification products were separated on an Applied Biosystems®3500 Genetic Analyzer using a 3kV, 5-second injection as described in the PowerPlex®Y23 System Technical Manual TMD035. Results presented as mean of peak heights for all 23 loci involved in Y chromosome profile. Obtained results showed significant difference considering both methods at ($P < 0.05$).

Lengths of the peaks are a strong indicator of the direct PCR method dexterity on the routine extraction method:

One hair samples that exposed to direct PCR technique showed preference in the scale of peak heights of most loci than the same samples those were extracted prior to exposure to the PCR, as shown in figure (1).

Differences between routine extraction method and direct PCR method through the lengths of the peaks in Y chromosome kit.

A significantly high scale of peak height in the studied 18 loci was observed in case of the direct PCR amplification of one hair samples compared to the routine extraction method (Fig. 2) (Table 1).

Lengths of the peaks were different when three hairs were loaded and compared by the two methods.

When the comparison between three hair samples in the two methods occurred, the scale of peak heights for the sample loaded directly in PCR be less than the same one that exposed to the routine extracted method except for three loci (DYS481, DYS437 and DYS391) that gave values 1000; 366 and 350 more than values in routine extracted method that were 630, 350 and 300 rfu respectively, as shown in figure (3) and table (1).

Significance differences between the two methods for three hairs samples.

Figure (4) demonstrated the significant differences between hair samples that loaded directly in the PCR and the same samples those were exposed to extraction step prior to PCR. There was a significantly high scale of peak heights in locus DYS481 for direct PCR sample than the same result in routine extracted method.

Table (1): showing the mean of peak heights for all loci involved in the PowerPlex®Y23 kit of both one and three hairs samples, the standard deviation errors. Data presented as mean ± standard error of means, (P <0.05) and Eta that represented the coefficient of spacing between samples involved in the test

Loci	Peak height (Mean ± S. E.)			
	Extracted sample PCR		Direct-PCR	
	One hair	3 hairs	One hair	3 hairs
DYS19	503.3±31.8	450.0±28.8	1016.6±44.1	283.3±16.6
DYS385 a	970.0±15.3	566.6±33.3	1283.3±101.4	326.6±37.1
DYS385 b	326.6±44.1	583.3±72.6	1200.0±57.7	583.3±44.1
DYS389I	433.3±16.6	383.3±44.1	1150.0±28.8	300.0±57.7
DYS389II	416.6±44.1	400.0±28.8	833.3±16.6	276.6±14.5
DYS390	716.6±44.1	616.6±44.1	2200.0±57.7	500.0±28.8
DYS391	133.3±33.3	366.6±33.3	3216.6±116.6	366.6±16.6
DYS392	633.3±16.6	466.6±16.6	1133.3±66.6	333.3±33.3
DYS393	1166.6±88.2	1066.6±44.1	5300.0±57.7	866.6±16.6
DYS437	433.3±33.3	350.0±50.0	2150.0±28.8	366.6±33.3
DYS438	500.0±28.8	566.6±33.3	1383.3±72.6	250.0±28.8
DYS439	983.3±16.6	716.6±60.1	750.0±28.8	316.6±44.1
DYS448	776.6±14.5	816.6±44.1	2066.6±66.6	500.0±57.7
DYS456	883.3±33.3	610.0±10.0	3066.6±88.2	516.6±44.1
DYS458	1116.6±44.1	1116.6±44.1	6583.3±220.5	966.6±16.6
DYS481	750.0±28.8	576.6±26.6	3366.6±66.6	1016.6±72.6
DYS533	1550.0±28.8	783.3±16.6	2816.6±16.6	516.6±44.1
DYS549	1083.3±44.1	716.6±44.1	716.6±44.1	466.6±33.3
DYS570	1900.0±57.7	1016.6±44.1	1966.6±33.3	500.0±28.8
DYS576	616.6±44.1	1066.6±33.3	1766.6±44.1	416.6±44.1
DYS635	1306.6±63.6	566.6±33.3	1266.6±60.1	300.0±28.8
DYS643	466.6±16.6	466.6±33.3	1400.0±50.0	283.3±44.1
Y-GATA-H	1483.3±72.6	733.3±33.3	1266.6±33.3	383.3±44.1
F	104.2	35.184	374.3	30.9
P-value	0.00	0.00	0.00	0.00
Eta	0.990	0.972	0.997	0.968
Eta^2	0.980	0.944	0.994	0.937

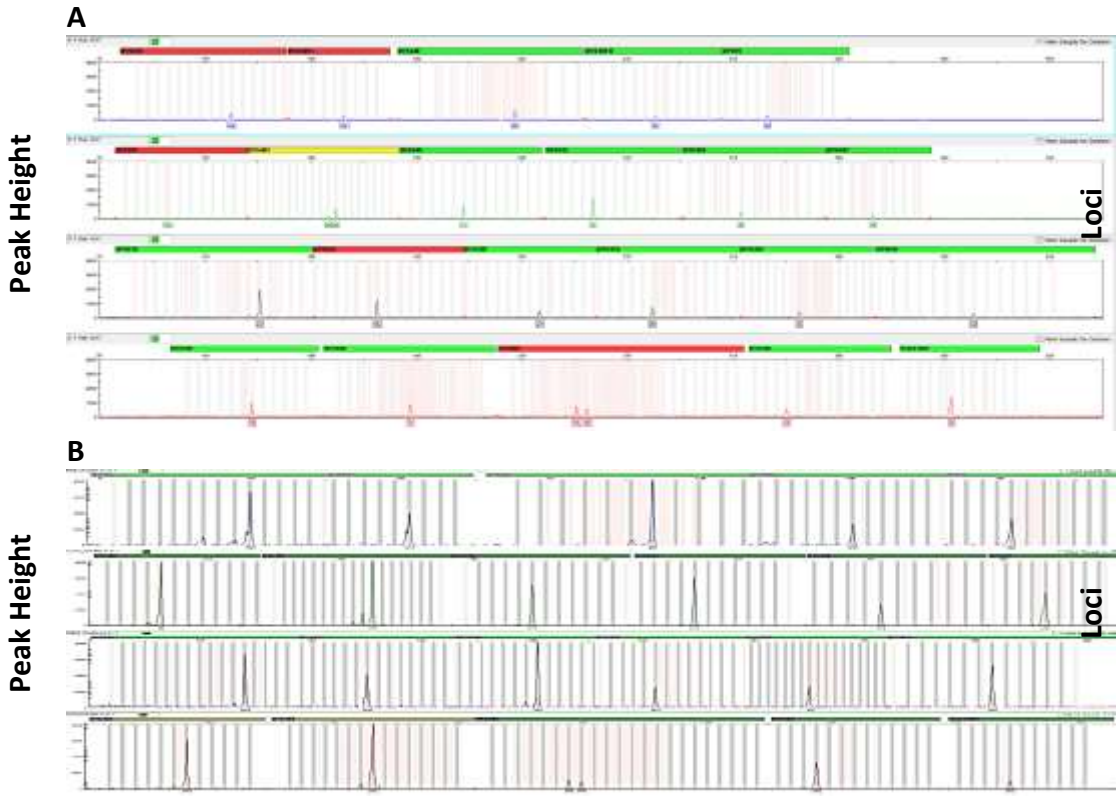

Fig.1: showing a comparison of the electropherogram of all dyes for the scale of peak heights between genotype of one hair sample for an individual; (A): one hair sample extracted by routine method, (B): one hair sample loaded directly in PCR reaction

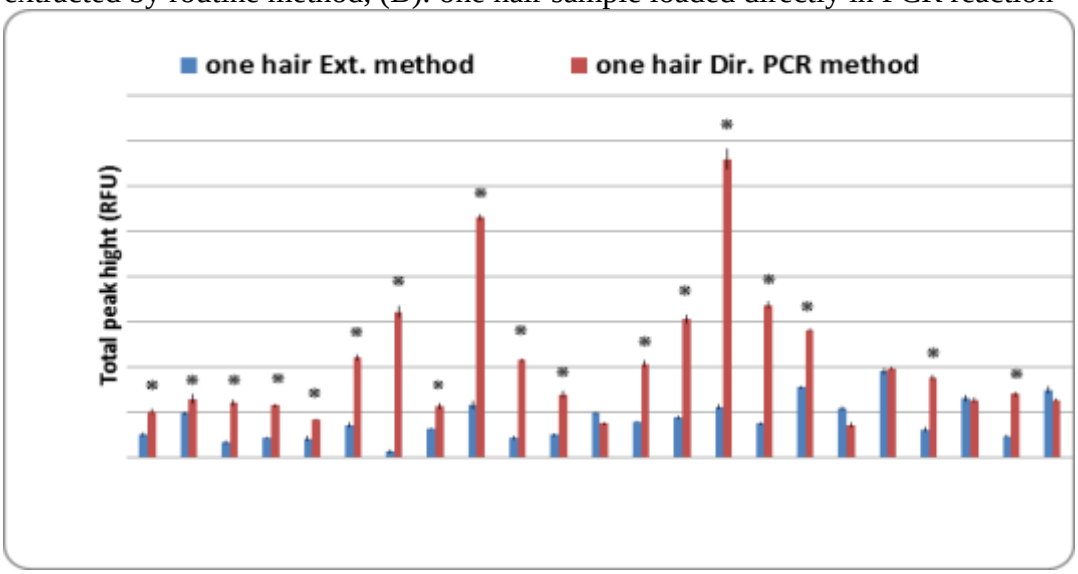

Fig.2: Chart showing a comparison between the mean of peak heights for all alleles of 23 loci in the Y chromosome of one hair samples when loaded directly in PCR reaction and when applied in routine extraction method. Data presented as mean ± standard error of means

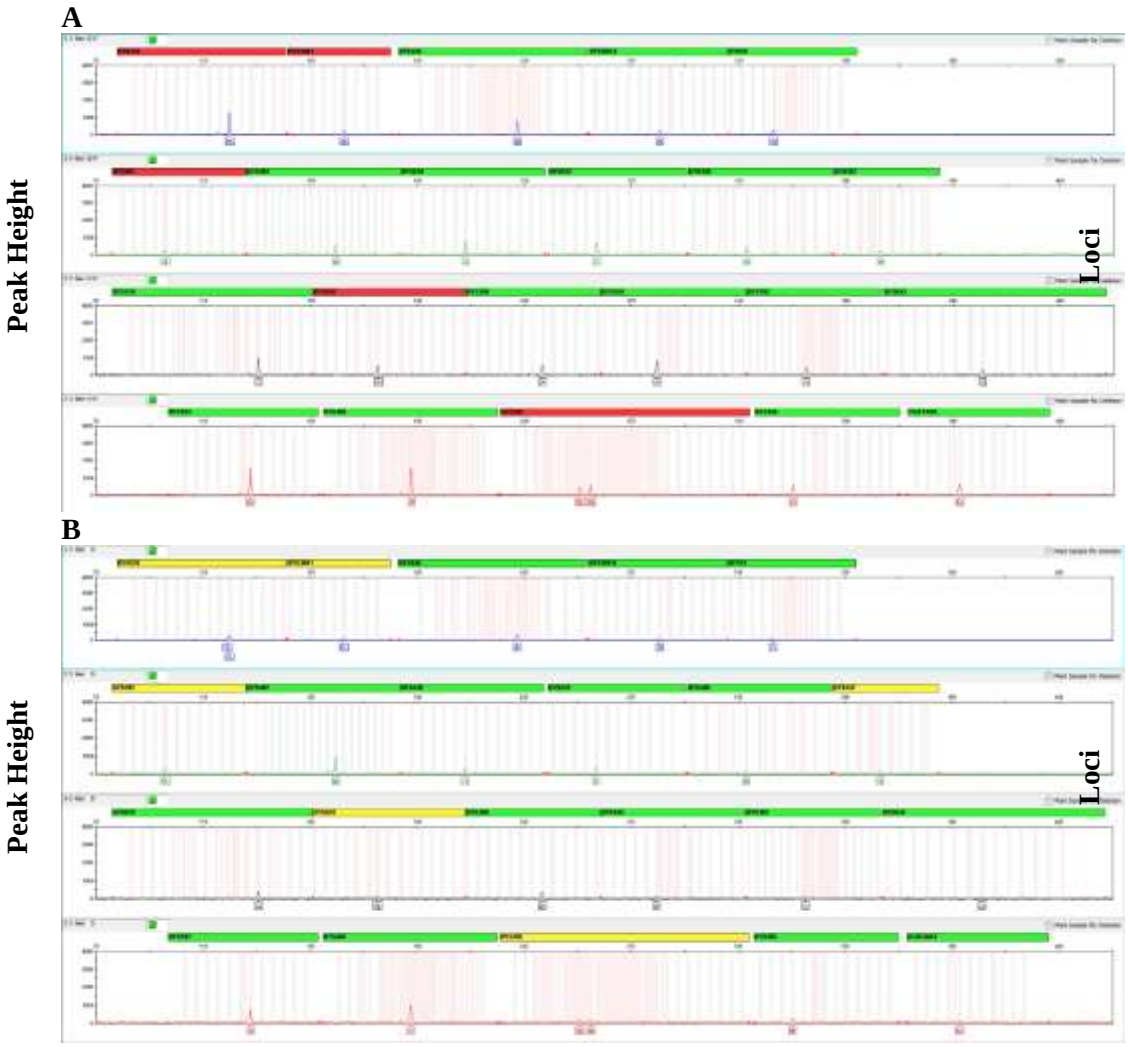

Fig.3: showing a comparison of the electropherogram for all dyes for the scale of peak heights between genotype of three hairs sample for an individual; (A): three hairs sample extracted by routine method, (B): three hairs sample loaded directly in PCR reaction

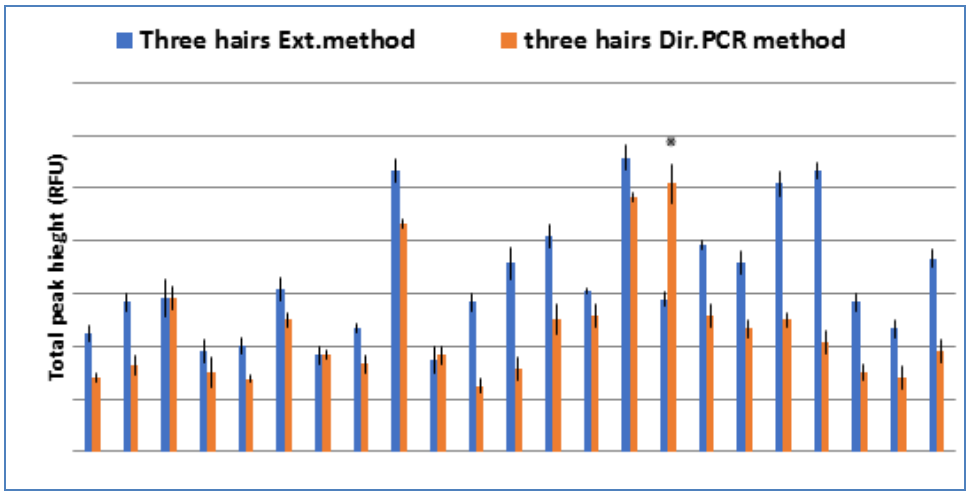

Fig.4: Chart showing a comparison between the mean of peak heights for all alleles of 23 loci in the Y chromosome of three hairs samples when loaded directly in PCR reaction and when applied in routine extraction method. Data presented as mean ± standard error of means

DISCUSSION

In the current study, obtained results showed clearly the vantage of using the direct PCR amplification technique over the traditional DNA extraction method when applied on one hair samples. Furthermore, the direct technique accorded distinguished results in the case of three hairs sample compared to the routine DNA extraction method, especially for individuals who don't use any hair waxes. In 2008, ***Bu et al.*** mentioned that direct PCR technique is considered of valuable use of low cost, simple process, time-saving operation and less cross-contamination. The whole process can be completed within two hours. Thus, the efficiency of clinical detection was improved greatly through PCR amplification that was directly carried out from whole blood without any DNA extraction step. In addition, Dohwan *et al.* (2016) showed that the Direct PCR reaction using human whole blood eliminates tedious DNA extraction and purification steps, and the use of the slidable plate enables fluid control without complicated microvalve systems.

Our results agreed with Templeton *et al*. (2015) who proved that direct PCR can be carried on scarce samples and significantly improved the profiling results from low-level DNA samples by increasing the yield of PCR product, negating the need for increased PCR cycle number. Direct PCR has the ability to effectively amplify low quantities of control DNA recovered from brass, glass, and plastic substrates. Further, obtained results coincided with Ottens *et al*. (2013) who succeeded to perform direct PCR technique on anagen hairs.

In conclusion, it is obvious that using direct PCR amplification technique can be favored over routine DNA extraction method and this is evident through the significant difference obtained in the scale of peak heights. These results are of considerable importance for forensic labs, as direct PCR saves sample processing time, reduces at least a quarter of the samples processing costs, and also enhance the detection efficiency for some of studied loci. Accordingly, we recommend the use of direct PCR technique in crime labs, as is always the condition in forensic laboratories.

Abbreviations:

RFUs: Relative fluorescence units

STR: Short tandem repeat

MANOVA: Multiple way analysis of variance

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ARABIC SUMMERY

تحديد تفاعل البلمرة المتسلسل المباشر للمواقع الجينية الخاصة بالتتابعات القصيرة المتكررة لكروموسوم الذكورة من عينات شعر آدمية

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لا يعتبر تفاعل البلمرة المتسلسل المباشر شائعًا في كروموسوم الذكورة في المعامل الجنائية فى مصر. تم إجراء الفحص الحالي لتقييم أولوية تقنية تفاعل البلمرة المتسلسل المباشر في اختبار (تتابعات متكرره قصيره لكروموسوم الذكورة) على طريقة الاستخلاص بالطريقة التقليدية. أظهرت النتائج أن تفاعل البلمرة المتسلسل المباشر على عينات الشعر التي تم الحصول عليها من خمسة أشخاص لا يستخدمون أي من مستحضرات التجميل الخاصة بالشعر، أعطت نتائج مرتفعة لأطوال القمم بالمواقع الجينية عن تلك التي خضعت لاستخلاص الحمض النووي قبل إدخالها إلى تفاعل البلمرة المتسلسل. بالإضافة إلى ذلك، تم الحصول على نتائج أفضل بكثير لتفاعل البلمرة المتسلسل المباشر عند تعرض شعره واحده لتفاعل البلمرة المتسلسل المباشر عن استخدام ثلاثة شعرات. هذه التقنية قد تحل بعض المشاكل التى تواجه مثل هذا العمل من ناحية الإسراع فى الوقت للحصول على نتيجة البصمة الوراثية، والحد من التكاليف للعينات، وكذلك قد تحسن فى جودة النتائج لبعض المواقع الوراثية.