IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 13, Issue 3 Ver. II (May. – June. 2018), PP 80-87
www.iosrjournals.org

Testing New PCR Additives for Enhancement of Direct PCR – Based Detection of Y chromosome Stars Loci (STRs loci) from Human Hair Samples

Hassab El- Nabi, S. E.[1]; Elroby, A. M.[2]
1 *Genetic Engineering and Molecular Biology Division, Department of Zoology, Faculty of Science, Menoufia University, Shebin El- Kom, Menoufia, Egypt*
2 General Authority of Forensic Evidence, Police Academy, Cairo, Egypt.
Corresponding Author: Hassab El- Nabi, S. E

Abstract: *In order to evaluate the effect of adding weak hair detergent on PCR buffer for further direct PCR technique, current investigation was carried out. Hair samples were collected from five different individuals and were subjected to direct PCR technique. Two types of commercial detergents and a physical treatment (gentle scratching of outer layer of hair follicle) were added to the PCR buffer. Results showed that Direct PCR over hair samples obtained from five persons who commonly use hair waxes recorded negative results. When the detergents and the physical treatment were applied, the scale of peak heights in all loci was significantly increased. In addition, results showed that soap foam detergent considered the best treatment. Much better results for direct PCR were obtained after treating three hairs by these methods, than using only one hair. These treatments may solve the problems of rapid and cost-reducing genotyping of forensic samples as well as improving the detection efficiency for some loci and also less contaminated DNA templates, especially since most of the convicted suspects are known to use hair waxes.*

Key words: *Direct PCR, DNA profiles, Forensics, detergents, hair.*

Date of Submission: 24-05-2018 Date of acceptance: 08-06-2018

I. Introduction

In order to obtain adequate amounts of DNA for downstream applications such as PCR, cloning, and DNA sequencing, DNA extraction is the first step in many molecular biology experiments (**Sambrook and Russel, 2001**). Extraction process impose cells to liberate DNA material via physical disturbance and/or chemical processing, which is then followed by a clean-up procedure in which unwanted cellular components are separated from the DNA (**Hajibabaei et al., 2005**). During sample extraction, DNA may be lost due to the methods used, which can affect the quality of the DNA profile obtained (**Sawran and Welch, 2012**). Direct amplification has gained increasing interest over the last few years. Newer, more robust amplification kits claim to perform direct amplification better and faster than kits previously available in the forensic science community. However, most of the commercially available amplification kits require pre-treatment of the body fluids with buffers or washing reagents prior to amplification (**Hall and Roy, 2014**). In 2010, **Shokralla *et al*.** hypothesized that a small amount of DNA oozes from the tissue into the preservation solution (usually ethanol), and that this DNA was amplifiable using a standard PCR protocol. They considered the preservative ethanol could be used as a source of genetic material for non-invasive analyses or when DNA analyses are required for specimens that have been consumed in prior experiments. They also succeeded to perform their hypothesis and performed amplification to DNA of the agave butterfly larvae, Hypopta agavis that presented in mescal (liquor). A primary advantage of direct amplification without purification of DNA is the reduced analysis time and higher throughput of databank samples (**Hall and Roy, 2014**). Current methods for detection of short tandem repeat (STR) profiles from reference samples can employ direct polymerase chain reaction (PCR) amplification of body fluids bound to swabs, FTA® and/or non-FTA® (fast technology for analysis) substrates. Most collection media for storing dried body fluid samples contain cell-lysing chemicals to preserve DNA within a sample that may contain PCR inhibitors (**Burgoyne, 1994**). Concerning human samples, it is currently limited to amplifying blood and buccal stained FTA® cards using specially designed multiplex kits in forensic DNA. These multiplex kits have improved buffer-polymerase systems which are more tolerant to inhibitors present on FTA® samples (**Swaran, 2014**). Furthermore, PPY23 System is configured with the majority of the highly discriminating loci with smaller amplicon sizes. Due to smaller amplicon sizes even, trace amounts of DNA can be amplified and the discrimination potential of partial profiles can be maximized (**Thompson *et al*., 2013**). The PPY23 System provides all the necessary materials to amplify the Y-STR regions of human genomic DNA (**Jain *et al*., 2016**).

In this study, we report a new method for Y-STR detection from hair samples picked from five different individuals using direct PCR after washing samples with weak detergents. This method kept the desired goal of saving cost and time required for obtaining DNA profile as well as having high quality of these DNA profiles. The results were compared on the basis of the scale of peak heights for the generated PCR profiles, between the hair samples that applied directly to the PCR only and the same samples that exposed to washing detergents before entering them to the PCR.

II. Materials and Methods

1- Sample collection and processing:

Current study was performed on five volunteers who donated their hairs. Hair samples (1 and 3) were taken from left and right side of the head of each person who commonly use hair waxes. Collected samples were then divided into four groups. First group was subjected to direct PCR. Hair samples in second group were separately placed (as 1 or 3 hairs for each one) in a 50 mL Falcon tube containing 25 mL of soap foam. The hairs were then transferred to another falcon tube containing 25 mL of double distilled water, shaken for washing for 3.5 min, and then this process of washing was repeated twice. The hairs were then picked, blotted dry in a clean filter paper, and used for direct application to PCR. Third group of hair samples was processed in the same way as the second group, but without using soap foam and replacing it with 10 mL of detergent mix (1 g sulphonic acid, 20% KOH, 15% sodium lauryl sulfate, 100 mL H_2O). The 4th group of hair samples was only scratched gently for their outer layer of hair root by a razor. In all sample groups no extraction process was applied.

2- Amplification of certain regions on Y chromosome via PCR:

PCR amplification of all samples was carried out using PowerPlex®Y23 System (Promega) in a final volume of 13 µL according to (Promega Corporation Technical Manual # DC2305 and DC2320). PowerPlex®Y23 kit specified for certain loci including (DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392 and DYS393), DYS438 and DYS439, in addition, DYS481, DYS533, DYS549, DYS458, DYS435, DYS448 and Y-GATA-H4) DYS570, DYS576 and DYS643. Gene Amp PCR System 9700 thermal cycler (AB/LT/Thermo) was used for sample amplification. Amplification set-up and cycling parameters, as described in the PPY23 Technical Manual (PP Y23 System Technical Manual, 7/12, available at: http:// www.promega.com/resources/protocols/technical-manuals/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 then initialized for loading in 3500 Genetic Analyzer through adding 1 µl of amplified sample or allelic ladder to 10 µl of Hi-Di Formamide and 0.5 µl of ILS provided with PPY23 system. Samples were denatured for 3 min at 95°C followed by a snap cool in an ice bath. For STR analysis in certain loci, GeneMapper ID v1.5 software (Applied Biosystems, UK) was used. Negative control was accompanied with amplification. Samples were stored at 4º C for further analysis. Data was obtained as electropherograms where each locus has one peak except two loci that may have one or two peaks that termed alleles. The height of peaks is directly proportional with the quality of DNA profile.

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

3- Statistical data analysis:

The Means of the peak heights for analyzed 23 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 $P \leq 0.05$.

III. Results

Results presented in **table (1 and 2)** and illustrated in **figures (1: 6)** showing the data of Y chromosome profiles of both one and three hairs samples for five individuals who exposed to direct PCR method and three specific treatments to overcome the negative outcome problem of direct PCR method. 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. The mean of peak heights for all loci involved in Y chromosome profile and standard deviation errors were obtained where data presented as mean ± standard error of means. Asterisks (*), refer to a significant difference between extracted and direct PCR results for the same locus ($P < 0.05$).

The effect of the detergents through the lengths of the peaks.

one and three hairs samples that applied directly in PCR only, didn't give any scale of peak height at all loci, but when these samples exposed to different washing detergents (soap foam, detergent mix and gently scratching of outer layer of the hair follicle treatment), gave high scale of peak heights in all of 23 loci. Also in three hairs samples that gave high scale of peak heights significantly (rfu) more than one hair samples, as illustrated in **figure (1 and 2)**.

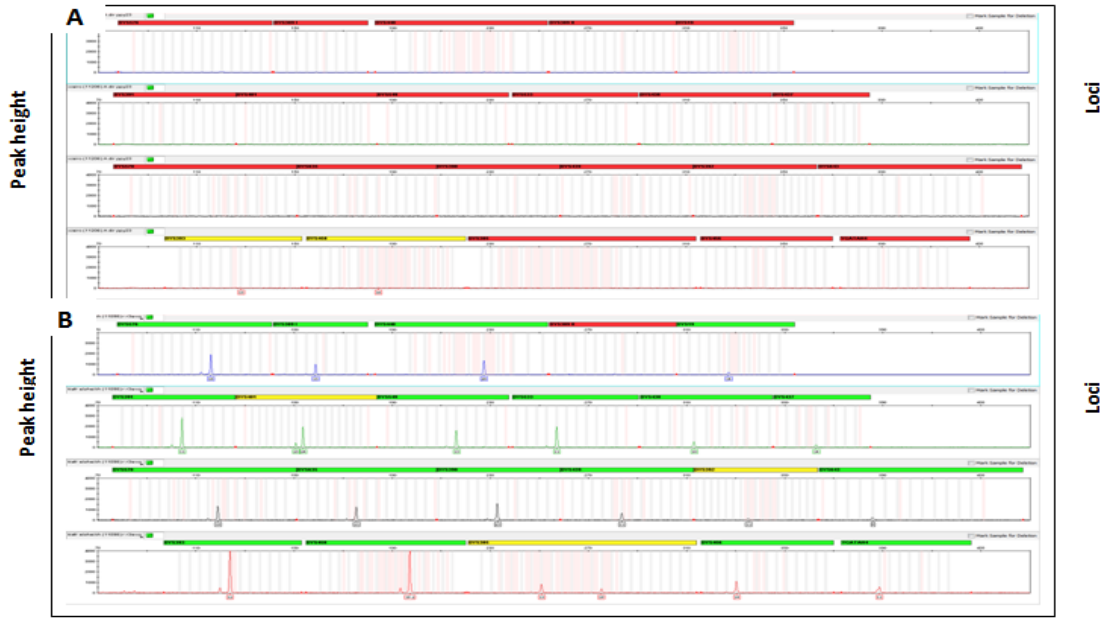

Figure (1): showing a comparison of the electropherogram all dyes for the scale of peak heights between genotype of one hair sample for an individual;

(A): one hair sample loaded directly in PCR reaction,
(B): one hair sample loaded directly after treatment with soap foam detergent.

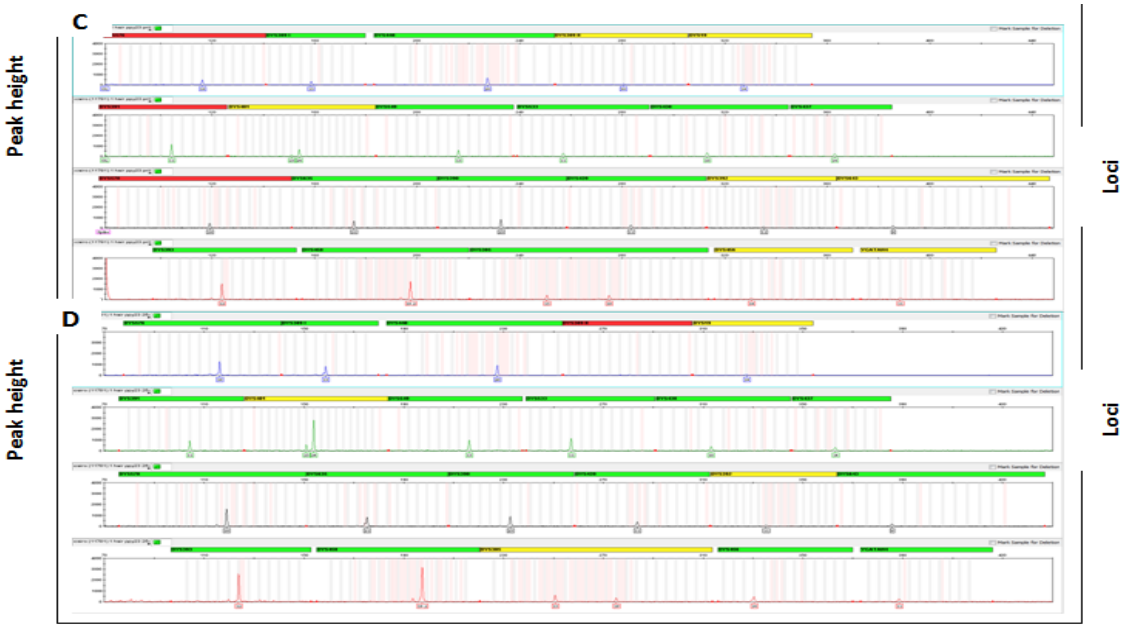

Figure (2): showing a comparison of the electropherogram all dyes for the scale of peak heights between genotype of one hair sample for an individual;

(C): one hair sample loaded directly after treatment with detergent mix solution,

(D): one hair sample loaded directly after treatment with scratching.

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

One hair samples of the five individuals that exposed to direct PCR after treatment with the washing detergents, gave a significantly fluorescence- based detection of all 23 loci, as shown in **table (1) and figures (1, 2)**.

Table (1): showing the mean of peak heights for all loci involved in the PowerPlex®Y23 kit of one hair samples exposed to direct PCR and the three detergents, 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	Treatments			
	Direct without	Foaming	Washing solution	Scratching
DYS19	0.00 ± 0.00	124.0 ± 35.7	40.0 ± 24.5	350.0 ± 83.7
DYS385 a	0.00 ± 0.00	290.0 ±131	290.0 ±135.0	660.0 ± 92.7
DYS385 b	0.00 ± 0.00	220.0 ± 58.3	120.0 ± 97.0	540.0 ± 136.0
DYS389I	5.00 ± 5.00	1290.0 ±323.0	4660.0 ± 1468.0	1020.0 ± 260.0
DYS389II	0.00 ± 0.00	70.0 ± 20.0	60.0 ± 29.2	370.0 ± 122.0
DYS390	0.00 ± 0.00	850.0 ± 202.0	2800.0 ± 696.0	1240.0 ± 291.0
DYS391	0.00 ± 0.00	1700.0 ± 414.0	5500.0 ± 1092.0	970.0 ± 173.0
DYS392	0.00 ± 0.00	120.0 ± 33.9	90.0 ± 40.0	510.0 ± 158.0
DYS393	5.0 ± 5.0	4180.0 ± 1038.0	5980.0 ± 1140.0	3020.0 ± 795.0
DYS437	0.00 ± 0.00	230.0 ± 80.0	70.0 ± 37.4	760.0 ± 258
DYS438	0.00 ± 0.00	3680.0 ±753.0	2880.0 ± 714.0	530.0 ±419.0
DYS439	0.00 ± 0.00	500.0 ± 105.0	600.0 ± 31.6	850.0 ± 251.0
DYS448	0.00 ±0.00	980 ±253	1740 ±129	1222 ±329
DYS456	0.00 ±0.00	660±144	240.0± 40.0	1080±306
DYS458	5.0 ± 5.0	3620±777	5900±1317	3610 ±939
DYS481	36.0 ±18.6	1440 ±320	3940 ±977	1740 ±420
DYS533	0.00 ±0.00	1300 ±318	3600 ±1017	1536 ±408
DYS549	0.00 ± 0.00	1080 ±238	4260 ±1028	1480 ±390
DYS570	0.00 ± 0.00	980 ±235	3100 ±727	1460 ±354
DYS576	5.0 ±5.0	1070 ±272	3160 ±681	1100 ±232
DYS635	0.00 ± 0.00	980 ±198	4480 ±1427	1230 ±322
DYS643	40.0 ±18.7	96.0 ±52.6	70.0 ±20.0	400 ±130
Y-GATA-H	0.00 ± 0.00	430 ±134	80.0 ±25.5	600 ±126
F-value	39.39			
P-value	0.00			
Eta	0.454			
Eta^2	0.206			

Y chromosome profiles from one hair samples showed high significance results.

Significance results were shown when direct PCR method for one hair samples compared with the same samples after exposed to the three detergent (soap foam, washing solution detergent mix and gently pull the outer layer of the hair follicle)

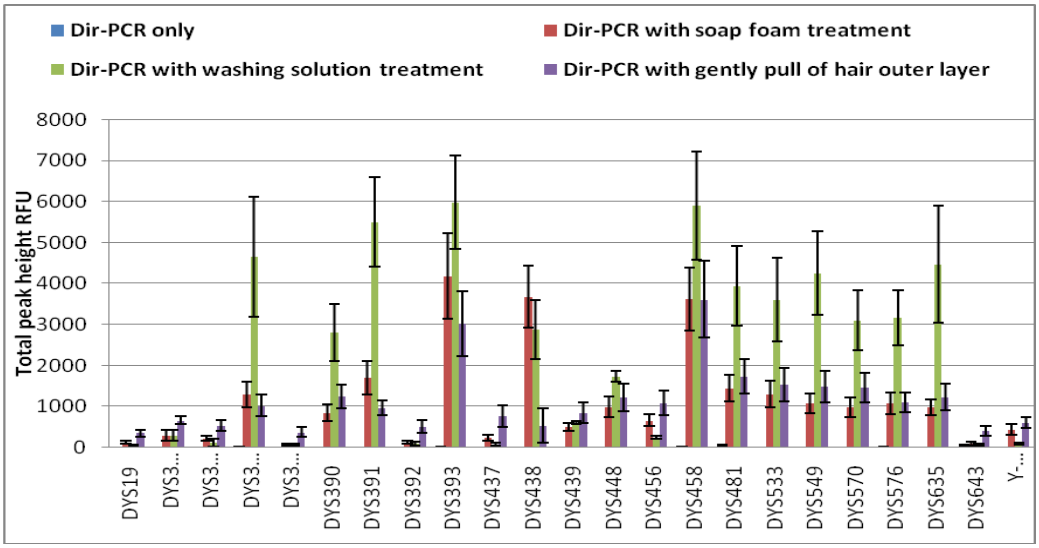

Figure (3): Chart showing a comparison between the mean of peak heights for all alleles of 23 loci in the Y chromosome of one hair samples when loaded directly in PCR reaction, treated with soap foam, treated with washing solution and when treated with gently pull of the outer layer of the hair follicle. Data presented as mean ± standard error of means

The effect of the detergents through the lengths of the peaks for three hairs samples.

Three hairs samples that applied directly in PCR not only didn't give any scale of peak height at all loci, but when these samples exposed to different washing detergents (soap foam, detergent mix and gently scratching of outer layer of the hair follicle treatment), gave a significantly high scale of peak heights in all of 23 loci more than one hair samples that exposed to the same conditions, as shown in **figures (4 and 5)**.

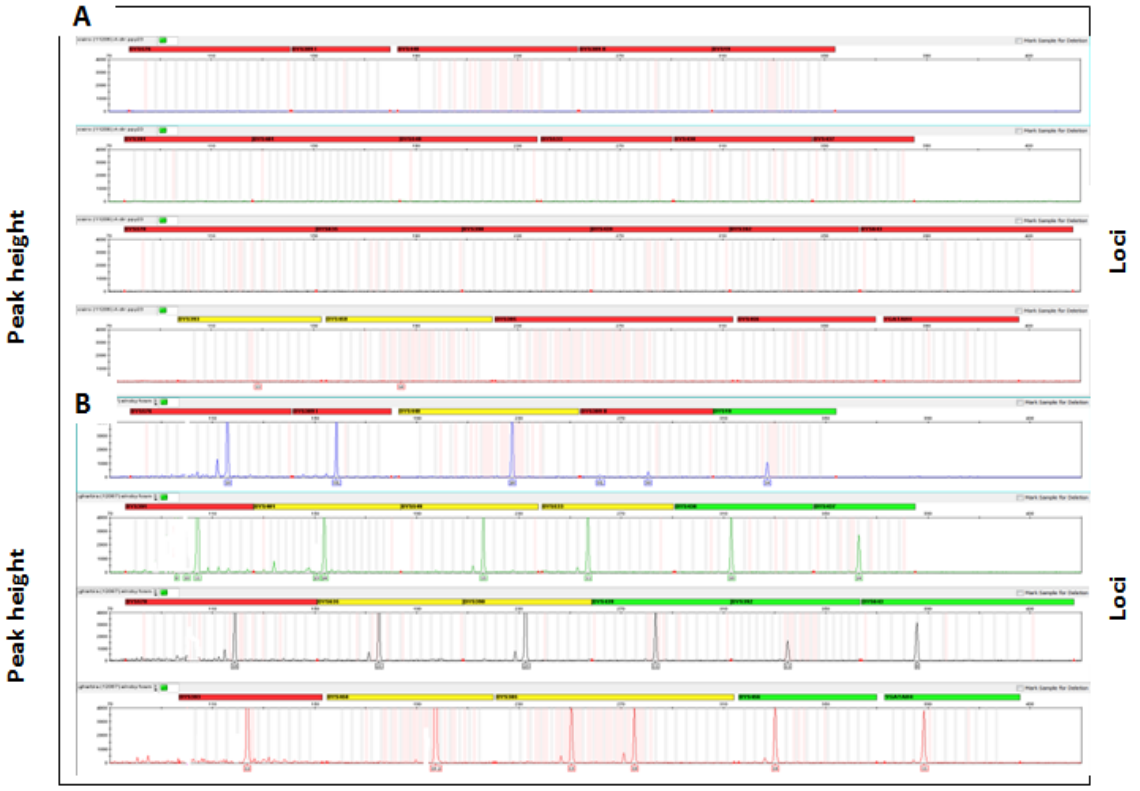

Figure (4): showing a comparison of the electropherogram all dyes for the scale of peak heights between genotype of three hairs sample for an individual;

(A): three hairs sample loaded directly in PCR reaction,
(B): three hairs sample loaded directly after treatment with foam soap detergent,

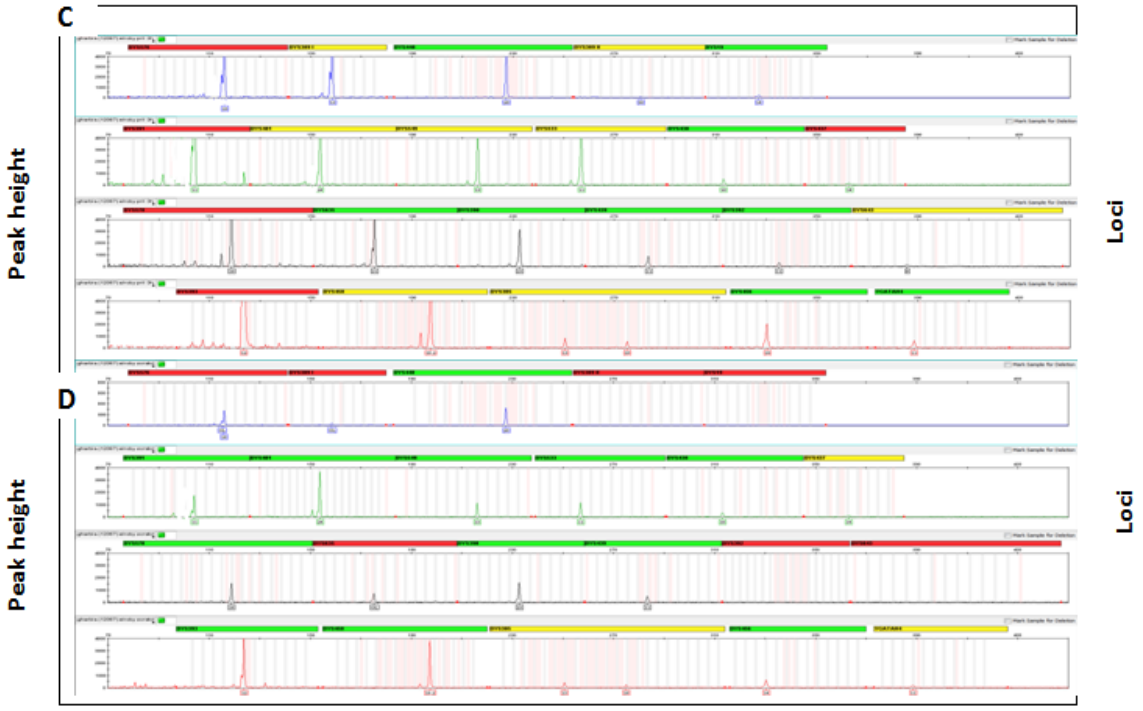

Figure (5): showing a comparison of the electropherogram all dyes for the scale of peak heights between genotype of three hairs sample for an individual;
(C): three hairs sample loaded directly after treatment with detergent mix solution,
(D): three hairs sample loaded directly after treatment with scratching.

Differences between direct PCR method and the treatment detergents through the lengths of the peaks for three hairs samples.

Three hairs samples of the five individuals that exposed to direct PCR after treatment with the washing detergents, gave a significantly fluorescence - based detection of all 23 loci even over the one hair samples from the same individuals as shown in **table (2) and figures (4, 5)**.

Table (2): showing the mean of peak heights for all loci involved in the PowerPlex®Y23 kit of three hair samples exposed to direct PCR and the three detergents, 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	Treatments			
	Direct without	foaming	Washing solution	Scratching
DYS19	0.00 ± 0.00	1660 ± 350.14	910±824.37	170±133.79
DYS385 a	0.00 ± 0.00	5400 ± 796.86	2360±540.92	780±432.89
DYS385 b	0.00 ± 0.00	4680 ± 482.07	1840±445.64	740±540.92
DYS389I	0.00 ± 0.00	6460 ± 1561.92	4500±1390.6	650±342.05
DYS389II	0.00 ± 0.00	1450 ± 379.47	540±441.13	160±92.73
DYS390	0.00 ± 0.00	7260 ± 1517.43	6940±1021.07	1140±429.65
DYS391	0.00 ± 0.00	8720 ± 149.66	8440±116.61	2420±1495.12
DYS392	0.00 ± 0.00	2360 ± 446.76	840±542.7	310±227.15
DYS393	0.00 ± 0.00	8200 ± 943.39	8360±92.73	3540±933.59
DYS437	0.00 ± 0.00	2200 ± 372.82	1120±676.31	480±430.58
DYS438	0.00 ± 0.00	3680 ± 753.25	2880±713.72	530±418.80
DYS439	0.00 ± 0.00	5120 ± 1058.01	5040±1538.37	720±348.42
DYS448	0.00 ± 0.00	6700±1401.78	6620±215.40	1020±370.67
DYS456	0.00 ± 0.00	5940±1230.28	2740±875.5	600±232.37
DYS458	0.00 ± 0.00	7880±1226.94	8320±73.48	2900±607.45
DYS481	48.0 ± 22.44	7100±1408.90	8240±81.24	2160±569.73
DYS533	0.00 ± 0.00	7160±1466.15	7380±395.47	1170±512.24
DYS549	0.00 ± 0.00	7140±1288.25	7460±426.14	1420±570.43
DYS570	0.00 ± 0.00	7600±1029.56	8400±130.38	1270±742.22
DYS576	0.00 ± 0.00	7240±1450.03	7900±232.37	940±318.74

DYS635	0.00 ± 0.00	7380±1476.95	7460±656.20	770±322.33
DYS643	40.0 ± 18.70	2560±593.80	660±585.32	200±176.06
Y-GATA-H	0.00 ± 0.00	5100±827.04	1080±932.41	810±674.24
F-value	150.76			
P-value	0.00			
Eta	0.706			
Eta^2	0.498			

Y chromosome profiles from one hair samples showed high significance results.

The three hairs samples that picked up from the second five persons and treated with different washing detergents, the scale of peak heights of all 23 loci have been increased significantly more than one hair samples from the same individuals in all particular loci, as shown in **figure (6)**.

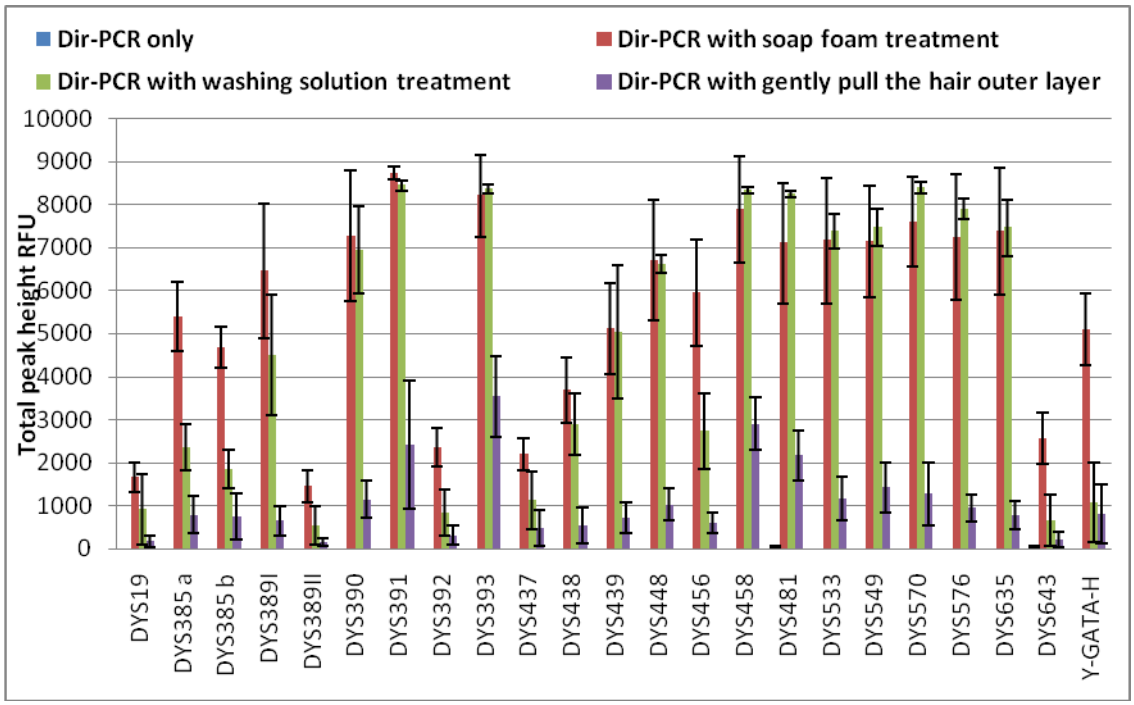

Figure (6): Chart showing a comparison between the mean of peak heights for all alleles of 23 loci in the Y chromosome of three hairs samples when loaded directly in PCR reaction, treated with soap foam, treated with washing solution and when treated with gently pull of the outer layer of the hair follicle. Data presented as mean ± standard error of means

IV. Discussion

In the present study, obtained results revealed that the use of common commercial hair waxes (e.g. gel, shampoo… etc.) can be considered as one of many reasons for hair weakness which it can affect DNA amplification process negatively. Obtained results also showed that either commercial hair detergents or even physical treatment by scratching can potently impact the direct PCR and can overcome such inhibition for direct PCR. It was found previously that the addition of a nondenaturing detergent; such as Triton X or Tween 20, in the PCR buffer can lyse robust cells and release DNA into solution without the need for a prior extraction process (**Linacre *et al.*, 2010**). This may be due to the high viscosity of the detergent and its hydrophobic and hydrophilic associated properties; thus, it may prevent DNA from adhering to the walls of the reaction vessel (**Rohland and Hofreiter, 2007; Brotherton *et al.*, 2013; Thomasma and Foran, 2013; and Templeton *et al.*, 2015**).

Furthermore, data showed that the genetic loci found at the end of each dye for hair samples profiles that were treated with the washing solution before being directly amplified, gave low scale of peak heights rather than those at the beginning of the same dyes. This may be attributed to the fact that the Taq-DNA polymerase was deactivated due to high molecular weight of these loci. In addition, this may be influenced by application of tested washing solution although they did not record the expected high result such as that in the beginning of each dye **(Pierce *et al.*, 2005)**. Thus, hair samples that treated firstly with the soap foam showed better results in direct PCR technique than those treated with the washing solution and gently scratching of outer layer of the hair follicle, because of the concentration of the surfactant found in soap was more suitable for direct PCR technique than others. Since the soap consists of a combination of ingredients (sulfonic acid and lauryl sulfate) that help to

properly treat the hair to give high results in the scale of peak heights. However, the ratio of the (soda), in the components may be represent the barrier to give positive results, this explains the perception that soap is better in giving positive results in all 23 loci than the washing solution, which has been significantly affected in the last loci in each dye.

From previously discussed results, it was found that using of weak detergent affected positively the results of direct PCR as expressed in peak height on different genetic loci on Y chromosome. The applied technique and addition of weak detergent have minimized time and cost and also improved the detection efficiency for some loci. Accordingly, we recommend the addition of detergent to PCR buffers in direct PCR technique for more accurate and less contaminated DNA templates.

Acknowledgement

The authors would like to thank the Forensic Science Laboratory Unit, Police Academy, Cairo, Egypt, for its support during the investigation and the Egyptian Ministry of Interior for its cooperation.

References

[1]. **Burgoyne, L. (1994)**. Safe collection, storage, and analysis of DNA from blood. Proceedings of the 5th International Symposium on Human Identification. Madison, WI: Promega Corporation.

[2]. **Brotherton, P.; Haak, W.; Templeton, J.; Brandt, G.; Soubrier, J.; Adler, C. J.; Richards, S. M.; Sarkissian, C. D.; Ganslmeier, R.; Friedderich, S.; Dresely, V.; van Oven, M.; Kenyon, R.; Van der Hoek, M. B.; Korlach, J.; Luong, K.; Ho, S. Y. W.; QuintanaMurci, L.; Behar, D. M.; Meller, H.; Alt, K. W.; Cooper, A.; and The Genographic Consortium. (2013)**. Neolithic mitochondrial haplogroup H genomes and the genetic origins of Europeans. Nat. Commun., 4: 1764. doi:10.1038/ncomms2656

[3]. **Hajibabaei, M.;** d**eWaard, J. R.; Ivanova, N. V.; Ratnasingham, S.; Dooh, R. T.; Kirk, S. L.; Mackie, P. M.; and Hebert, P.D.N. (2005)**. Critical factors for assembling a high volume of DNA barcodes. Philos. Trans. R. Soc. Lond. B Biol. Sci. 360: 1959-1967.

[4]. **Hall, D. E. and Roy, R. (2014)**. An evaluation of direct PCR amplification. Croatian medical journal, 55(6): 655-661.

[5]. **Jain, T.; Shrivastava, P.; Bansal, D. D.; Dash, H. R.; and Trivedi, V. B. (2016)**. PowerPlex Y23 System: A fast, sensitive and reliable Y-STR multiplex system for forensic and population genetic purpose. J. Mol. Biomark. Diagn., 7(3): 287-293.

[6]. **Linacre, A.; Pekarek, V.; Swaran, Y. C.; and Tobe, S. S. (2010)**. Generation of DNA profiles from fabrics without DNA extraction. Forensic science international: genetics, 4(2): 137-141.

[7]. **Pierce, K. E.; Sanchez, J. A.; Rice, J. E.; and Wangh, L. J. (2005)**. Linear-After-The-Exponential (LATE)-PCR: primer design criteria for high yields of specific single-stranded DNA and improved real-time detection. Proceedings of the National Academy of Sciences of the United States of America, 102(24): 8609-8614.

[8]. **Rohland, N. and Hofreiter, M. (2007)**. Comparison and optimization of ancient DNA extraction. Biotechniques, 42(3), 343-352.

[9]. **Sambrook, J. and Russell, D. (2001)**. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

[10]. **Swaran, Y. C. (2014)**. Direct PCR in forensic science–an overview. Malaysian J. Forensic Sci., 5: 6-12.

[11]. **Sawran, Y. C. and Welch, L. (2012)**. A comparison between direct PCR and extraction to generate DNA profiles from samples retrieved from various substrates. Forensic Sci. Inter. Genetics, 6(3): 407-412.

[12]. **Shokralla, S.; Singer, G. A.; and Hajibabaei, M. (2010)**. Direct PCR amplification and sequencing of specimens' DNA from preservative ethanol. Biotechniques, 48(3): 305-306.

[13]. **Thomasma, S. M. and Foran, D. R. (2013)**. The influence of swabbing solutions on DNA recovery from touch samples. Journal of Forensic sciences, 58(2): 465-469.

[14]. **Thompson, J. M.; Ewing, M. M.; Frank, W. E.; Pogemiller, J. J.; Nolde, C. A.; Koehler, D. J.; Shaffer, A. M.; Rabbach, D. R.; Fulmer, P. M.; Sprecher, C. J.; and Storts, D. R. (2013)**. Developmental validation of the PowerPlex® Y23 System: a single multiplex Y-STR analysis system for casework and database samples. Forensic Sci. Inter.: Genetics, 7(2): 240-250.

[15]. **Templeton, J. E.; Taylor, D.; Handt, O.; Skuza, P.; and Linacre, A. (2015)**. Direct PCR improves the recovery of DNA from various substrates. Journal of forensic sciences, 60(6): 1558-1562.

[16]. **PP Y23 System Technical Manual, 7/12,** available at: http:// www.promega.com/resources/protocols/technical-manuals/101/ powerplex-y23-system-protocol.

IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) is UGC approved Journal with Sl. No. 5012, Journal no. 49063.

Hassab El- Nabi, S. E Testing New PCR Additives for Enhancement of Direct PCR –Based Detection of Y chromosome Stars Loci from Human Hair Samples ." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 13.3 (2018): 80-87.