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Validation of Half-Reaction Volumes of the Promega PowerPlex® Forensic Amplification Kits (PowerPlex® 18D Systems, PowerPlex ® 21System, PowerPlex® Fusion System and PowerPlex® Y23 System) in STR Analysis

التحقق من فاعلية استخدام نصف حجم التفاعل في مجموعة محاليل تكثير الحمض النووي الجنائي الخاص بـ Promega PowerPlex® (PowerPlex®18D Systems, PowerPlex®21 System, PowerPlex® Fusion System and PowerPlex® Y23 System) المستخدمة في تحليل التكرارات المترادفة القصيرة

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Abstract

DNA amplification is known to be the most expensive step during forensic DNA analysis. This study evaluated the half-reaction amplification protocol (12.5 µL PCR product) using DNA amplification kits from Promega PowerPlex® (PowerPlex® 18D System, PowerPlex® 21System, PowerPlex® Fusion System and PowerPlex® Y23 System), which might aid in reducing sample analysis cost by half and allow the analysis of more samples.

A sensitivity study (15 samples) along with testing of various blood stain samples (*n*=100) that were submitted to the Medico-Legal Directorate laboratory for DNA testing was accomplished to compare the DNA profiles resulting from half-reaction volume procedure to those with full-reaction volume procedure, using three differed methods along with standard protocol to evaluate the effect of half reaction volume with some variables.

Results demonstrated the use of half-reaction amplification protocol preceded by washing step for all afore-

Keywords: *Forensic Science, DNA Amplification, Promega PowerPlex®, Half Reaction Volume, Blood, DNA Profile*

المستخلص

من المعروف أن تفاعل البلمرة المتسلسل (PCR) هو الخطوة الأكثر تكلفة في عملية تضخيم الحمض النووي للفحوصات العدلية. وقد قيمت هذه الدراسة طريقة العمل باستخدام نصف حجم التفاعل المطلوب لإجراء مضاعفة ال(12.5 DNA ميكرولتر) باستخدام عدد مختبرية مختلفة تابعة لشركة Promega Power-Plex®، والتي قد تساعد في تقليل تكاليف تحليل العينة بمقدار النصف وتسمح بتحليل المزيد من العينات. وتم إجراء دراسة حساسية (15 نموذجًا) إلى جانب اختبار مختلف عينات بقع الدم (100 نموذج) التي تم استلامها من قبل مختبر شعبة العائدية والنسب في دائرة الطب العدلي في بغداد من أجل مقارنة البصمة الوراثية الناتجة عن إجراء نصف حجم التفاعل مع تلك التي نتجت باستخدام الطريقة القياسية، وتم اختبار 3 طرق جنبًا إلى جنب مع بروتوكول قياسي لتقييم تأثير حجم رد فعل نصف التفاعل مع بعض المتغيرات. وتوضح النتائج أن استخدام نصف التفاعل مسبوقا بخطوة الغسل لجميع

الكلمات المفتاحية: علوم الأدلة الجنائية، تضخيم الحمض النووي، طقم *Promega PowerPlex* ®، نصف حجم التفاعل، الدم، السمات الوراثية

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mentioned DNA amplification kits gave a robust and reliable amplification result that aid to increase the number of samples analyzed and decreased the test cost for each kit without compromising the quality of 3DNA profiles obtained.

العدد المختبرية المستخدمة لإجراء تفاعل البلمرة المتسلسل يعطي نتائج قوية وموثوقة تساعد في زيادة عدد العينات التي تم تحليلها وبكلفة أقل لكل مجموعة دون المساس بجودة البصمة الوراثية التي تم الحصول عليها.

1. Introduction

Short tandem repeat (STR) DNA analysis is the main method used to resolve the identity, kinship and paternity disputes. The use of DNA amplification by Polymerase Chain Reaction technique (PCR) is a powerful tool to amplify minute amounts of DNA that are used for forensic tests and other purposes due to its unprecedented sensitivity [1, 2]. STR analysis is a powerful tool used to compare specific DNA loci from two or more samples. Polymorphic STR segments increase the uniqueness of DNA profiles that are applied for forensic testing [3].

Several multiplex DNA amplification kits are available for forensic testing. Promega PowerPlex® 18D System, PowerPlex® Fusion System, and PowerPlex® Y23 System DNA amplification kits are commonly used in-forensic laboratories for different forensic case-works, paternity, kinship, and genealogical testing.

PowerPlex® 18D System is widely utilized for human identification applications. allows analysis of the 13 CODIS loci and Amelogenin in addition to four new loci: D3S1358, D8S1179, TPOX, TH01, D21S11, D18S51, Penta E, D7S820, D16S539, D5S818, D13S317, CSF-1PO, Penta D, vWA, FGA, D19S433, and D2S1338 that were developed in a way that aided direct DNA amplification, especially from FTA paper, as well as its rapid thermal cycling protocol [4]. PowerPlex® 21System has two more loci than PowerPlex® 18D System: D12S391 and D1S1656, while PowerPlex Fusion System permits concurrent amplification for all loci found in PowerPlex® 21System accompanied with additional 4 loci: D2S441, D10S1248, DYS391, and D22S1045, which raises the discrimination power, also it supports data sharing between different databases [5, 6].

Y-STR markers are found on the non-recombining region (NRY) of the Y chromosome, they create a haploid profile for male DNA samples, the firm paternal inheritance of Y-STRs clarify their importance in paternity and kinship tests. PowerPlex® Y23 System is used to analyze 17 Y-STR loci that are popular in other kits (DYS385a/b, DYS458, DYS390, DYS635, DYS438, DYS456, DYS19, DYS448, DYS439, DYS392, DYS389II.I, DYS437, GATAH4, DYS389I, DYS393, DYS391) along with 6 more new loci (DYS481, DYS533, DYS549, DYS570, DYS576, and DYS643) having higher gene diversities [7].

Due to the precarious security situation of the country, the need for paternity and kinship testing has increased enormously. The person-in-charge of the Paternity and Kinship Division needs to be careful to provide appropriate laboratory materials from reliable sources. Given the increasing number of cases received by the division in recent years, there was an urgent need to perform as many tests as possible along with reducing the cost of each test using modified methods for each kit without compromising the results quality. DNA amplification step is the most suitable to cut the test costs because it is the most expensive step in DNA analysis. Each one of the above mentioned kit recommended a PCR master mix reaction volume of 25µL for each sample. However, the volume of the DNA amplification reaction can range from 5 to 100µL [8]. The decrease of the reaction volume from 25µL to 12.5µL may provide a sustainable utility of cost-saving without undermining the quality of the results. Previous publications reported effective use of half volume of PCR mix in obtaining good quality results that were similar to original full-reaction data [9-11].

The present paper reports the results of using half of the actual PCR volume of three different DNA amplifications kits. The general purpose was to optimize and evaluate the achievement of a half-reaction amplification protocol using Promega PowerPlex® 18D System, PowerPlex® 21System, PowerPlex® Fusion System, and PowerPlex® Y23 System DNA amplification kits.

2. Materials and Methods

Fifteen in-house known samples were enrolled in this study for sensitivity and comparison purposes by which each sample was treated with different procedures to

compare the resulted profiles. Blood samples were taken and placed on Direct™ Classic Card- FITZCO, USA and Whatman® FTA® card, UK and left for air dry for 24 hours.

For each DNA amplification kit used for routine DNA tests in our laboratories (PowerPlex® 21System, PowerPlex Fusion System and PowerPlex® Y23 System)four different procedures were used and are shown in Table-1, which also summarizes a comparison between standard and modified methods used in DNA amplification kits PowerPlex® 18D System, PowerPlex® 21System, PowerPlex Fusion System and PowerPlex® Y23 System. Direct amplification was performed in a GeneAmp® PCR System 9700 thermal cycler according to the manufacturer protocol for each kit. PCR products were run on 3130 xl Genetic Analyzer® (Applied Biosystems, USA) as mentioned in the technical manual and the data were analyzed with Gene Mapper ID Analysis Software (Applied Biosystems, USA). Method-3 was applied on 100 blood samples that were submitted to Paternity and Kinship Laboratory/ MLD, for analysis and research. These samples were deposited on blood storage cards in the same way as the known samples enrolled in this study. The DNA profiles were analyzed to compare the results of these different methods.

For each method reagents were added subsequently in enumerated tubes; first, Amplification Grade water was added to the tube, then PowerPlex® Master Mix followed by (50%) PowerPlex® Primer Pair Mix. FTA® card punches (1.2mm) were added as the last step (with an exception of method 3).

For method-3 modification; FTA® card punches were placed in enumerated tubes,10 µL of nuclease free water was added then samples were placed in the incubator at 37°c for 5 minutes, then water was aspirated and reagents were added next. Statistical calculations were made for the DNA profiles obtained from sensitivity test (full and half reaction volume results) using One-way ANOVA (Analysis of variance) with post-hoc Tukey HSD (Honestly Significant Difference) Test Calculator for comparing multiple treatments (https://astatsa.com/OneWay_Anova_with_TukeyHSD/).

3. Results and Discussion

Different samples were used to evaluate the performance of half-reaction amplification, a comparison between DNA profiles obtained using full (25 µL) and half (12.5 µL) amplification volumes was made as well as full assessment for the quality of the resulting profiles (i.e. Stutters allele drop out, pull-up, off-scale data, etc.). DNA profiles obtained by using Method 4 were analogous to the DNA profiles obtain using standard method (method 1), while in Method 3; the use of washing step for the FTA punch before adding the PCR reagents gave the best DNA profiles for all amplification kits as shown in the figures, (Figure-1 and 2- shows DNA profiles obtained by using PowerPlex® Y23 System, PowerPlex Fusion System, PowerPlex® 21System, and PowerPlex® D18 System with half-reaction volume/ wash step procedure). This might happen because hydrolysis aids to overcome the inhibition caused by heme carryover [12,13]. On average an FTA punch gives a DNA concentration of 50 – 360 ng (depending on white blood cell count); this concentration was accepted and beneficial using half-reaction volume procedure.

All the modifications that were applied to the three methods mentioned in Table-1, were successful to provide dependable DNA profiles that were analogous to the profiles resulted by using standard method for all samples. Statistical analysis for the sensitivity samples (15 samples) was made using One-Way Anova Analysis of Variance with post-hoc Tukey HSD test. ANOVA-based peak heights (relative fluorescence units (RFUs)), Table-2 average rfu for the five methods applied for sensitivity samples), showed significant differences between half-reaction volume/with wash step (total average of 4 amplification kits) and full reaction volume profiles (method 3 to method 1) regarding peak height ($p<0.01$). ANOVA results (Table-3) and (Table-4) shows significant differences between the half-reaction volume methods [for method 2, 3 (PowerPlex y-23 and PowerPlex fusion) and method 4] DNA profiles. The overall results indicated that DNA profiles obtained by using half reaction volume for DNA amplification are dependable and approvable. No inhibition was observed; as well, half-reaction methods showed a high sensitivity for containing high DNA quantity, by exhibiting great peak heights (rfu). The average peak heights were increased as total amplification volume decreased; peak problems (stutters, drop-out, etc.) were evaluated for all the tested samples and noticed to be decreased.

Paternity and kinship division receives dozens of samples need to be examined daily, and the urge to provide more work solutions is constantly increasing, and the more samples were analyzed using the wash/ half-reaction method (method 3), the more it was proved to be

Table 1- *Comparison between standard and modified methods used for DNA amplification kits PowerPlex® 18D Systems, PowerPlex® 21System, PowerPlex Fusion System and PowerPlex® Y23 System.*

PCR Amplification Mix Component	Method 1	Method 2	Method 3	Method 4
Modifications	Standard Method	Using half-reaction reagents	Using wash step and half-reaction reagents	Using 5X Amp Solution™ Punch Solution™ and half-reaction reagents
Water/Amplification Grade	15 µL	7.5 µL	7.5 µL	1.5 µL water 5+µLPunchSolution™
PowerPlex® Master Mix	5 µL	2.5 µL	2.5 µL	2.5µL
PowerPlex® Primer Pair Mix	5 µL	2.5 µL	2.5 µL	2.5
TOTAL	25 µL	12.5 µL	12.5 µL	2.5 µL

Table 2- *Average rfu for the five methods applied for sensitivity samples.*

Method		Average RFU
Standard Method		1122.92
Using half-reaction reagents		3708.33
Using 5X Amp Solution™ Punch Solution™ and half-reaction reagents		3343.48
Using wash step and half-reaction reagents/ Total Avarage		2363.75
Using wash step and half-reaction reagents	PowerPlex 18	1594.87
	PowerPlex 21	1523
	PowerPlex Y-23	3570.45
	PowerPlex 24	2766.67

effective and robust. The resulted DNA profiles were dependable and more samples were analyzed using the same kit which is very important in our laboratories due to the increased numbers of the cases referred to the Paternity and Kinship division as a result of the precarious situations of the country.

4. Conclusion

In conclusion, this study demonstrates that the reduction of the total reaction volume (without modifying reaction components ratio) for DNA amplification is very effective and helps with small amounts of DNA template. Also, this method aid to generate DNA profiles that were comparable to the full-reaction data. The most influential effect for using a half-reaction protocol is the lessening of the operational cost which lead to amplify twice the number of the samples with the same PCR kit.

Acknowledgement

We would like to thank all the members of the Paternity and Kinship Laboratory/ MLD for accomplishing in the study.

Table 3- *ANOVA calculation results.*

PCR Mix	Sum of Square (SS)	Degrees of Freedom (V)	Mean Square (MS)	F Statistic	*p*-value
Treatment	184,321,032.86	6	30,720,172.14	27.7119	1.11e-16
Error	215,059,917.39	194	1,108,556.28		
Total	399,380,950.25	200			

Table 4- *Post-hoc Tukey HSD Test results.*

Treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inferfence
Standard vs half-reaction reagents	12.0298	0.0010053	** p<0.01
Standard vs 5X Amp and Half reaction	10.2216	0.0010053	0.0010053
Standard vs wash step and half-reaction PowerPlex 18	1.9623	0.7827834	insignificant
Standard vs wash step and half-reaction PowerPlex 21	2.4435	0.5846798	insignificant
Standard vs Using wash step and half-reaction PowerPlex y23-	11.1379	0.0010053	** p<0.01
Standard vs Using wash step and half-reaction PowerPlex 24	8.5102	0.0010053	** p<0.01

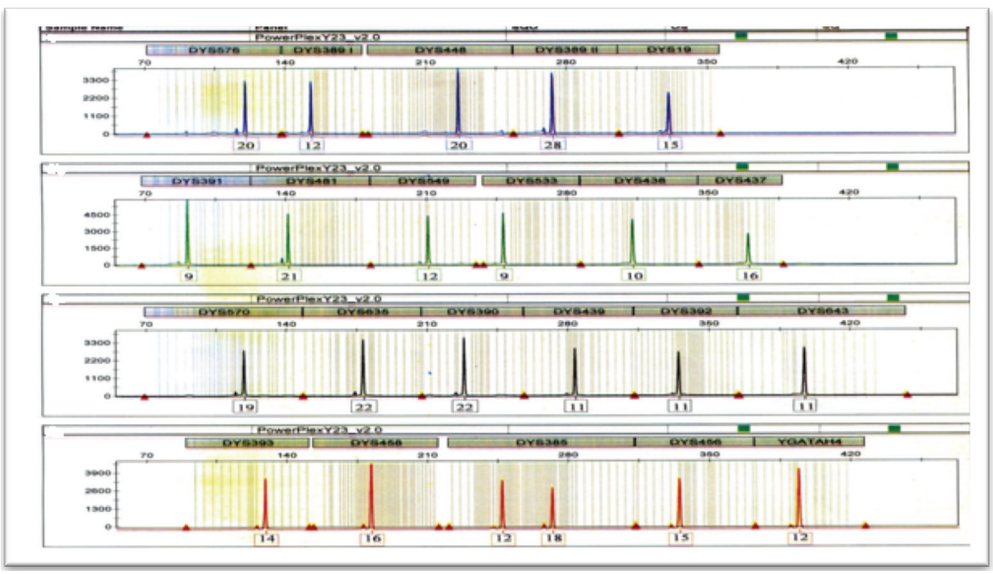

Figure 1- *DNA profile obtained by PowerPlex® Y23 System using half-reaction volume / wash step procedure*

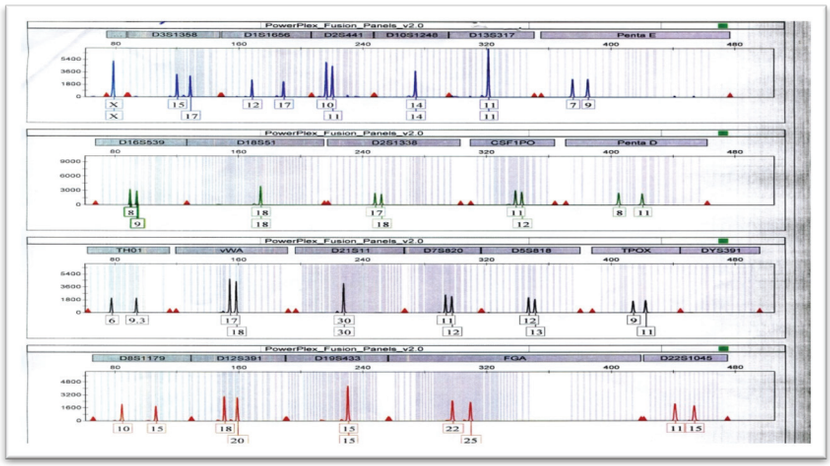

Figure 2- *DNA profile obtained by PowerPlex® Fusion System using half-reaction volume / wash step procedure.*

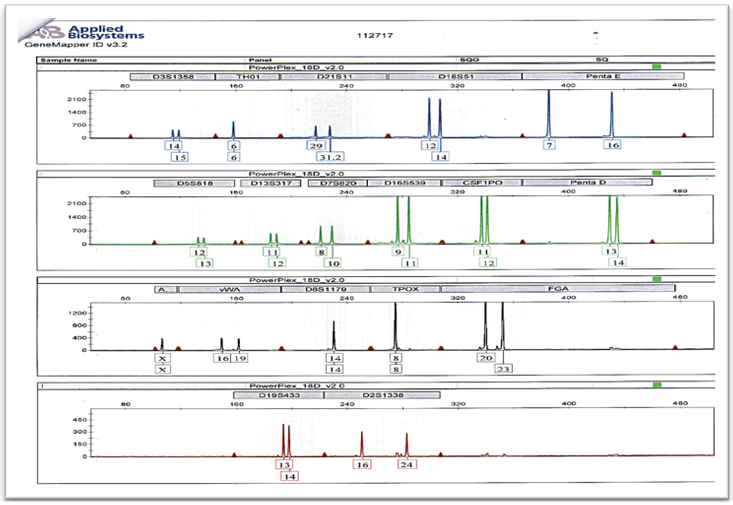

Figure 3- *DNA profile obtained by using PowerPlex® 21System, using half-reaction volume / wash step procedure*

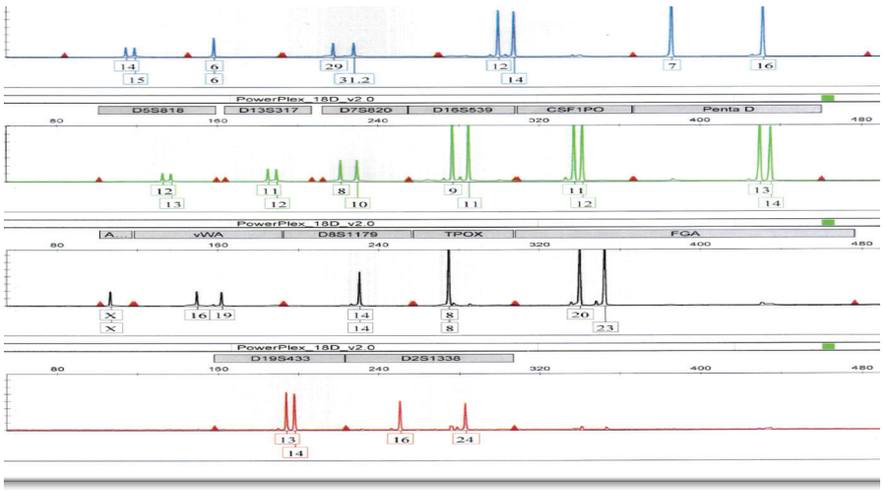

Figure 4- *DNA profile obtained by PowerPlex® 18D System using half-reaction using half-reaction volume / wash step procedure.*

Conflict of Interest

The authors declare no conflict of interest.

Ethical Approval

This research was conducted based on Article 2 of the Iraqi Forensic Medicine Law of 2013.

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