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Research article

Genetic analysis of fingerprints—Could WGA or nested-PCR be alternatives to the increase of PCR cycles number?

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Abstract

In this work we aimed to compare the application of increasing PCR cycle number, whole genome amplification and nested-PCR on fingerprints genetic analysis. Results were compared for correct alleles, allele dropin and allelic dropout. We concluded that increasing the number of PCR cycles is yet the best way to attain the required sensitivity.

Keywords: LCN; Fingerprints; WGA; Nested-PCR; Forensic genetics

1. Introduction

In forensic casework, scientists often need to analyze samples that contains only trace amounts of genomic DNA (less than 100 pg), known as low copy number (LCN). Good examples are fingerprints. However, it is often very difficult to obtain complete genetic profiles of these samples using standard STR amplification methods. Increasing cycle number is widely used to improve results, though sometimes resulting profiles are difficult to interpret. Other alternative methods, such as whole genome amplification (WGA) and nested-PCR, have been described. Most WGA methods use random primers and low stringency annealing conditions to amplify large sections of the genome, in order to increase the quantity of the starting DNA template, prior to any downstream analysis. Some authors have shown the potential of the application of WGA to genotyping LCN and degraded samples for forensic use [1]. However, it has not been thoroughly examined. Nested-PCR has also been suggested as a method to improve sensitivity in LCN samples [2]. This method reduces the amount of non-specific products and can analyze minute amounts of DNA, but requires transference of PCR products into a separate tube, which is disadvantageous. The present study aims to compare the application of increasing cycle number, WGA and nested-PCR on fingerprints genetic analysis.

2. Materials and methods

Forty-five fingerprints left on slides were divided in 15 groups of 3. Each group was swabbed firstly with a sterile swab moistened with sterile distilled water. Afterwards the same procedure was carried with a dry swab. DNA was extracted with phenol:chloroform:isoamyl alcohol, and concentrated with Microcon® spin columns. DNA was eluted in 67 μl, approximately three times more normal volume for one fingerprint. Samples were analyzed with AmpFlSTR® Identifiler™ (Applied Biosystems) kit using both 28 (Ident28) and 34 Ident34) cycles (normal and LCN condition, respectively) and REPLI-g (Qiagen) (according to the manufacturers' instructions) followed by PCR amplification with Identifiler™ using 28 cycles to perform WGA. After amplification with Identifiler™ kit using 28 cycles, nested-PCR was performed with a miniSTR heptaplex (Table 1). PCR products were separated by 3100 AB Prism Genetic Analyzer; results analysis was performed by using Genescan v.3.7 and Genotyper v.3.7 analysis software. Results were compared for correct alleles, allele dropin and allelic dropout.

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Table 1
MiniSTR primers used to perform nested-PCR

Locus	GenBank	Primers (5′–3′)
D7S820	AC004848	for **[VIC]**-GAACACTTGTCATAGTTTAGAACG rev TCATTGACAGAATTGCACCAC
D16S539	AC024591	for **[VIC]**-CTCTTCCCTAGATCAATACAGAC rev GCATGTATCTATCATCCATCTCTG
D18S51	X91254	for **[NED]**-TCTGAGTGACAAATTGAGACCTT rev CTTCTCTGGTGTGTGGAGATG
CSF1PO	X14720	for **[NED]**-ACAGTAACTGCCTTCATAGATAG rev GTGTCAGACCCTGTTCTAAGTA
TH01	D00269	for **[6FAM]**-CCTGTTCCTCCCTTATTTCCC rev GGGAACACAGACTCCATGGTG
FGA	M64982	for **[6FAM]**-GGCATATTTACAAGCTAGTTTCT rev ATTTGTCTGTAATTGCCAGC
Amel.	M55418	for **[6FAM]**-CCCTGGGCTCTGTAAAGAATAGTG rev ATCAGAGCTTAAACTGGGAAGCTG

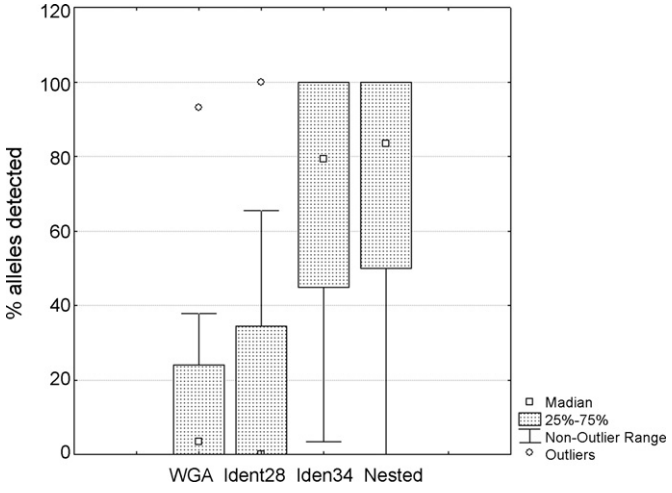

Fig. 1. Box-Whiskers plot for comparison concerning percentage of correct alleles between tree methods used.

3. Results and discussion

Some authors often refer that WGA seems to be very efficient for LCN samples. However, these results are usually obtained when human cell lines are analyzed (high quality DNA). In the present study, data showed that WGA is not as efficient as increasing cycle number or nested-PCR in genetic analysis of fingerprints. With Ident34, median of correct alleles detected is 80% higher than with WGA (Fig. 1). Results of WGA are very similar to Ident28 concerning the percentage of correct alleles detected, with no statistical differences ($p > 0.05$) (paired samples t-test). These results can derive from the fact that apoptotic phenomenon causes the breakup of DNA into fragments of low molecular weight which is much less adequate than using high molecular weight DNA; as it is recommended by manufacturers to obtain efficient whole genome amplification. Nested-PCR gave identical results as Identifiler™ using 34 cycles concerning percentage of alleles detected (Fig. 1), allele dropin and allelic dropout. With nested-PCR was also possible to analyze LCN samples, in accordance with other author results [2], however the drawbacks of this technique makes increasing the number of PCR cycles the best way to analyze LCN samples.

The results obtained in this study and those from other authors suggest that increasing cycle number is more efficient than nested-PCR and WGA for genetic analysis of fingerprints. Nevertheless, to analyze other LCN samples (non-degraded) it is possible that WGA can also provide good results although it seems that stochastic effects cannot be avoided.

Conflict of interest

None.

References

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