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Validation of a reaction volume reduction protocol for analysis of Y chromosome haplotypes targeting DNA databases

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ABSTRACT. The use of Y chromosome haplotypes, important for the detection of sexual crimes in forensics, has gained prominence with the use of databases that incorporate these genetic profiles in their system. Here, we optimized and validated an amplification protocol for Y chromosome profile retrieval in reference samples using lesser materials than those in commercial kits. FTA® cards (Flinders Technology Associates) were used to support the oral cells of male

individuals, which were amplified directly using the SwabSolution reagent (Promega). First, we optimized and validated the process to define the volume and cycling conditions. Three reference samples and nineteen 1.2 mm-diameter perforated discs were used per sample. Amplification of one or two discs (samples) with the PowerPlex® Y23 kit (Promega) was performed using 25, 26, and 27 thermal cycles. Twenty percent, 32%, and 100% reagent volumes, one disc, and 26 cycles were used for the control per sample. Thereafter, all samples (N = 270) were amplified using 27 cycles, one disc, and 32% reagents (optimized conditions). Data was analyzed using a study of equilibrium values between fluorophore colors. In the samples analyzed with 20% volume, an imbalance was observed in peak heights, both inside and in-between each dye. In samples amplified with 32% reagents, the values obtained for the intra-color and inter-color standard balance calculations for verification of the quality of the analyzed peaks were similar to those of samples amplified with 100% of the recommended volume. The quality of the profiles obtained with 32% reagents was suitable for insertion into databases.

Key words: Forensic genetics; Uniparental markers; Genetic profiles

INTRODUCTION

In forensic genetics, experts attempt to identify the origins of biological samples with maximum certainty (Jobling and Gill, 2004). Towards this purpose, several techniques for recovering forensic analysis-grade DNA from samples are used. Standardization of sample analysis using short tandem repeats (STR) has been one of the primary objectives of the forensic community for several years (Butler, 2005).

Among the samples that can be recovered through STR, the Y chromosome repeat regions are important for cases involving male individuals (Jarreta, 1999). These regions are used in standard criminal analyses, and are also included in genetic profile databases to store information for the future (Kayser, 2007).

Genetic profiling databases are used in many countries worldwide to store and organize genetic information about criminals and suspects. Among these, the FBI database, which uses the CODIS system for data storage, is particularly important (Miller et al., 2003).

With the implementation of Law 12.654/12 establishing the Criminal Profiles Database in Brazil, criminal experts have started collecting and organizing information from individuals in the prison system. Considering the high number of criminals with characteristics that fit the law, forensic genetics laboratories are required to insert these profiles in the system.

Examinations involving DNA analysis for forensic purposes are expensive because of the high standard and specificity requirements of these genetic profiles. To reduce the expenses associated with the generation of high quality genetic profiles, we aimed to optimize and validate an amplification protocol for the recovery of Y chromosome profiles in reference samples by reducing the amount of materials commonly recommended in commercial kits.

MATERIAL AND METHODS

Preparation of the sample

The FTA® cards (Flinders Technology Associates) of the EasiCollect collection devices (GE Healthcare, Little Calford, UK) for 270 samples were used for supporting buccal cells of male subjects. Initially, a validation process was performed to define optimal cycling conditions and reduced volumes.

Three reference samples, collected from FTA® cards, were used, with nineteen 1.2 mm-diameter discs were manually drilled with the Uni-Core Punch 1.2 mm drill (GE Healthcare) and placed in 200 µL MicroAmp® capped reaction microtubes (Applied Biosystems, Foster City, CA, USA) or MicroAmp® optical 96-well reaction plate (Applied Biosystems) containing SwabSolution direct amplification reagent (Promega, Madison, WI, USA) without prior extraction.

Optimization of amplification

We optimized the amplification protocol of the PowerPlex® Y23 kit (Promega) based on the manufacturer's recommendations. Reactions with 25, 26, and 27 thermal cycles were performed for one and two 1.2 mm-diameter discs, which we named samples 1 and 2, for each cycle. Two different concentrations of the mix with reduced volume were used: 1.6 µL PowerPlex® Y23 5X master mix (equivalent to 32% of the recommended amount), 0.8 µL PowerPlex® Y23 10X primer pair mix (equivalent to 32% of the recommended amount), and 5.6 µL ultra-pure water (equivalent to 32% of the recommended amount); 1.0 µL PowerPlex® Y23 5X master mix (equivalent to 20% of recommended amount); 0.5 µL PowerPlex® Y23 10X primer pair mix (equivalent to 20% of the recommended amount), and 3.5 µL ultra-pure water - 3.5 (equivalent to 20% of the recommended amount). Eppendorf Research® 0.5-10 µL, 10-100 µL and 100-1,000 µL micropipettes were used to dispense liquids manually, and thermocycling was performed in a Veriti thermal cycler's 96-well format (Applied Biosystems). In addition, controls with 100% reaction volume were used along with the samples.

Polymerase chain reaction (PCR) amplification

After the optimum amplification parameters were established, all samples (N = 270) were amplified using 27 PCR cycles with a 1.2 mm disc and 32% amplification reagents distributed in three 96-well plates. The sample distribution in the 96-well plate was as follows: 90 wells contained unknown samples, two wells contained positive and negative controls (Low Tris-EDTA - Applied Biosystems), respectively, and four wells contained the YFiler™ Allelic Ladder (Applied Biosystems), one for every three columns.

A mixture containing 9.5 µL Hi-Di™ formamide (Applied Biosystems) and 0.5 µL CC5 internal lane standard (ILS) 500 Y23 was distributed manually using micropipettes into the wells of the electrophoresis plates (Promega). One microliter PCR product was added to this mixture in each well.

Capillary electrophoresis

The ABI 3500 8-capillary genetic analyzer (Applied Biosystems) was used with

the POP-4™ polymer (Performance Optimized Polymer) (Applied Biosystems). Injection parameters specified by the PowerPlex® Y23 kit (Promega) were used.

The Y chromosome STR fragments were analyzed using the GeneMapper ID-X v 1.2.1 software (Applied Biosystems, 2009) after importing the PowerPlex® Y23 panels, bins, and stutter text files. The previously validated value of 100 rfus (Relative Fluorescence Units) was used as the peak height analysis threshold.

RESULTS

Three separate samples were first submitted to optimization in the following categories: one disc in a reaction with 32% of the original reagent volume, two discs in a reaction with 32% of the original volume, one disc in 20% of the original volume, and two discs in 20% of the original volume. In addition, amplifications with 100% reagent volume, one disc, and 26 cycles were used for the control per sample.

Triplicates of each of the three samples selected were submitted to different cycling conditions during the amplification, such as 25, 26, and 27 cycles of PCR. For reactions containing 32% reagent volume, the best genetic profile was generated using 27 PCR cycles and a single disc.

For reactions containing 20% of the recommended reagents, the best series was obtained with 26 cycles and one 1.2 mm disc. Sample recovery was difficult from reactions containing two discs because a significant quantity of the reaction liquid was absorbed by the FTA paper, which reduced the amount of the aspirated sample.

Results of the validation experiments showed that amplification of single disc with 32% reagent volume yielded the best result. Thereafter, 270 samples were submitted to genotyping. Of the 270 samples amplified with 32% reagent volume, 267 generated complete and qualitative genetic profiles. To verify the quality of the remaining three samples, amplification reactions were performed with 100% reagents, which did not yield complete genetic profiles, a result similar to that obtained with reduced amount of reagents. This could be caused by errors during sample collection, for example, negligible amount of material obtained in the buccal smear, or because of incorrect adhesion of the collecting sponge to the FTA paper.

Data was analyzed using a study of equilibrium values between fluorophore colors. In the samples amplified with 32% reagents, the intra-color and inter-color standard balance were calculated to verify the quality of the analyzed peaks for the four fluorophores present in the kit. We obtained a minimum of 0.005499 and a maximum of 0.308852 with a median of 0.142877 for the inter-color balance. The intra-color representation values for samples with 32% reagent volume are shown in Figure 1. The comparative data of profiles generated with 20, 32, and 100% reagent volumes are shown in Figures 2 and 3.

For samples amplified using 20% reagents, a considerable imbalance was observed in intra- and inter-dye peak heights, rendering interpretation of results for insertion into the database difficult (Figure 2).

DISCUSSION

The amplification of Y chromosomes haplotypes is an important tool in forensic genetics, as it may be the only source of relevant information in cases of sexual crime or confrontation of vestiges. Generally, female victims are the largest donor of genetic

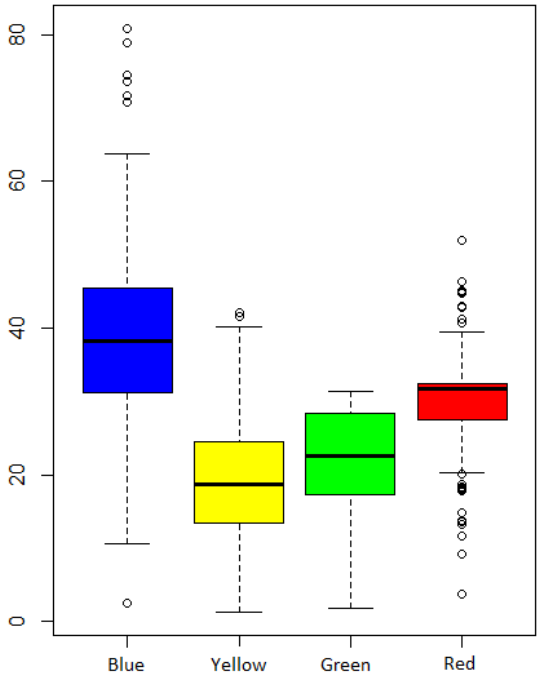

Figure 1. Peak height intra-color balance for each fluorescent dye in 8 µL final volume of the amplification reaction. The X-axis indicates the fluorescence colors and the Y-axis indicates the intra-color balance of peak height in percentage.

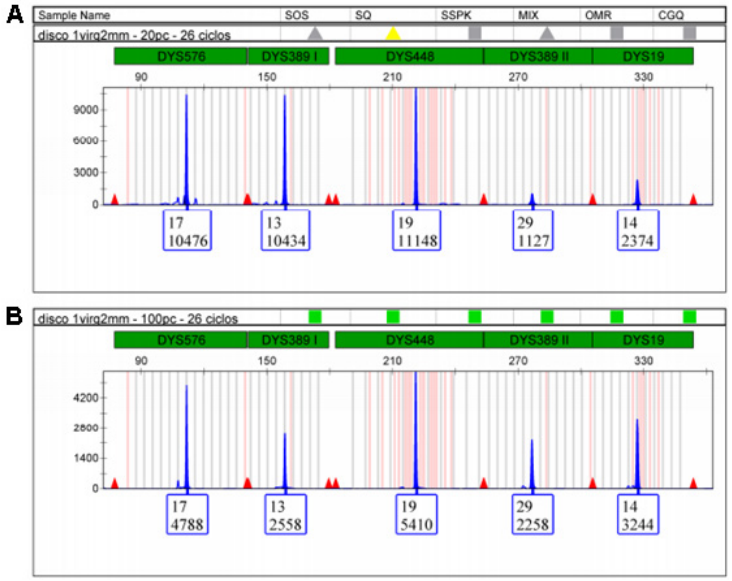

Figure 2. Electropherograms of chromosome haplotypes. **A.** Reaction with 20% reagents. **B.** Full reaction (100%).

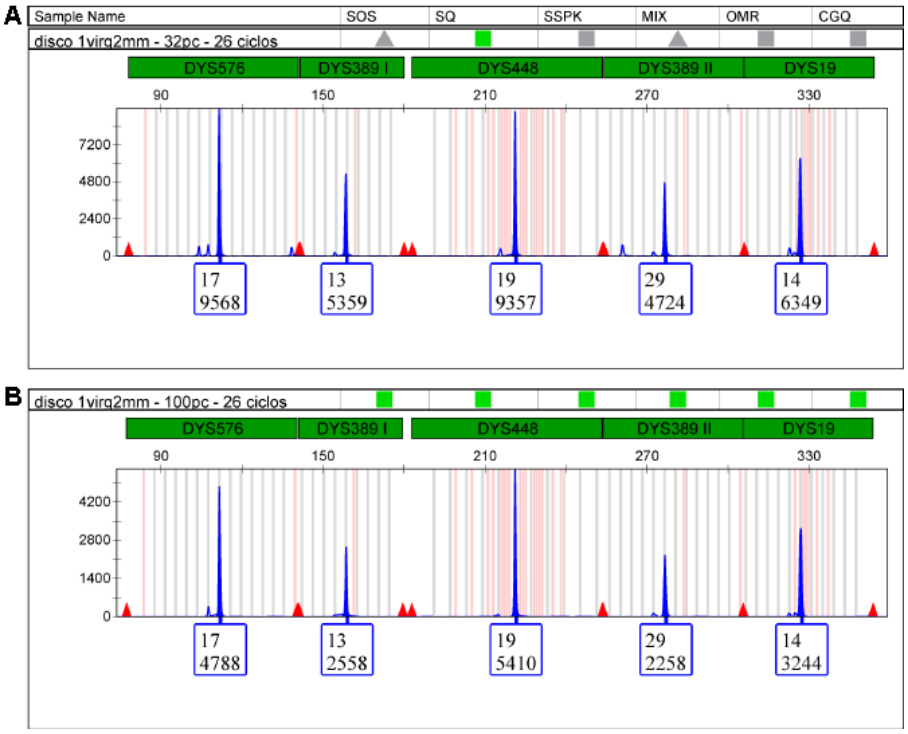

Figure 3. Electropherograms of chromosome haplotypes. **A.** Reaction with 32% reagents. **B.** Full reaction (100%).

material in sex crimes (for example, there are ten parts of a woman's genetic material and one part of that of a man), making identification of autosomic STRs difficult. Therefore, leading genetic profile databases should maintain the Y chromosome haplotypes for future comparison purposes. The high demand for analysis and storage of samples in these banks necessitates the development of rapid and less expensive means of performing these analyses. It is worth noting that in the majority of cases, collections are made on saliva swabs stored on FTA paper, which yields high amounts of good quality DNA that may be used to develop new protocols with reduction and readjustment of the inputs.

To improve the effectiveness of the haplotype analysis techniques, studies have focused on enhancing the quality of the profiles, as well as reducing the costs associated with the use of commercial kits. Here, the reduction in the amount of the commercial reagents to 32% of the recommended volume did not affect the quality of the DNA recovered from the reference samples. However, with greater reduction in reagent volumes (as with 20% of the recommended reagents) the quality of the samples was affected.

The profiles of the samples processed with 32% of the recommended reagent amounts were suitable for incorporation in data banks compared to the profiles of samples generated by full reactions, which increased the reliability of the generated profiles.

Contrary to the data published by Raziel et al. (2015), the profiles of samples processed with 20% of the recommended reagents were not suitable for incorporation into the databases.

This discrepancy may have occurred due to differences in the laboratory procedures used in this study versus that by Raziel et al. (2015); in contrast to the automated laboratory procedures used by Raziel and coworkers, manual procedures were adopted in this study.

Since the Y chromosome harbors a set of uniparental markers, its haplotype is also used for ethnic and ancestral research, mapping of origins, and population development. Research involving Y chromosome haplotypes utilize a large sample size for providing larger statistical reason during data analysis. Therefore, the use of a reduced and effective protocol is important for analyses.

Research towards improving and standardizing methods for generating Y chromosome profiles and validation of reductionist protocols are currently underway in various laboratories worldwide. This work presents an economical and effective method of obtaining haplotypes and provides a basis for extension of these studies.

Conflicts of interest

The authors declare no conflict of interest.

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