

Validation of the AmpF ϕ STR[®] Yfiler[™] kit

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Abstract. The use of Y chromosome STR markers is of great utility in forensic cases, especially in sexual assault investigations. Recently it has been great improvement in commercial kits that offer large multiplex reactions in a single step, systems with high discrimination power and reliable and reproducible results. The AmpF ϕ STR[®] Yfiler[™] kit is the most recent commercial product of Applied Biosystems that offers 17 STR from human Y chromosome DYS19, DYS385a, DYS385b, DYS389 I, DYS389 II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS448, DYS456, DYS458, DYS635, Y GATA H4, DYS438 and DYS439. In this work several aspects were assayed: differences in equipment used for PCR amplification, sensitivity and specificity and application on forensic cases with low concentrations of human genomic DNA. © 2006 Published by Elsevier B.V.

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1. Introduction

The analysis of Y chromosome STR's systems in the forensic field is of great utility especially for the analysis and interpretation of mixtures. In female/male mixtures where there is prevalence of female cells, the analysis of the nuclear STR's masks the male profile; the study of Y-STR's systems is of great help to establish the male profile present in the mixture. In male mixtures, it is useful to establish the number of unrelated male individuals contributing to this mixture, which is very important for the interpretation of the results and statistical calculations. In this work, we carry out the evaluation of the kit AmpF ϕ STR[®] Yfiler[™] developed by Applied Biosystems, which include 17 Y chromosome STR's markers.

2. Materials and methods

Twenty DNA extracts were analyzed: 15 from blood samples, 3 from epithelial cells and 2 from postcoital samples, and also the Applied Biosystems 9947A and 007 cellular

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Table 1

Comparison of the results obtained in the two thermal cyclers used

Thermal cycler	Total number of analyzed samples	Obtained results	
		No. of samples completely typed	No. of allelic losses
9700	16	9	17
PTC-100	16	5	57

lines. The PCR conditions were those recommended by the manufacturer. The fragments were examined in genetic analyzers ABI PRISM 310 and ABI PRISM 3100.

3. Thermal cycler comparison

Sixteen different kind of samples (blood, epithelial cells, postcoital samples and cellular lines) were amplified under the same reaction conditions in two different thermal cyclers: Applied Biosystems 9700 and MJ Research PTC-100. When compared the obtained results, it was observed that the signals obtained in the AB 9700 were more uniform than those obtained in the PTC-100. Additionally in the PTC-100 more allelic losses were presented, 57 in the PTC-100 compared with 17 of the 9700 (Table 1). These findings are compatible with the recommendations of the manufacturer.

4. Sensitivity and specificity

Different concentrations from the Applied Biosystems 007 cellular line were used (10, 5.0, 1.0, 0.5, 0.1, 0.05, 0.025 and 0.01 ng). In the concentrations from 5.0 to 0.05 ng good signals were obtained with values above 75 rfu, results clearly interpretable and without artifacts. When the concentrations of 0.025 and 0.01 ng were used, allelic losses and low signals (below 75 rfu) were observed. For the 10 ng concentration, the stutter signals were increased, therefore we concluded that the optimum sample concentration for AmpF/STR® Yfiler™ kit is among 0.05 up to 5.0 ng (see Fig. 1).

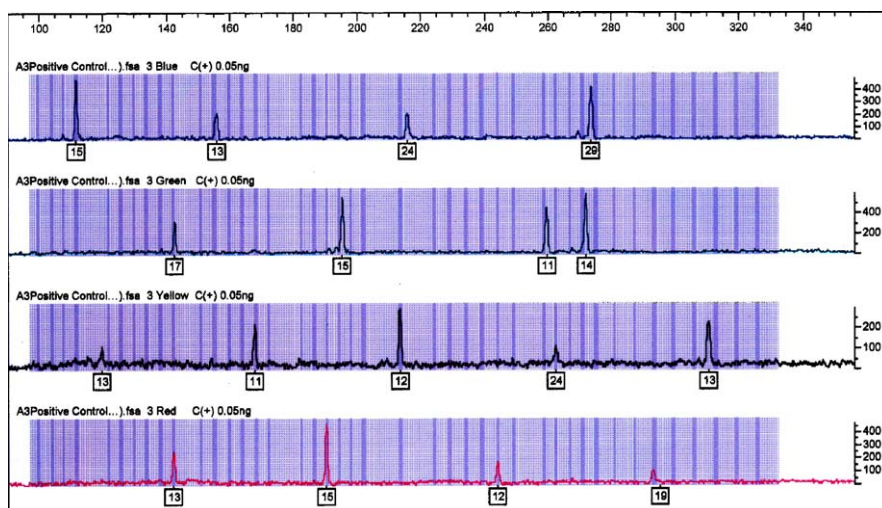


Fig. 1. Male control DNA 007 (0.05 ng).

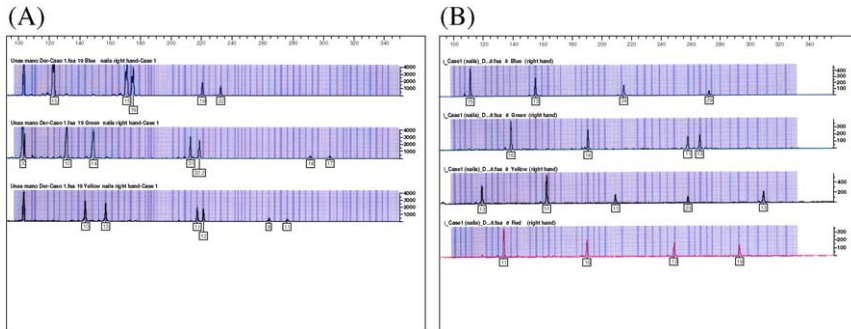


Fig 2. (A) Forensic casework subungual sample nuclear STR's and (B) Y-STR's results.

We also analyzed 3 female DNA samples in different concentrations from 5 to 100 ng and no signals were observed. With these results we can conclude that this kit is very sensitive and has a great specificity for male samples, which makes it very useful for use in forensic samples.

5. Forensic casework

Three different forensic casework samples were analyzed. In the first one the subungual samples taken from a homicide victim were analyzed. In the preliminary results of nuclear STR's a mixture was detected in samples taken from the left hand with prevalence of the victim's female profile. In the sample taken from the right hand, only the victim's profile was observed (see Fig. 2A). When analyzing these samples for the Y-STRs systems the male profile was detected in both hands (see Fig. 2B).

In the other two cases of sexual assault, samples with a count of 2 sperm cells in the whole preparation (more than 100 microscopic fields of 40 \times) and partial results for nuclear STR's systems were examined. When analyzing these samples for Y-STR's, signals above 75 rfu were obtained in all the 17 systems, clearly interpretable and without artifacts. These genetic profiles match with those obtained from the reference samples of the suspects. These results demonstrate the great utility of the use of YfilerTM AmpF ϕ STR[®] kit for this type of samples once again.

Finally we analyzed the inter laboratory samples from GEP-ISFG-2005 with the AmpF ϕ STR[®] YfilerTM kit, we obtained similar results from 44 participant laboratories for 11 Y-STR systems and the other systems from the six participant laboratories.