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TECHNICAL NOTE

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The Successful DNA Typing of Samples Following a Thermal Cycler Power Loss*

ABSTRACT: An approach for generating DNA profiles when critical samples have been consumed and a power outage occurs during the polymerase chain reaction (PCR) amplification reaction is described. This study demonstrates that a complete and accurate DNA short tandem repeat profile can be obtained: (1) when single source DNA samples are amplified for 26, 27, or 28 cycles using the Profiler Plus and COfiler Amplification Kits after an interruption in amplification, (2) from mock samples when PCR amplification has been interrupted early (after five cycles) or late (after 18 cycles) and the sample is subjected to an additional round of amplification, even after incubation of the sample at room temperature overnight, and (3) from nonprobative casework samples interrupted after approximately 18 cycles of amplification, an overnight incubation at room temperature and subjected to one or two additional rounds of PCR amplification for approximately 26 total cycles. Samples interrupted before five completed cycles and subjected to additional PCR cycles yielded variable results.

KEYWORDS: forensic science, DNA typing, polymerase chain reaction, COfiler, Profiler Plus, product gel

Polymerase chain reaction (PCR)-based test systems have been used in forensic science since the early 1990s (1), are highly sensitive (2,3), and can often yield results from biological evidence that was previously unsuitable for DNA analysis with Southern blotting and restriction fragment length polymorphism (RFLP) techniques (4). Because of the increased sensitivity of PCR-based systems, biological evidence of very limited quantity is often submitted for DNA analysis and consumed for a single test. Thus, a critical disruption of the testing process may prevent the analysis of an item of evidence where no sample or DNA remains for retesting.

On rare occasions power outages have occurred in our laboratory that disrupted the PCR amplification of samples. Normally, this results in the re-extraction of the remaining samples and/or amplification of remaining DNA. However, if an evidence sample is consumed during extraction and amplification, reanalysis is not possible. We have developed an approach for generating DNA profiles when a power outage has interrupted amplification and insufficient sample remains for retesting or there is a requirement to conserve any remaining sample. In this study we address whether samples interrupted early or late during PCR amplification, and then incubated overnight at room temperature, can be accurately typed and then apply this approach to nonprobative casework samples to illustrate its value in a forensic laboratory.

Materials and Methods

DNA Extraction, Quantitation, and PCR Amplification

Chelex® (Bio-Rad Laboratories, Hercules, CA) extractions were performed on nonsemen-containing samples according to standard procedures (5). Semen-containing samples were differentially extracted using a standard organic extraction procedure (6) and removal of the substrate using a piggy-back spin. Reagent blank controls were included with each extraction batch.

Samples were quantitated using the QuantiBlot® Human DNA Quantitation System (Applied Biosystems, Foster City, CA) as described in the user's manual. Approximately 0.75 ng of DNA were amplified for up to 28 cycles in 25 μL reactions in the Perkin Elmer 480 thermal cycler (Applied Biosystems), which was attached to an uninterruptible power supply (Franek, Tustin, CA), using the AmpFlSTR® Profiler Plus™ and COfiler™ PCR Amplification Kits (Applied Biosystems) and following the manufacturer's recommendations, except the final extension at 60°C was performed for 90 min and the final soak was at 15°C. Samples prematurely interrupted during amplification, either by power outage or experimentally, were incubated overnight at room temperature. Additional amplification cycles were performed on a Perkin Elmer 480 thermal cycler as described above. Some samples were amplified together postinterruption but for differing numbers of cycles. Samples were either amplified together and then stopped at various cycle numbers by removal from the thermal cycler and put

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on ice until their return to the thermal cycler for the extension step, or the samples were added to the thermal cycler in a staggered manner to achieve the correct total number of cycles for each sample before the extension step. Positive and negative amplification controls were included with each amplification.

Agarose Product Gel Electrophoresis

Four microliters of PCR-amplified products in 2 µL of 6X PCR gel loading mix (0.025% bromophenol blue, 30% w/v glycerol, and 25 mM EDTA) were electrophoresed in 1X TBE (89 mM Tris, 89 mM boric acid, and 2 mM EDTA) at 100–150 V for approximately 45 min on a 4% Nuseive™ 3:1 agarose gel (Cambrex, East Rutherford, NJ). 0.01% Gelstar® (Cambrex) staining solution was used to visualize the PCR products. Two hundred nanograms of the Gibco BRL (Gaithersburg, MD) 123 bp ladder was also electrophoresed in a separate lane as a molecular weight standard.

Capillary Electrophoresis and Analysis

PCR-amplified samples were analyzed on either the ABI Prism 310 or 3100 Genetic Analyzer. One to four microliters of PCR product from the samples and the positive amplification control, 1 µL GeneScan-500 ROX size standard (Applied Biosystems), and 24 µL deionized formamide (Applied Biosystems) were injected for 5 or 10 sec and electrophoresed using standard operating procedures. The intensity of the products on the product gel relative to the positive amplification control was used to determine the volume of product to use during electrophoresis and to determine if a 5 or 10 sec injection would be used. Four microliters of reagent blank controls and negative amplification controls were injected. Samples were analyzed using GeneScan 3.1 and Genotyper 2.5 software (Applied Biosystems). A threshold value of 60 relative fluorescence units (RFUs) was used as a minimum peak height definition for determining the presence of an allele.

All positive amplification controls gave complete profiles, except where noted in the text. All reagent blank controls and all negative amplification controls gave negative results devoid of any amplification artifacts.

Calculation of PCR Cycles Completed Before Power Outage

The following equation was used to estimate the number of PCR cycles completed before the power outage occurred:

$$\text{Approximate (number symbol) of cycles completed} = \frac{[(T - t) + T_U] - 15\,\text{mins}}{T_C}$$

where T is the time the power outage occurred, t the time the thermal cycler program was started (recorded by analyst at start of program), T_U the time (in minutes) the uninterruptable power supply ran (estimated by doing a mock amplification on the thermal cycler, unplugging the thermal cycler, and timing how long the thermal cycler continues to run), 15 min is the length of ramp time before the first cycle begins, T_C the length of time of one complete cycle (in minutes), taken as an average of several complete cycles.

Results/Discussion

Following a power loss to a thermal cycler and the resulting disruption of PCR amplification, partially amplified samples may be exposed overnight at room temperature. To determine: (1) if additional PCR cycles could be performed on the interrupted samples to obtain a reliable profile, (2) if the overnight room temperature incubation affects the integrity of the samples typing results, and (3) how many cycles must be completed to obtain a complete Profiler Plus profile with single source DNA samples, a mock power outage was carried out during the amplification of three neat DNA samples. After 18 PCR cycles were completed, the thermal cycler was unplugged to simulate a power outage. The samples were then incubated overnight at room temperature in the thermal cycler. The following day 7, 8, 9, or 10 additional cycles were completed for a total of 25, 26, 27, or 28 cycles, respectively. No additional reagents or *Taq* DNA polymerase were added before restarting the amplification. These samples were analyzed on an agarose product gel, and the appropriate volume was injected into an ABI 3100 Genetic Analyzer. The DNA profiles were compared with those obtained from the same three samples amplified for 28 uninterrupted cycles in accordance with the standard operating procedures (SOPs) of this laboratory. Full and concordant profiles were obtained using Profiler Plus and COfiler when 26–28 total cycles of PCR were performed and a nearly full profile was obtained with only 25 cycles (Fig. 1). Importantly, the heterozygous peak height ratios were maintained in all the samples tested regardless of total cycle number completed and no atypical (aberrant) results or artifacts were observed (Fig. 1). These results demonstrate that DNA samples can be readily and reliably typed following an overnight incubation at room temperature, and that the disruption late in the amplification process does not have an adverse effect on the quality of DNA typing. Moreover, complete or almost complete profiles were obtained with as few as 25 cycles. This indicates that the estimation of the number of cycles completed before a power outage does not have to be precise to obtain accurate full or partial profiles.

One would predict if a disruption of amplification has any adverse effect on PCR product generated in subsequent cycles, that a disruption early in the amplification of samples might have the most dramatic effect on the quality of DNA profiles as fewer amplification cycles would have been completed. To determine if samples disrupted early in the amplification process could also be

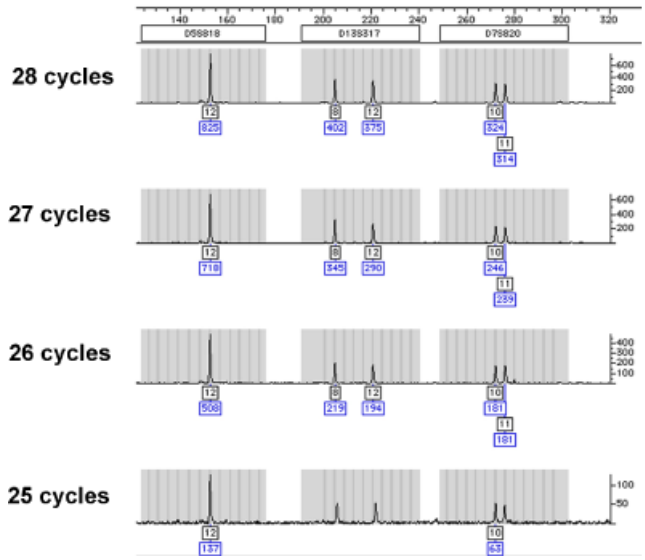

FIG. 1—*Representative profiles of the NED-labeled Profiler Plus ® amplified products typed on a 3100 Genetic Analyzer after 28, 27, 26, and 25 total amplification cycles. Fully concordant results were obtained from the FAM and JOE-labeled products, without any allelic dropout (data not shown). Allele designations and relative fluorescence units are shown in the boxes below the peaks.*

accurately typed, two neat samples and two mixed samples were disrupted following 1, 3, and 5 completed PCR cycles. These samples were incubated overnight at room temperature in the thermal cycler and then 27, 25, and 23 additional cycles performed, respectively, for a total of 28 cycles. The profiles obtained from these samples were compared with those obtained from the same DNA samples amplified according to our laboratory's SOPs. Both single-source samples amplified with COfiler and one single-source sample amplified with Profiler Plus interrupted after one or three completed cycles gave complete and correct profiles. One single-source sample amplified with Profiler Plus gave a correct but partial profile after interruption at one or three cycles (six and seven loci, respectively). Variable results were obtained for the two mixed profiles. One sample did not amplify well with Profiler Plus after one or three cycles; however, the COfiler profile was almost complete. The second mixed sample had a full primary profile when amplified with Profiler Plus after one or three cycles, and almost complete primary and secondary profiles when amplified with COfiler. The Profiler Plus positive amplification control interrupted after one cycle gave a partial profile (five loci); all other positive amplification controls gave complete profiles (data not shown). The only significant artifact observed was substantial –A in a few samples, but it did not interfere with the assignment of alleles. There was no consistent pattern of –A at one, three, or five cycles or within a sample. A very small off-ladder multipeaked artifact was observed in three samples (two samples at one cycle and one at both one and three cycles) at D5S818 between alleles 8 and 9. The samples amplified for five complete cycles before disruption of amplification yielded complete and accurate profiles without significant artifacts. Importantly, the peak height ratios at each locus were maintained in these samples (Fig. 2). These results indicate that samples disrupted early in the amplification process and incubated overnight at room temperature can also be reliably typed, and that five completed PCR cycles may be a critical point to ensure complete profiles.

To address the utility of this approach in the forensic laboratory for casework, nonprobative casework samples were tested. Four casework samples were differentially extracted yielding a non-sperm fraction (NSF) and a sperm fraction (SF). During the amplification of these samples in a Perkin Elmer (PE) 480 thermal cycler (Applied Biosystems) an unexpected power outage occurred after an unknown number of cycles, and the samples were at room temperature overnight. Fortunately, sufficient extracted DNA remained for amplification and subsequent reporting of the data. This series of events permitted a direct comparison of the profiles obtained from the samples amplified in accordance with our SOPs to the profiles obtained when the amplification was disrupted and additional amplification cycles were performed experimentally.

The number of completed PCR cycles is stored in the memory of more recently manufactured thermal cyclers such as the PE 9700. Unfortunately, this is not a feature of the older PE 480 thermal cycler, and no printer was attached to the 480 to record the cycles when the power outage occurred. Hence, it was necessary for us to establish a method to estimate the number of PCR cycles completed. Using the equation described in "Materials and Methods," the completed cycle number was estimated to be 18. As our laboratory has not validated the use of >28 cycles of amplification for these kits and to ensure the standard 28 cycles were not exceeded due to the possible inaccuracy in the estimated completed cycles, samples were targeted for 26 total PCR cycles. Thus, eight additional cycles were performed on the NSFs without any additional reagents or *Taq* polymerase, and the samples were analyzed on an agarose gel along with a standard curve of samples amplified for 25, 26, 27, and 28 cycles (Fig. 3*a*). Products were observed for all samples, suggesting that a sufficient number of cycles had been completed to obtain a useful profile.

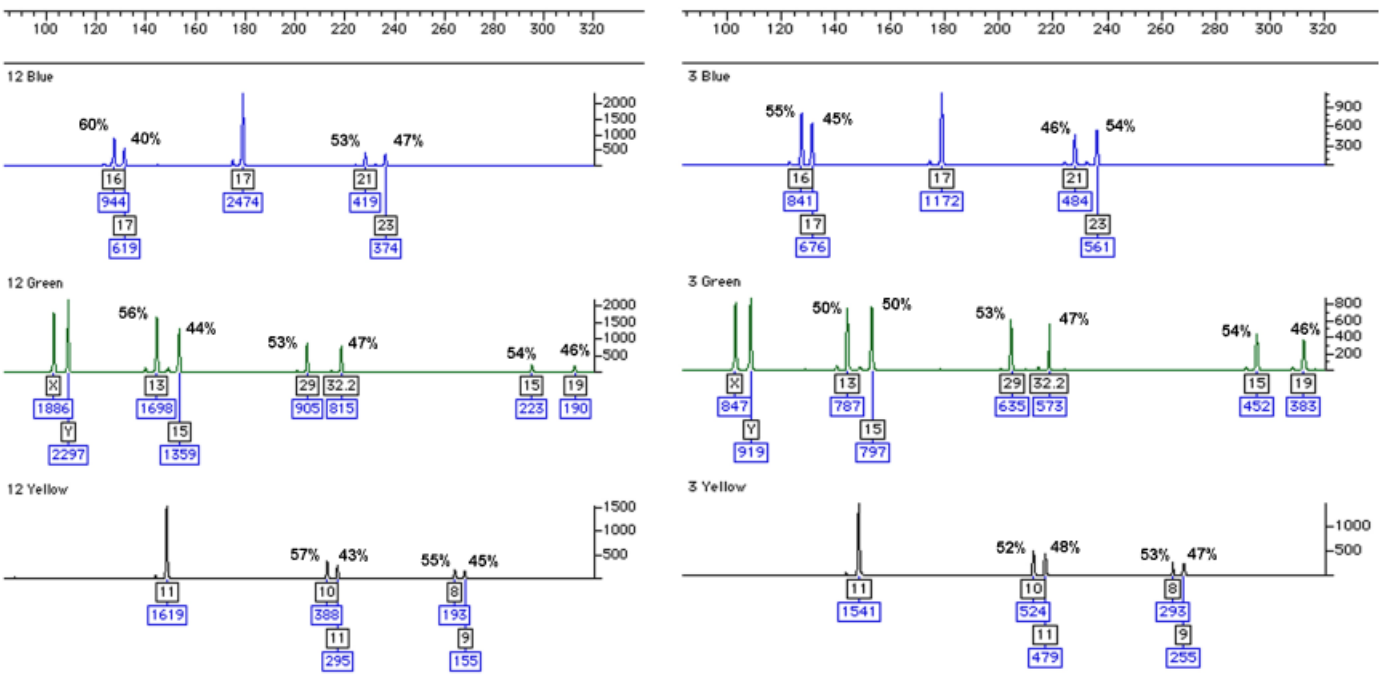

FIG. 2—*DNA profile using the Profiler Plus® Amplification kit obtained from a sample interrupted after five completed PCR cycles (left), incubated at room temperature overnight, and amplified for 23 more cycles the next day, and an aliquot of the sample amplified for 28 cycles without interruption according to the laboratory standard operating procedures (right). The percentages indicate the proportion of total relative fluorescence units (RFUs) at the locus. Allele designations and RFUs are shown in the boxes below the peaks.*

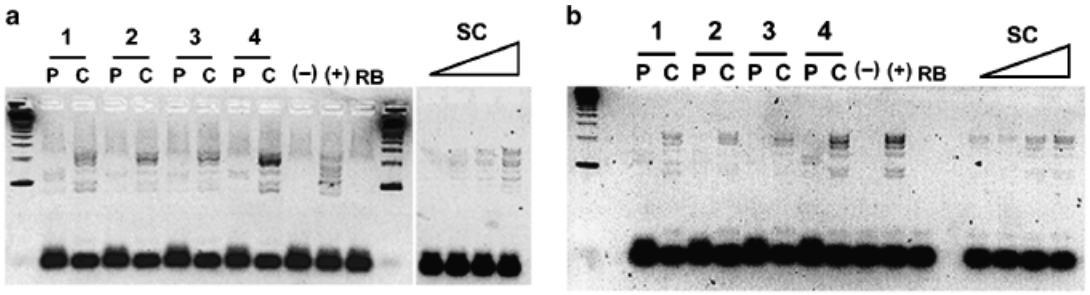

FIG. 3—*Product gel of nonsperm fraction samples 1–4 (a) and sperm fraction samples 1–4 (b) amplified with AmpFlSTR Profiler Plus (P) and COfiler (C). The standard curve (SC) includes samples amplified for 25, 26, 27, and 28 cycles, left to right, respectively, using Profiler Plus (left) and COfiler (right). (−) is the negative amplification control, (+) is the positive amplification control, and RB is the reagent blank control. The controls were amplified with Profiler Plus (left) and COfiler (right). The Gibco BRL 123 bp ladder was used as a size standard and is shown in the first lane of 3a and 3b, and between the RB and SC lanes in 3a.*

However, if the total cycle number was significantly underestimated and no product was observed on the product gel after one additional amplification session, it is unlikely that interpretable results would be obtained from the samples. To determine if a second round of additional amplification cycles could be performed while maintaining the integrity of the DNA profile results, an experiment was designed where the additional cycles were performed on two occasions. The SFs had four additional cycles performed first with an overnight incubation at 15°C. The samples were then analyzed on an agarose gel to illustrate that the necessary amplification had not been achieved as no product was observed (data not shown). Another four cycles of PCR were performed on the SFs and the samples analyzed on a second agarose gel before typing (Fig. 3*b*). Comparison of the product intensities of the experimental samples to the standard curve indicated that both the SF and NSF samples amplified robustly with COfiler® and less so when amplified with the Profiler Plus® system. The number and the combination of the primers involved in the amplifications may be partly responsible for this difference, however the actual basis is unclear. The intensity of the NSF and SF samples on the agarose gel was used as a guide for determining the injection volume and injection time on the ABI 3100 Genetic Analyzer.

Full and accurate DNA profiles were obtained from the single-source SF samples amplified for approximately 26 cycles in two additional amplification sessions with either the COfiler® or Profiler Plus® system (Fig. 4) and the single-source NSF samples amplified with COfiler in one additional amplification session. Partial profiles (four or seven loci) were obtained for the single-source NSF samples amplified with Profiler Plus. The heterozygous peak height ratios within loci were maintained in these samples (Fig. 4), demonstrating that this approach is applicable to forensic samples. The success with typing the SF samples also demonstrates that additional cycles can be performed on more than one occasion.

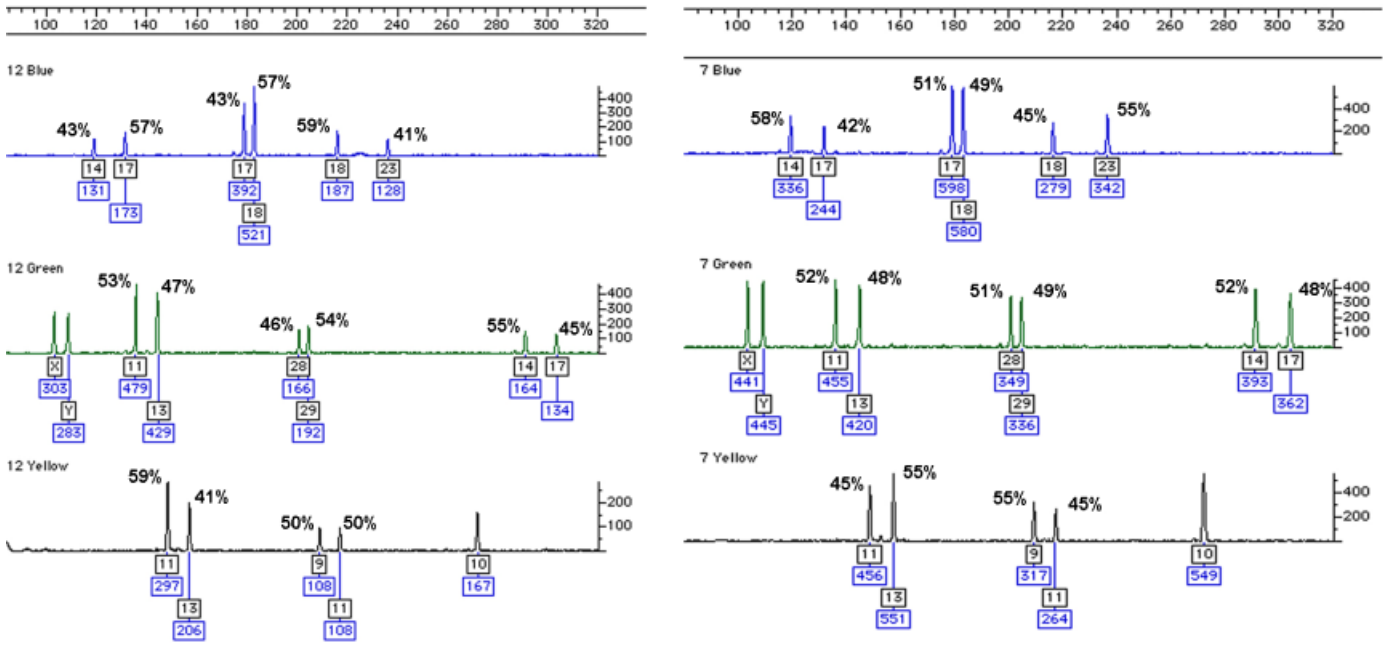

FIG. 4—*Representative DNA profile obtained using the Profiler Plus® Amplification Kit from a sperm fraction sample interrupted after approximately 18 completed polymerase chain reaction cycles (left), incubated overnight at room temperature and amplified for an additional four cycles with interruption and an additional four cycles (estimated at approximately 26 total cycles), and another aliquot of the same DNA sample amplified in accordance with the laboratory standard operating procedures for 28 total cycles (right). The percentages indicate the proportion of total relative fluorescence units (RFUs) at the locus. Allele designations and RFUs are shown in the boxes below the peaks.*

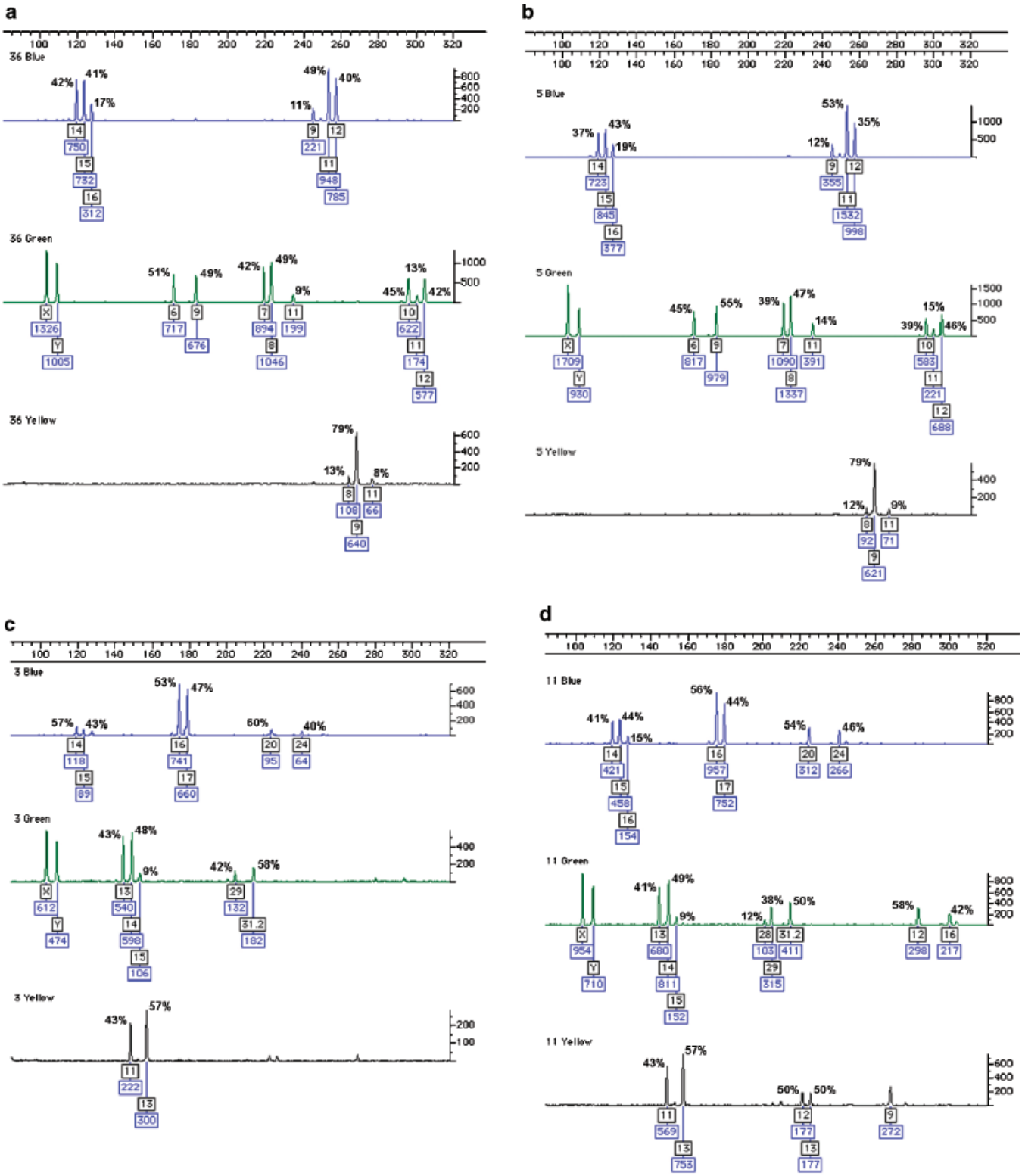

FIG. 5—*Representative DNA profile obtained using the COfiler® (a, b) and Profiler Plus® (c, d) Amplification Kits from a sperm fraction sample interrupted after approximately 18 completed PCR cycles (a, c), incubated overnight at room temperature and amplified for an additional four cycles with interruption and an additional four cycles (estimated at approximately 26 total cycles), and another aliquot of the same DNA sample amplified in accordance with the laboratory standard operating procedures (b, d). The percentages indicate the proportion of total relative fluorescence units (RFUs) at the locus. Allele designations and RFUs are shown in the boxes below the peaks.*

Full and accurate DNA profiles were also obtained from the mixture NSF and SF samples amplified with the COfiler® system (Figs. 5*a* and *b*). However, when these samples were amplified with the Profiler Plus® system, partial mixture profiles were generally obtained (Figs. 5*c* and *d*). It is important to note that the partial profile obtained from these samples was consistent with the primary DNA profile of the mixture; there were not any discordant types detected, and in general, the secondary types were lost. This

loss of types is probably due to the high likelihood that the samples were not amplified for the full 28 cycles.

These studies have demonstrated that complete or almost complete profiles can be obtained with 25–28 cycles and that reliable types can be obtained from samples that have been interrupted early or late during PCR amplification and then incubated overnight at room temperature followed by additional cycles of PCR on one or two occasions for a total of $\leq$28 cycles. Concordant single-source profiles were obtained with both the Profiler Plus® and COfiler® systems. Concordant profiles were also obtained when mixture samples were amplified with the COfiler® system while partial profiles containing no discordant types were obtained when mixtures were amplified with the Profiler Plus® system. Relative peak height ratios were maintained in both single-source and mixture samples. Although these studies were performed with the Profiler Plus and COfiler kits only, it is likely that similar results would be obtained for other commercially available multiplexed amplification kits, such as the AmpF*l*STR Identifiler Amplification Kit and the various PowerPlex Amplification kits (Promega, Madison, WI).

Additionally, this study demonstrates that a product gel may be used as an aid for estimating when a sufficient number of cycles have been completed to ensure a DNA profile after injection on a Genetic Analyzer. If products are not observed on the product gel for a sample and its corresponding positive amplification control, then it is likely that 24 or fewer cycles have been completed, and the samples could be amplified for a minimum of four more cycles. If the input DNA for amplification is less than the standard amount amplified in the laboratory, then the positive amplification control should be relied upon for estimating the total number of cycles completed. In all cases, the reagent blank control(s), and the positive and negative amplification controls should be treated in the same manner as the corresponding samples, and should be analyzed to determine that the appropriate results are observed as verification of the amplification process.

The application of the PCR to DNA profiling has enabled the forensic scientist to analyze minute evidentiary samples. On the rare occasion when a disruption to the testing process occurs, retesting is often the best decision, but retesting may not be an option if a sample is consumed or must be conserved. When reanalysis is not possible the approach described above offers a viable option.

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