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Improved STR Genotyping Results from Challenging Casework Samples in Germany Using the AmpF ℓ STR[®] SEfiler Plus[™] PCR Amplification Kit

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Due to the overwhelming success of forensic DNA typing in routine criminal cases, more and more evidence samples are collected from crime scenes and submitted to the laboratory. Initially, only typical biological stains such as blood, semen, and saliva traces that could be clearly identified as potentially suitable for DNA typing were taken. The increased sensitivity of PCR-based typing methods using fluorescence-based detection methods of amplified STR fragments has increasingly changed this focus to include all types of contact stains from objects which might have been touched by hands, face or even the ear of a perpetrator, e.g. an earprint from a door where someone might have listened. Typically, the area of the presumed skin contact is swabbed using a sterile dry or moistened cotton swab, which is subsequently dried and sent to the laboratory.

The only way to find out whether such a swab is suited for STR typing is to perform a DNA extraction and STR typing. Although a significant number of contact stains fail to yield a sufficient amount of DNA, there is a relevant proportion of samples generating a complete STR profile suitable to be entered into a criminal DNA database. This is illustrated in Figure 1 displaying the typing success of 471 consecutive crime scene samples which were subjected to STR analysis in our laboratory. Full STR profiles for the eight German DNA database loci (comprised of the seven loci from the European standard set, i.e. THO1, VWA, D21S11, FGA, D8S1179, D3S1358, D18S51 as well as ACTBP2, also known as SE33) were obtained for almost 90% of blood stains and cigarette butts, and between 48 and 56% of clothing items and bottle necks. For the challenging contact stains, which represent the largest number of samples of this survey (n=254), 11% gave a full profile from a single person suitable for the database, whereas an additional 37% gave partial or mixed profiles not suitable for the database, but still useful for direct comparisons with suspect profiles. Thus, useful results could be obtained for almost 50% of all contact stains analyzed. On the other hand, more than 50% of these stains as well as significant proportions of up to 18% of the other stain types gave no results, or poor results that could not even be used for a direct comparison. One reason for this is of course due to the fact that either no or only a low amount of DNA was available from the swab, or that the DNA was too degraded for standard STR analysis. Another important reason could be the presence of PCR inhibitors on the swabbed surface preventing the efficient amplification of DNA from the epithelial cells.

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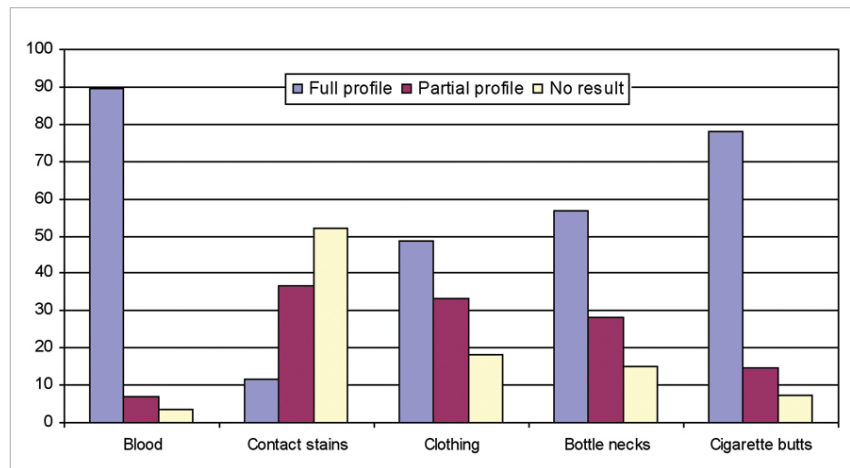


Figure 1: STR typing success for different types of crime scene samples using the SEfiler™ kit (in %). Data were obtained from 471 consecutive samples as follows: blood stains (n=57), direct contact stains (n=254), clothing items such as gloves, caps, sweaters, socks (n=66), bottle necks or other drink containers (n=53), and cigarette butts (n=41).

In the context of a validation study, we have tested the newly released SEfiler Plus™ kit. Based on developments for the AmpF/STR® MiniFiler™ PCR Amplification Kit, the SEfiler Plus™ kit contains a modified buffer system to achieve a more uniform and balanced amplification, more robustness in the presence of inhibitors, as well as enhanced sensitivity. Furthermore, as for the MiniFiler™ kit, the recommended number of amplification cycles has been increased from 28 to 30. Our results indicate that the overall performance has been improved compared to the current version of the SEfiler™ kit, although this kit was already working well for our routine casework samples, as described above. The sensitivity and uniformity of amplification is illustrated in Figure 2: two-fold dilutions of control DNA starting from 250 pg down to 31.25 pg have been tested, and the STR fragments of the “blue systems” are shown in the figure. Peak heights for the 250 pg and 125 pg samples are sufficiently uniform and acceptable. For the 62.5 pg sample, peak balance and height are more diverse, and allelic drop-out becomes evident in the 31.25 pg sample (VWA). Based on the results of four replicates for each of the different dilutions, no drop-out was observed for the 250 and 125 pg samples, occasional drop-out occurred for the 62.5 pg sample (only one of a total of 96 alleles was lost), and more frequent drop-out was found for the 31.25 pg sample (12 of 96 alleles at 6 different loci). Clearly, stochastic effects are the cause for this loss of information, as there is no evidence that the drop-out was locus-dependent.



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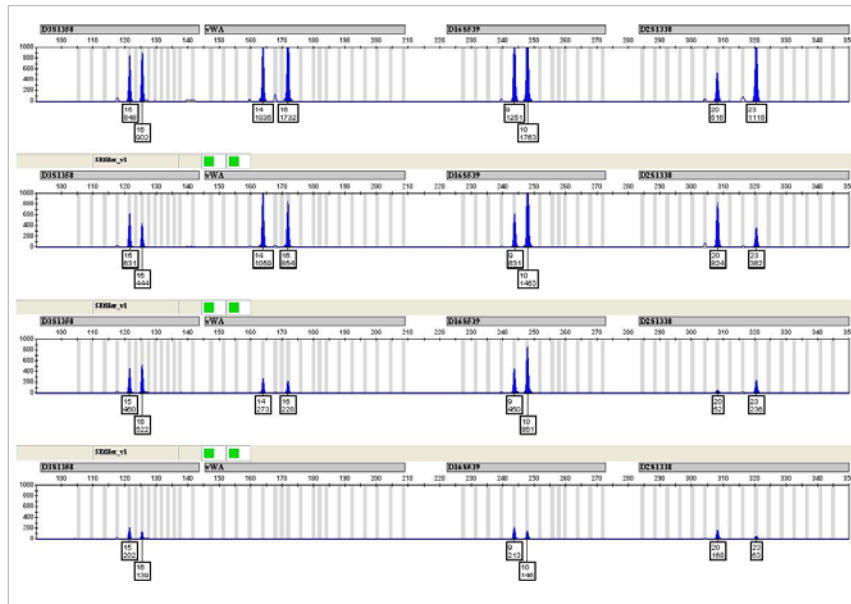


Figure 2: STR typing results of DNA dilutions using the SEfiler Plus™ kit. Human control DNA 007 was subjected to PCR using 250 pg, 125 pg, 62.5 pg, and 31.25 pg, respectively (panels from top to bottom); the scale has been adjusted to 1000 rfu for all panels.

Routinely, we subject stain samples with poor DNA yield and/or quality to 30 PCR cycles. This normally increases the total number of successfully typed alleles, but also the apparent peak height imbalance between alleles. However, such results are only reported when confirmed in a second experiment. Figure 3 illustrates an example of a case where alleles were detected for all eight database loci using the standard SEfiler™ kit with 30 cycles, although there was clear evidence for the occurrence of locus and allele dropout (possibly affecting D16, D2, FGA, D18, top panel). The subsequent analysis using the SEfiler Plus™ kit confirms all alleles observed in the first experiment, and reveals further alleles for some of the affected loci (bottom panel). A possibly spurious third allele is detected at THO1, but could also be due to a low level mixture. For D18S1, only a single prominent allele is present, so that drop-out of a second allele becomes less likely. Overall, the peak heights within each fluorescent dye panel are more uniform and balanced. The typical “ski slope” effect of amplification signals characterized by decreasing peak heights with increasing fragment sizes, as depicted by the standard SEfiler™ results for this sample, is not found. Based on our observations from the validation study, the SEfiler Plus™ kit generates more consistent and reproducible STR profiles in particular from challenging samples with low DNA content, or containing potentially inhibitory substances reducing the amplification efficiency.



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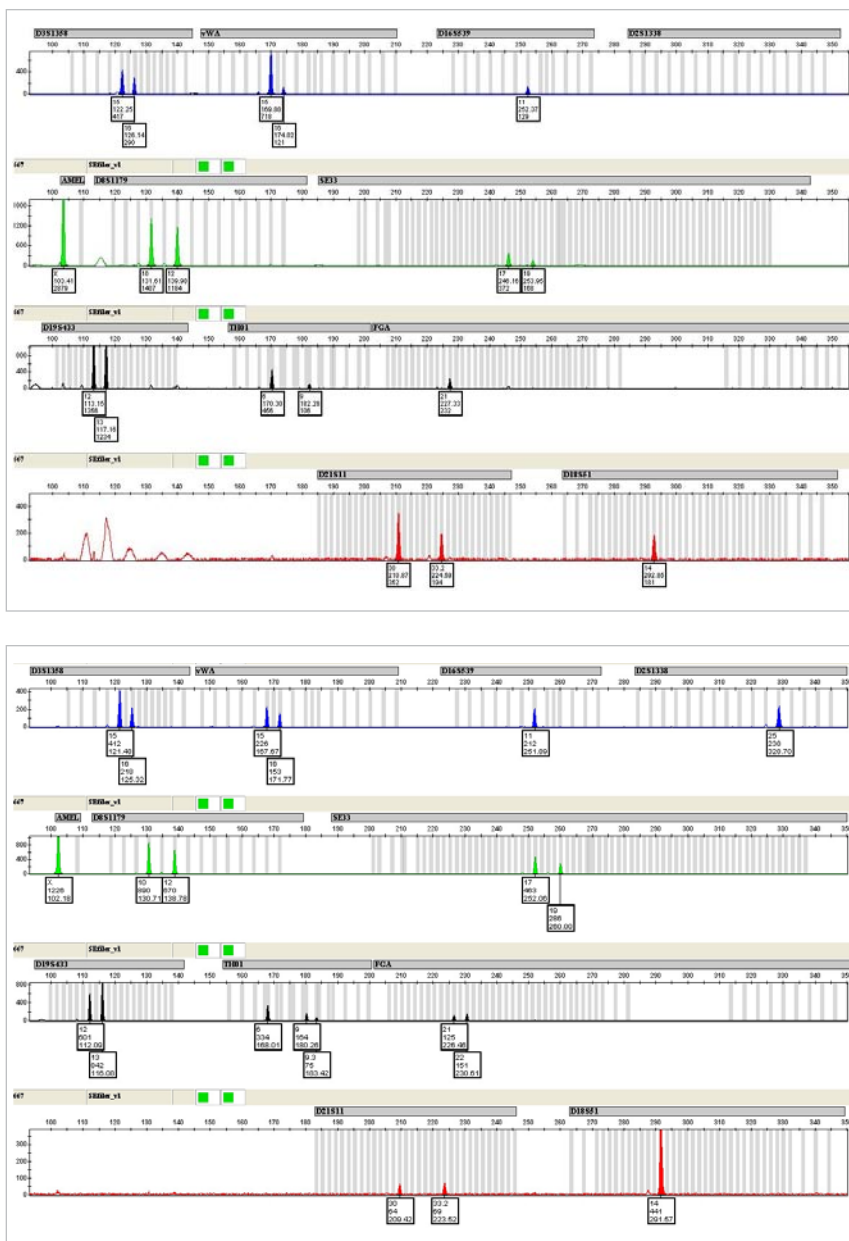


Figure 3: Comparison of standard SEfiler™ kit and SEfiler Plus™ kit amplifications of a forensic sample. DNA was extracted from a cigarette end, and approximately 150 pg (as determined by Quantifiler PCR) were subjected to a 30 cycle PCR for both the standard SEfiler™ kit (top) and the SEfiler Plus™ kit (bottom).

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Nevertheless, there is a sensitivity limit for obtaining reliable DNA typing results. When all biochemical parameters are optimized and fine-tuned to achieve a nearly 100% amplification efficiency, a minimum number of template molecules is still required to ensure a complete typing result. Below this limit, stochastic effects leading to preferential amplification of one allele and subsequent drop-out of another allele will influence the typing results and their interpretation (Gill et al., 2005, 2006). Typically, DNA samples with concentrations of less than 150-200 pg, or samples with more total DNA but containing a minor component in this range, will be affected, and results from such samples have to be interpreted with great caution. The improved chemistry of the SEfiler Plus™ kit will help to better understand and control these effects which we are currently observing more often in routine casework samples.

References

1. Gill P, Curran J, Elliot K; A graphical simulation model of the entire DNA process associated with the analysis of short tandem repeat loci, *Nucleic Acids Res* 33 (2005) 632-643.
2. Gill P, Brenner CH, Buckleton JS, Carracedo A, Krawczak M, Mayr WR, Morling N, Prinz M, Schneider PM, Weir BS; DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci Int.* 160 (2006) 90-101.