B14 Increasing the Efficiency of STR Profiles Through Amplified Product Concentration

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This presentation will impact the forensic community and/or humanity by providing an alternative to re-amplification which preserves sample extract and increases the possibility of generating a profile in the absence of any remaining extract.

In forensic nuclear DNA analysis, DNA profiles for unknown case samples are generated using one of several commercially available short tandem repeat (STR) kits. A common occurrence with inhibited or degraded samples is the generation of a partial profile (low-intensity alleles), or no profile at all. However, there may not be enough extract or specimen remaining to re-amplify these samples. The goals of the experiments described in this presentation were to determine if the concentration of amplified STR products by vacuum evaporation could increase the relative fluorescence units (RFUs) of the alleles that were originally below the reporting threshold, and if so, to validate this concentration method for use at the Armed Forces DNA Identification Laboratory (AFDIL).

In order to validate the concentration procedure, 6 samples of 1.0 ng positive control DNA were amplified and analyzed with the ABI AmpFlSTR® Profiler Plus and AmpFlSTR® Cofiler kits on the ABI 377. All of the alleles from these samples were over AFDIL's 50 RFU reporting threshold. One of the positive DNA control amplification products from each kit was serially diluted as follows: (a) 1:4, (b) 1:8, (c) 1:16, (d) 1:32, (e) 1:16, (f) 1:32, (g) 1:64, and the dilutions were analyzed on the ABI 377. Dilutions that generated either no STR results or partial STR profiles (profiles that had allele peaks below 50 RFUs) were used as the standard by which nine of the remaining ten STR amplification products were diluted. Results demonstrated that 120 out of 150 possible allele peaks were below 50 RFUs for Profiler Plus and 104 out of 120 possible allele peaks were below 50 RFUs for Cofiler.

To test whether concentration of the diluted product would increase the allele peak RFUs, the diluted amplification products (described above) were transferred to 1.7 mL tubes and dried down completely in a Jouan HetoVac for 1 hour. The concentrated amplification products were then re-suspended in a loading solution containing 4 uL distilled water and 5 uL formamide loading buffer (formamide, GS500, and Bromophenol blue) and reanalyzed. The resulting STR profiles were compared to their corresponding diluted profiles for increases in peak RFUs. On average, the peak heights for Profiler Plus increased 4-fold and for Cofiler 6-fold. After concentration, 116 out of the 120 Profiler Plus peaks previously below AFDIL's 50 RFU cut off were now above the reporting threshold. Likewise, 96 out of 104 allele peaks that were below reporting thresholds for Cofiler were now above 50 RFUs. In no instance were additional extraneous peaks observed for any sample after concentration.

The concentration method was then applied to non-probative casework reagent blanks, negatives, and substrate controls to determine if any minor contaminates that were not detectable prior to concentration were evident after concentration. A total of 39 controls consisting of 16 Profiler Plus negatives and 19 Profiler Plus reagent blanks and 1 Cofiler negative, 1 Cofiler reagent blank, and 2 Cofiler substrate controls that had no visible peaks prior to concentration were concentrated. After concentration, 32 of the 39 controls had no visible peaks; however, the remaining seven controls exhibited 1 or 2 peaks that Genotyper called as true alleles. The concentrated profiles were then compared to the original sample profiles from their respective cases and in no instance did the concentrated peaks match any of the original case sample profiles, suggesting that these peaks were most likely the result of concentrating a low-level PCR contaminant that was present in the unconcentrated sample.

As the final validation step, twenty-two casework samples from Qiagen BioRobot extracted bloodstain cards that had exhibited partial Profiler Plus profiles were concentrated and analyzed as described above. Results demonstrated that 80% of the peaks, or 74 out of 92 possible allele peaks increased in RFUs after concentration. Of the 74 peaks that increased in RFUs, 52% or 48 peaks were brought above AFDIL's 50 RFU-reporting threshold. The average peak height increased 3.5fold. In all instances but one, the post-concentration peaks were in concordance with the original STR profiles generated during case processing. In one sample, an extraneous peak was observed at the FGA locus. This peak, however, was not present after the sample was reloaded, arguing that the peak was most likely due to a gel artifact.

In conclusion, concentration of STR amplification products has proven to be effective in improving the chance of obtaining an STR profile when no profile or a partial profile was originally generated after amplification. Concentration provides an alternative to re-amplification, which preserves the sample extract and increases the possibility of generating a profile in the absence of any remaining extract. In addition, the baseline background for the samples is not elevated after the concentration process, which suggests that this process does not introduce artifacts into the samples that are not already present at low levels. However, it is recommended that all negatives, reagent blanks, and substrate controls be concentrated along with their

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corresponding casework samples for comparison on a case-by-case basis.

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STR, Partial Profile, PCR Product Concentration

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