STR DNA Typing: Increased Sensitivity and Efficient Sample Consumption Using Reduced PCR Reaction Volumes for Database Analysis, and Casework Implications

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With the advent of the PCR technology and availability of highly discriminative megaplex kits, only a few nanograms of template DNA are required for successful typing to be achieved in a single PCR amplification reaction. Still, crime scene samples where DNA extraction yields less than this minimal requirement are encountered in forensic casework. This can lead to low intensity profiles in STR analysis, and to consumption of all available template, neither of which is desirable in a forensic setting. Although cognizant of the potential pitfalls associated with amplifying too few targets in PCR or in too small a volume, we have sought to explore the potential benefits of using microfluidics to:

1. enhance detection and sensitivity;
2. reduce consumption of irreplaceable crime scene samples.

In a first experiment, two control and four casework samples presenting some level of difficulty in interpretation, essentially weak/degraded samples or mixtures, were subjected to amplification under 15 different "amount of template DNA" (0.063 – 2 ng)/volume (5 – 40 µl) ratios in a Perkin-Elmer 9600 Thermal Cycler, with 200 µl string-cap MicroAmp tubes. Results showed that a concomitant four-fold reduction for both reaction volume and template DNA (down to 10 µl and 0.125 ng) produced identical profiles with same characteristics of signal intensity and allele ratios at heterozygous loci (HR). An eight-fold reduction (down to 5 µl and 0.063 ng) provided the same signal intensity but HR values were slightly affected in one to three STR loci for 80% of control samples and 50% of casework samples. Loss of moisture appeared to be the cause of this artifact. (It should be noted that attached-cap tubes were also tested and shown to permit even more significant evaporation of solute.) Stochastic effects were not observed in the tested range.

In a second experiment, 20 additional casework samples were tested on a restricted range of "amount of template DNA"/volume ratios and 14 more with a single 2 ng/5 µl condition. Similar results were observed

In summary, with appropriate amplification tubes, 0.5 ng of DNA in a 10 µl reaction produces an identical profile to a 2 ng sample of the same DNA in a 40 µl reaction. The same profile is obtained still with 0.25 ng of DNA in a 5 µl reaction. Although quite suitable for a database application, profiles generated with 5 µl reaction volumes may potentially be problematic in mixture ratio analysis situations of casework samples. We conclude that PCR reaction volume reduction can enhance detection and sensitivity while reducing consumption of:

1. irreplaceable crime scene samples and
2. costly reagents