B155 Comparing Minifiler™, Identifiler™, and Powerplex® 16 Performance With Challenging Samples

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The goal of this presentation is to illustrate considerations and comparative advantages of use of MiniFiler, Identifiler, and PowerPlex 16 for DNA casework.

This presentation will impact the forensic science community by providing insight in STR kit selection for casework analysis.

In processing remains from the World Trade Center site, Bosnian and Mexican graves, and many other sample sources, our laboratory has become interested and expert in evaluation of challenging remains. In this work, we compared performance of the recently introduced Amp*Fl*STR™ MiniFiler PCR Amplification Kit by Applied Biosystems with other commercial multiplexes for use with challenged samples.

The MiniFiler Users Guide indicates the product "is an assay optimized for genotyping degraded and/or inhibited DNA samples." Recent evaluation of MiniFiler performance confirms it can be used to identify some of the larger missing loci absent in Identifiler amplifications in some particularly challenged samples (Eisenberg). However, Hill et al. recently published that the primers sequences selected display 27 instances of discordant allele calls among 1308 tested individuals when comparing profiles between this system and the Identifiler system.

This 2% discordance in sample matching prompted us to investigate whether there are ways to "optimize" existing Identifiler and/or PowerPlex 16 kits to meet the needs of profiling challenged samples and to avoid this discordance in results versus CODIS- searchable profiles. To investigate this possibility, first the amplification reaction volume was lowered to 6µl with each kit to generate stronger signals. Next, 32 cycle amplification for PowerPlex 16 (at the top end of the Manufacturer's recommended number), 32 cycle amplification for Identifiler (four cycles above the Manufacturer's recommended number), and 30 cycles for MiniFiler (equal to the Manufacturer's recommended number) were selected to provide more even signal.

Multiplex performance was then compared under these modified conditions while challenging the systems with:

1) Small amounts of DNA Template.
2) Degraded DNA templates created by digestion with DNAse
3) UV-treated DNA substrates
4) Inhibition by addition of increasing amount of indigo, humic acid, and hematin to the amplification reactions.

Under these conditions, all three multiplex systems generally performed similarly with small amounts of template, with degraded template, and with UV-treated template. MiniFiler provided the strongest profiles in the presence of indigo and humic acid, but differences were not dramatic. PowerPlex 16 displayed the greatest resistance to hematin, again with no dramatic differences among systems.

In conclusion, with optimized conditions for all systems, Identifiler, PowerPlex 16, and MiniFiler all work well with challenging samples. The greater concordance among Identifiler, PowerPlex 16, and CODIS entries, and the ability to obtain more profiled loci with the megaplex systems suggest that modifying the protocols with use of these systems may be preferred to use of MiniFiler as a first follow up when obtaining a poor profile with a challenged sample. Minifiler would provide a second backup.

References:

Eisenberg AJ, October, 2006. Presentation at 17th International Symposium on Human Identification, abstract published at http://www.promega.com/geneticidproc/ussymp17proc/oralpresentations/Eisenberg.pdf.

Hill C, et al., *J Forensic Sci.* July 2007, 52;4:870-873.

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Inhibitors, Degradation, MiniFiler

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