B154 Principles of STR Multiplex Amplification You May Not Know

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The goal of this presentation is to relay the principles that provide quality STR profiling under a variety of amplification conditions.

This presentation will impact the forensic science community by demonstrating how the implementation of these principles can save sample material and reagent expense to produce high quality DNA profiles.

STR multiplex amplification has been studied to understand general principles relating effects of changes in template amount, multiplex primer concentration, amplification cycle number, and amplification volume. The results reveal that, except for stochastic variation with limited template amounts, the same quality, intensity, and accuracy of DNA profiles can be obtained while varying each of these parameters over a broad range. In fact, often these parameters can be modified, but balanced to produce identical results under very different conditions.

Previous authors (Gaines et al., Leclair et al., Frégeau et al.) have shown that in specific instances with specific multiplexes that amplification volume can be lowered to 12.5µl and even 5µl reaction volumes with no loss in multiplex performance. This workcan be extended to demonstrate that there is a systematic inversely proportional relationship between amplification reaction volume and amplification product intensity for all multiplexes. That is to say with the same amount of template, a 2µl amplification reaction is stronger than a 6µl reaction which in turn is stronger than a 25µl reaction which again in turn is stronger than a 50µl reaction.

A low volume amplification reaction approach not only saves reagent material, it also limits the requirement for sample consumption. Increased intensity is also shown of lower volume amplification reactions can be offset by use of either lower primer concentration or fewer amplification cycles during thermal cycling, or both. Thus, a balanced profile indistinguishable from that using the manufacturer's recommended reagent concentrations and amplification protocol can be generated with the same reliability with lowered cost in both reagent and sample consumption.

It is illustrates that these principles apply universally across all NDIS-approved commercially available multiplex sets including PowerPlex 16, COfiler, Profiler Plus, and Identifiler. This knowledge has important implications that will be discussed regarding evaluation of low copy number samples.

References:

Gaines ML et al. *J Forensic Sci* 2002;47(6):1224-1237. Leclair B et al. *J Forensic Sci* 2003;48(5):1001-1013. Frégeau CJ et al. *J Forensic Sci* 2003;48(5):1014-1034.

STR, Low Copy Number, PCR

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