B77 An Examination of Primer Binding Site Mutations Occurring Between miniSTRs and Commercial Multiplex Kits

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After attending this presentation, attendees will learn information on the concordance between the Miniplex reduced-size primer sets and commercial STR kits as well as the sources of any non-concordance.

This presentation will impact the forensic community and/or humanity by identifying possible sources of non-concordance in DNA profiles in a variety of population samples by sequencing the region between the Miniplex kits and standard STR kits.

The goal of this study is to utilize population studies involving miniSTRs and standard multiplex STR kits in order to identify primer binding site mutations between the different STR kits.

Miniplexes are a set of four multiplex STR loci which contain 3-6 reduced size STR amplicons per set. The primers are re-designed to be placed as close as possible to the end of the repeats to reduce amplified product size.[1] To avoid overlap between loci only one dye lane is used for each locus. The primer sets include 12 out of 13 of the Combined Index System (CODIS) core STR loci. Miniplexes 1 and 3 can be combined to create a six (6) loci kit called Big Mini. The Miniplex kits allow reduction in size of amplified products (amplicons) of up to 299 base pairs, with most amplicons ranging in size from 60-200 base pairs. The primers are tagged with fluorescent dyes (6FAM, VIC and NED) for detection. Because of the reduced size of the PCR products, the MiniSTRs provide a valuable tool in the analysis of samples where allele dropout and reduced sensitivity of larger alleles occurs due to degradation of the DNA.

Concordance studies are used to demonstrate that these new primers will produce consistent genotypes when compared to those generated by commercial kits. Concordance studies have been performed for Asian, African American, Caucasian, and Hispanic population samples. Results generally show a 98% concordance between MiniSTRs and commercial STR multiplexes with most of the differences occurring at two loci, vWA and D13S317.[2] To perform these studies, DNA concentrations ranging from 200 to 300 pg were amplified with Big Mini, Mini 2 and Mini 4 kits in a 25 µl reaction volume for 33 cycles. Lower amounts of template were necessary due to the increases sensitivity of the MiniSTRs. The analysis of the samples was performed using organic extractions with real time PCR for quantification. Amplification and typing were performed using the Identifiler™ and Miniplex Kits.

Primer binding site mutations can lead to allele drop out or low sensitivity of one allele during DNA analysis. Even though the presences of many polymorphisms were taken in consideration during development of the Miniplex kits, unknown problems may still emerge. Among the samples where lack of concordance was found, representative samples from different loci were chosen to be sequenced. Previously unknown polymorphisms within the primer binding sites were detected in certain samples, leading to allele loss and non- concordant homozygous profiles. Deletions between the primer binding sites for the Miniplexes and the commercial kits were another source of non-concordance, resulting in allele calls that were one repeat unit larger than those produced by the commercial kits.

Interestingly, in these studies instances where redundancy in sequence permitted primer binding in spite of the presence of deleted sequences were found. Overall, the result of this work demonstrate a high degree of concordance between the different amplicons and the sequencing data reveals the direct effect of primer binding mutations on allele calls.

1. *Butler, J.; Shen, Y.; McCord, B. The Development of reduced size STR amplicons as tools for analysis of degraded DNA.* Journal of Forensic Sciences, ***2003,*** 48(5)*, 1054-1064.*
2. Drábek, J.; Chung, D.; Butler, J.; McCord, B. Concordance study between Miniplex assays and a commercial STR typing kit, *J. Forensic Sciences*, 2004, *49(4)*, 859-860.

Miniplexes, Mutations, Concordance

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