A110 Concordance Testing Comparing STR Multiplex Kits With a Standard Data Set

Carolyn R. Hill, MS, National Institute of Standards and Technology, 100 Bureau Drive, MS8312, Gaithersburg, MD 20899; Margaret C. Kline, MS, National Institute of Standards and Technology, 100 Bureau Drive, Building 227 Room B226, MS 8311, Gaithersburg, MD 20899-8311; David L. Duewer, PhD, and Peter M. Vallone, PhD, National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899-8311; and John M. Butler, PhD, National Institute of Standards and Technology, 100 Bureau Drive, Mail Stop 8311, Gaithersburg, MD 20899-8311*

After attending this presentation, attendees will understand the value of concordance testing using commercial forensic DNA short tandem repeat (STR) multiplex kits to detect allelic dropout or "null" alleles present in a standard data set.

This presentation will impact the forensic science community by demonstrating that null alleles do occur within a standard data set when comparing STR multiplex kits with different primer sequences. These "null" alleles have been sequenced to confirm the results and determine the cause (primer binding site mutations) and are reported to the forensic community.

Concordance evaluations are important to detect allelic dropout or "null alleles" present in a data set. These studies are performed because there are a variety of commercial STR multiplex kits with different configurations of STR markers available to the forensic community.[1] The electrophoretic mobility of the markers can vary between kits because the primer sequences were designed to amplify different polymerase chain reaction (PCR) product sizes. When multiple primer sets are used, there is concern that allele dropout may occur due to primer binding site mutations that affect one set of primers but not another.[2] These null alleles become evident only when data sets are compared. Null alleles are a concern because this could result in a false-negative or incorrect exclusion of two samples that come from a common source (only if different PCR primers are used). A base pair change in the DNA template at the PCR primer binding region can disrupt primer hybridization and result in a failure to amplify and detect an existing allele.[2]

Multiple concordance studies have been performed at NIST with a standard sample set (~1450 in-house U.S. population samples) using various STR multiplex kits including Applied Biosystems Identifiler®, MiniFiler™, NGM™, SGM Plus™, and Profiler Plus™ kits, as well as Promega PowerPlex® 16, ESX 17 and ESI 17 Systems.[3-8] Various discordant results have been identified using concordance software developed at NIST, confirmed by DNA sequencing, and reported to the forensic community on the null allele web page of STRBase.[9]

To test for concordance between data sets, the current strategy at NIST is to use standard samples, software, sequencing, and STRBase, or the four "S"s of concordance. Ultimately, the information can be used by kit manufacturers when designing new STR multiplexes to either add an extra (degenerate) primer or redesign primers away from primer binding site mutations in the final kit configurations. Some kit manufacturers decide not to change the primer sequences and rely simply on the documentation or publication of the reported null alleles.[3]

Even though concordance studies are important to find null alleles between data sets, discordant results rarely occur for most primer sets. At NIST, concordance evaluations have been performed for over 150,000 allele comparisons. In the MiniFiler concordance study,[4] 99.7% full concordance was observed and in the PowerPlex ESX 17/ESI 17 study,[5] full concordance was seen for >99.8% in all comparisons performed.

In addition, comprehensive STR multiplex evaluations to determine and characterize kit performance were completed with a subset of the aforementioned kits, including NGM™, SGM Plus™, Profiler Plus™, PowerPlex® ESX 17, and ESI 17 Systems. The thorough examinations include heterozygote peak height ratio and stutter percentage calculations, as well as the determination of allele frequencies and population statistics heterozygosities and probability of identities).

A summary of the results, including discordance and kit performance results, will be shown in order to help assess the benefits of performing concordance testing using a standard data set with STR multiplex kits that have different primer sequences for the same markers.

References:

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 ** Presenting Author*