



Pathology/Biology Section - 2016

H13 Internal Validation of the Promega® PowerPlex® Y23 Amplification Kit for Use in Forensic Casework

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After attending this presentation, attendees will understand Y-chromosomal Short Tandem Repeat (Y-STRs), the Promega® PowerPlex® Y23 PCR amplification kit, and the benefits the kit can provide if implemented in conjunction with traditional STR testing in forensic casework.

This presentation will impact the forensic science community by demonstrating the improved capabilities of Promega's® newest Y-STR system which includes greater efficiency and discrimination. The system can be used for both casework and direct amplification applications with a shortened amplification time at an hour and a half, as well as demonstrate a large degree of sensitivity, even in the presence of excessive female DNA.

Y-STR systems can be an effective tool in a variety of ways within the forensic DNA community. Most commonly, Y-STRs have demonstrated their usefulness in sexual assault evidence where the female DNA contribution overwhelms the male DNA contribution or when a differential extraction cannot effectively separate the male and female components. Generation of male profiles with Y-STRs can also assist in the identification of missing persons and human remains within the Combined DNA Index System (CODIS) in conjunction with traditional autosomal STR testing. Additionally, Y-STR testing can help in distinguishing paternally unrelated male contributors in complex autosomal DNA mixtures as well as potentially excluding male contributors in samples containing minor male components.

The PowerPlex® Y23 System is the most recent Y-STR system developed by Promega® to replace the previous Y-STR system, PowerPlex® Y. The PowerPlex® Y23 amplification kit contains 11 more loci than the previous Promega® Y system and includes two rapidly mutating loci which allows for potentially greater discrimination between paternally related males. The system can be used for both casework and direct amplification applications with a shortened amplification time of an hour and a half, providing a more efficient analysis process. The Y23 kit demonstrates a large degree of sensitivity, even in the presence of excessive female DNA.

The North Carolina State Crime Laboratory (NCSCL) Forensic Biology section does not currently use a Y-STR kit in casework processing. An internal validation was performed on the PowerPlex® Y23 PCR Amplification kit in accordance with the Scientific Working Group for DNA Analysis Methods (SWGDM) validation guidelines and the Federal Bureau of Investigation (FBI) Quality Assurance Standards for Forensic DNA Testing Laboratories September 2011 revision. Automated extractions were performed through the project using the Qiagen® EZ1 Advanced® Robot. Quantitation was performed on the Applied Biosystems® 7500 Real-Time Polymerase Chain Reaction (PCR) instrument using the Quantifiler® Duo quantification kit and the GeneAmp® 9700 thermal cycler was utilized for PCR amplification. Capillary electrophoresis was performed on the Applied Biosystems® 3500xL Genetic Analyzer and all data were analyzed using GeneMapper® ID-X v 1.4.

Internal validation studies included the following: precision; sensitivity; concordance; reproducibility; contamination; stochastic; mixtures (to include male/male and male/female scenarios); stochastic evaluation of the DYS385 locus; minimum threshold assessment; and, non-probative/mock sample studies. Sensitivity results demonstrated that the Y23 system could consistently generate full profiles at concentrations of 0.03125ng and full male profiles were also observed in several samples at concentrations as low as 0.0156ng. Male/female mixture study results indicated that full male profiles could consistently be obtained at ratios as extreme as 1:16,000, illustrating the specificity of the Y23 kit for male DNA amplification. Additionally, a study was performed to explore the viability of the Y23 PCR product over a period of several weeks. These studies identified the most efficient and appropriate operating procedures for the PowerPlex® Y23 amplification kit that meets the laboratory's needs and requirements.

This internal validation demonstrates the potential benefit of implementing the PowerPlex® Y23 kit in other forensic casework laboratories and will assist the NCSCL's Forensic Biology section in evaluating the addition of Y-STR analysis in the analysis of sexual assault evidence. The NCSCL Forensic Biology section will perform future studies to include stutter assessment, half-reactions, and alternate injection times. Other studies looking at the correlation between high mutation rate loci and related male samples may also be performed. The implementation of the PowerPlex® Y23 system will expand the testing capabilities of the forensic biology section.

DNA, Y-STRs, Y23

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