B107 Evaluating the Utility of Highly Sensitive Male Quantitative Polymerase Chain Reaction (qPCR) Targets for Simple, Low-Cost, and Rapid Screening of Sexual Assault Samples

Andrew Loftus, PhD, Innogenomics Technologies, LLC, 1441 Canal Street, Ste 307, New Orleans, LA 70112; Gina Murphy, MS, Innogenomics, 1441 Canal Street, Ste 307, New Orleans, LA 70112; Jonathan S. Tabak, InnoGenomics Technologies, 1441 Canal Street, Ste 307, New Orleans, LA 70112; Anne H. Montgomery, MS, InnoGenomics Technologies, LLC, 1441 Canal Street, Ste 307, New Orleans, LA 70112; and Sudhir K. Sinha, PhD, InnoGenomics Technologies, LLC, 1441 Canal Street, Ste 307, New Orleans, LA 70112*

After attending this presentation, attendees will understand the determination of the optimal genomic targets for the detection of male DNA in sexual assault cases.

This presentation will impact the forensic science community by evaluating the comparative utility of several male qPCR targets as a potentially valuable tool to improve a forensic laboratory's capacity to screen a high volume of sexual assault case samples.

The reduction of Sexual Assault Kit (SAK) backlogs has become of top concern and priority for many jurisdictions across the country. In 2011, the National Institute of Justice (NIJ) published a special report in response to recent discoveries of thousands of untested SAKs in police evidence rooms nationwide. In this report, one of the priorities identified for crime laboratories is to "create a plan to handle work if large numbers of previously untested SAKs are suddenly sent to the crime lab." This plan must include a more streamlined process for triaging SAKs to identify the presence of testable male DNA in these samples. Historically, serological screening using color immunological tests, chemical tests, or microscopic analyses have been the laboratory method of choice for initial processing of SAKs; however, these conventional methods have several drawbacks, including false positives and negatives, difficulty implementing automation, subjective interpretation, and the length of time the tests take in the laboratory. In the past decade, advancements in forensic quantitation systems based on real-time PCR have greatly improved the sensitivity and reliability of sample detection, allowing for higher throughput screening of sexual assault samples. Screening for male DNA (Y-screen) enables an objective, quantitative analysis of the presence of male DNA in a sexual assault sample. The process is easily automatable, using a 96-well format allowing for the screening of a high number of samples simultaneously using a real-time PCR-based method. These methods provide a downstream correlation with the profiling results obtained with commonly employed Short Tandem Repeat (STR) and Y-chromosomal Short Tandem Repeat (Y-STR) genotyping systems.

This study evaluates the utility of two different qPCR targets for detection and screening of male DNA in sexual assault and other casework samples. The methods studied include the use of a quick and simple lysis step to non-differentially lyse the cells in conjunction with two high-copy targets located on the Y chromosome. Studies performed include sensitivity, precision/reproducibility, male:female mixtures, and correlation with downstream STR and Y-STR results of mock sexual assault samples.

Results indicate that both tested targets are adequate for use in the accurate detection of male DNA, with the high copy target enabling the most sensitive detection of male DNA in sexual assault samples. Sensitivity studies using a dilution series conducted in triplicate with the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 2372 Component A indicated the ability to consistently detect male DNA at quantities below

one picogram. Mixture studies demonstrated the ability to consistently detect low quantities of male DNA (10pg) in all male:female mixtures tested, including 1:8000 male:female mixtures. Additionally, a quantitation threshold value that may reliably be used as a stopping point was determined for STR and Y-STR analysis, although this value may require adjustment based upon the sensitivity of the genotyping systems and interpretation criteria employed in each forensic laboratory. This study demonstrates the utility of real-time PCR male-specific targets in high throughput and fast screening of sexual assault cases. These methods will undoubtedly improve a laboratory's capacity to screen a high volume of sexual assault samples.

SAKs, Male DNA, QPCR