E98 Optimizing Sensitivity and Validating the Illumina® Infinium Assay for Genotyping of Forensically Relevant Sample Types for Investigative Lead Generation

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Learning Overview: The goal of this presentation is to demonstrate a validated forensic workflow using the Illumina® Infinium assay capable of producing accurate Single Nucleotide Polymorphism (SNP) genotyping data for investigative lead generation with a higher sensitivity to lower DNA amounts and geared toward forensic-centric sample types.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by pioneering a standard validation for a forensic workflow for genome-wide SNP genotyping. Additionally, this presentation highlights the capabilities of this assay to be more sensitive, overcoming the 200ng standard input and essentially shifting the applicability of this technology from clinical laboratories to the forensic community.

Forensic genealogy applies enhanced genetic processing techniques (array-based genome-wide SNP genotyping) combined with traditional genealogical research techniques to produce new leads in cases that have gone cold or where traditional investigative means have been exhausted. The use of this technology in investigative forensics has skyrocketed since the 2018 arrest of Joseph DeAngelo as the Golden State Killer. Most microarray-based genome-wide SNP genotyping takes place under clinical research, providing services that are not adapted to forensically relevant sample types. Furthermore, Direct To Consumer (DTC) laboratory tests require high quality and quantity DNA. The Infinium assay workflow is a genome-wide microarray genotyping assay that utilizes the BeadChip platform.[1] This accurate and flexible microarray technology allows for the ability to interrogate a large number of SNPs through unlimited loci multiplexing.[2-4] However, overcoming the 200ng standard input for this assay is essential for forensic genomics as it is rare to obtain DNA at such high quantities from forensic samples.

Here is described a validation of Illumina's® Infinium assay using the Infinium Global Screening Array to show successful optimization and validation down to DNA input levels more consistent with forensic sample types and shifting the applicability from clinical laboratories to the forensic community. In an effort to set a standard for validating a forensic workflow to generate genome-wide SNP genotyping data, the validation design, where applicable, is guided by the current Federal Bureau of Investigation (FBI) Quality Assurance Standards (QAS) for DNA testing laboratories and the Scientific Working Group on DNA Analysis Methods (SWGDAM) Validation Guidelines for DNA Analysis Methods.[5,6]

Precision and accuracy of the assay will be evaluated using the National Institute of Standards and Technology (NIST) standard reference material that have been extensively characterized.[7] The sensitivity study will be carried out using blood, buccal/saliva, and semen samples. These common sample types will be extracted, quantified, and diluted in a series ranging from the manufacturer-recommended input target of 200ng down to 1ng of total DNA input—an input amount more consistent to forensic samples. Currently, there are no computational methods to parse mixtures to individual genotypes. Given that mixtures are a possibility, a mixture study will be conducted to observe how data are presented at different mixture ratios. Identifying mixtures is also critical when assessing for possible contamination. Reagent blanks will be incorporated at extraction and carried throughout the workflow. Additionally, during the precision and accuracy study, the plate layout will be designed in a way to identify possible contamination during the assay. This validation will demonstrate a forensic workflow using the Illumina® Infinium assay capable of producing accurate SNP genotyping data for investigative lead generation with a higher sensitivity to lower DNA amounts and geared toward forensic-centric sample types.

Reference(s):

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Microarray, SNP Genotyping, Investigative Lead Generation

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