H26 A Quantitative Assay for Accurate 16S DNA Quantification for High-Throughput Sequencing (HTS) Library Preparation of Microbial Samples

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After attending this presentation, attendees will better understand how to quantify microbial DNA from human samples for improved performance of microbial HTS applications.

This presentation will impact the forensic science community by presenting a new method that will improve performance of microbial sequencing in their laboratories.

Sequencing and classification of microbial taxa within forensically relevant biological fluids has varied applications from individualization to body fluid identification. A distinct advantage as a method for body fluid identification is that it can be easily implemented into a comprehensive HTS panel for human identity. The quantity of bacterial DNA from human samples is currently estimated based on the quantity of total DNA isolated from the sample. This method overestimates the quantity of bacterial DNA due to the mixed nature of the sample and consequently makes library preparation unreliable and variable. The purpose of this project was to develop a reliable assay that can accurately and specifically quantify microbial DNA within a mixed sample for reliable 16S library preparation in advance of high-throughput metagenomics sequencing.

Venous blood, saliva, semen, vaginal and menstrual secretions, urine, and fecal matter were extracted using standard DNA isolation protocols. A quantitative Polymerase Chain Reaction (qPCR) method was developed using universal 16s rDNA primers, and cycling conditions were adapted for qPCR. A commercially available microbial community DNA standard consisting of pooled genomic DNA from eight bacterial and two fungal species was used to develop an accurate, precise standard curve. Five samples of each of the previously mentioned body fluid samples were quantified to determine the dynamic range necessary to detect bacterial communities within all body fluids. Following qPCR optimization, 16S libraries were amplified and evaluated from several samples at various DNA concentrations in order to determine the precise amount of microbial DNA needed for successful HTS library prep. DNA extracts were quantified using a standard human DNA assay to calculate the average ratios of human DNA to bacterial DNA in each biological fluid. Last, the body fluids were subjected to HTS to evaluate the success of the chosen microbial DNA quantity optimal for library preparation.

Bacterial concentrations between body fluids ranged from 5.12ng/µl to 0.001ng/µl. Saliva, vaginal secretions, and menstrual secretions proved to have a higher abundance of bacteria compared to blood and urine, which are considered to be “sterile” in healthy individuals, and beyond dormant bacteria or bacteria collected from skin surfaces upon exit of the respective fluids. A sample was considered negative if quantified below 10pg/µL. Results from gel electrophoresis reveal that successful HTS sequencing can be expected with 20pg of microbial DNA as quantified using this novel method.

This research has shown that quantification of microbial DNA from DNA extracts of forensically relevant body fluids is successful and increases library preparation reliability and success.

Body Fluid Identification, 16S rDNA, Microbiome

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