B197 The Evaluation of a Nanopore Sequencing Platform for Concordance and Reliability in Short Tandem Repeat (STR) Analyses

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After attending this presentation, attendees will better understand current nanopore sequencing technology and its potential utility in the forensic science community, particularly with forensic STR analysis.

This presentation will impact the forensic science community by attempting to evaluate and determine the potential utility of a revolutionary small-scale nanopore sequencing platform for obtaining High-Throughput Sequencing (HTS) data.

The question in the forensic science community is not whether HTS will be implemented, but how and when. It is crucial to evaluate all potential avenues of HTS that can bring the forensic science community up to speed in the most cost- and time-efficient manner possible. This becomes increasingly imperative with the expansion of both the number of samples and the number of biomarkers per sample being employed in forensic laboratories today. This research works to evaluate and determine the potential utility of a revolutionary small-scale nanopore sequencing platform for obtaining HTS data for the forensic science community.

Research was conducted using the only nanopore platform currently commercially available: Oxford Nanopore's MinION™ nanopore system. Samples were extracted and amplified using STR amplification techniques currently used in forensic science laboratories. By evaluating the nanopore platform with commonly used, commercially available STR primers for amplification, implementation would potentially require fewer validations, implementation into the workflow would be more easily achieved, and the Combined DNA Index System (CODIS) data entry could be streamlined. In the MinION™ platform, DNA passes through a nanopore protein channel for sequencing. The unique amount of current blocked as each nucleotide moves through the channel is translated into a base call. It was posited that the 5' fluorophores incorporated during STR amplification would hinder this process and possibly block ligation of the necessary adapters for sequencing during the library preparation. Thus, STR amplicons were pretreated with ExoSAP-IT® reagent in an effort to remove the fluorophores for improved ligation efficiency and higher quality sequencing.

Products were analyzed by Capillary Electrophoresis (CE) after ExoSAP-IT® treatment to determine if the fluorophores were removed by the treatment. Data revealed incomplete cleavage of the 5' fluorophore mobility complex from all amplicons. One specific locus, D8S1179, had STR alleles detected on the CE, indicating the continued presence of the fluorophore; however, sequence data was still obtained upon analysis on the MinION™ nanopore sequencing device. These results demonstrated that the removal of the fluorophore is not necessary, and STR sequencing data is obtainable with fluorophores still attached. Alternatively, it is possible that the majority of the sequence data is originating from the non-tagged strand.

Overall, sequencing with the nanopore device yielded a total of 3,053 sequence reads less than 500bp in length,

with the following loci identified: CSF1PO, FGA, TH01, TPOX, D5S818, D7S820, and D13S317. Additional sequence reads were identified for CSF1PO, TH01, and D7S820 but did not pass Quality Assurance (QA) thresholds. Only six of the identified loci were concordant with reference profiles obtained by CE analysis. Thus, an accuracy rate of less than 50% was observed. While these results are promising, they also illustrate that some additional work is needed to fully evaluate this HTS method. Continuing work will include replicate runs on an updated MinION™ platform, which includes a redesigned nanopore protein flowcell and improved bioinformatics software to address these exact error and accuracy issues. Also, additional concordance and accuracy data will be analyzed and compared to reference data obtained from CE, which may serve to further streamline and improve MinION™ data analysis.

This research works to provide information to the forensic science community about a small, portable HTS platform that is potentially both time- and cost-efficient. The preliminary data determines that, with further improvements in error and accuracy rates, the potential uses of nanopore sequencing devices in forensics are tremendous.

Nanopore, Short Tandem Repeat, High-Throughput Sequencing