FORENSIC AUSTOSOMAL STR PROFILING USING THE PROMEGA POWERSEQ KIT ON OXFORD NANOPORE TECHNOLOGIES' MINION DEVICE

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The high variability characteristic of short tandem repeat (STR) markers is harnessed for human identification in forensic investigations. Despite the power and reliability of current typing techniques, sequence-level information both within and around STRs are masked in the length-based profiles generated. Forensic STR profiling using next generation sequencing (NGS) has therefore gained attention as an alternative to traditional capillary electrophoresis (CE) approaches.

In this proof-of-principle study, we evaluate the forensic applicability of the newest and smallest NGS platform available, the Oxford Nanopore Technologies (ONT) MinION device. Although nanopore sequencing on the handheld MinION offers numerous advantages, including on-site sample processing, the relatively high error rate and lack of forensic-specific analysis software has prevented accurate profiling across STR panels in previous studies. Here we present STRspy, a streamlined method capable of producing length- and sequence-based alleles designations from noisy, long-read data. To demonstrate the capabilities of STRspy, seven reference samples (female: n = 2; male: n = 5) were amplified using the Promega PowerSeq 46GY System and sequenced on the ONT MinION device in triplicate. Basecalled reads were processed with STRspy using a custom STR database containing alleles reported in the STRSeq BioProject NIST 1036 dataset. Resultant STR allele designations and flanking region SNP calls were compared to the manufacturer-validated genotypes for each sample. STRspy generated robust and reliable genotypes across all autosomal STR loci using 500pg of input DNA amplified with 30 PCR cycles, achieving 100% concordance based on both length and sequence. Furthermore, we were able to identify flanking region SNPs with >90% accuracy.

These results demonstrate that nanopore sequencing platforms are capable of revealing an additional level of variation in and around STR loci with sufficient read coverage. As the first method to successfully profile the entire panel of autosomal STRs amplified by a commercially available kit using an ONT platform, STRspy significantly increases the feasibility of using Nanopore sequencing in forensic applications.