DEVELOPMENTAL VALIDATION OF A METHOD FOR QUANTITATIVE HIGH-THROUGHPUT FORENSIC MICROSATELLITE (STR) SEQUENCING

Seth A. Faith, Melissa R. Scheible, Sarah Bailey, NC State University

Forensic science is poised to adopt new methods in DNA analysis utilizing next-generation sequencing (NGS) to obtain finer resolution and higher bandwidth in genetic analysis. To date, NGS workflows for forensic short tandem repeat (STR) sequencing do not afford a strict quantitative analysis (e.g., input ≅ output), which would be beneficial for applications such as mixture and low copy analysis. Forensic samples in NGS workflows are routinely normalized for library input quantities and molar library concentrations prior to sequencing. Furthermore, the current NGS methods are laborious, having numerous steps for introduction of operator error. Here, we present developmental validation in optimizing a streamlined forensic NGS workflow for STR sequencing that is non-normalized for quantitative analysis and automated for precision and accuracy. The workflow first amplifies STR loci with a balanced multiplex PCR reaction (PowerSeq, Promega Corp.). The post-PCR product is bead purified and the entire product is used for NGS library construction with a high efficiency adaptor ligation protocol (HyperPrep kit, KAPA Biosystems) operationalized on an Eppendorf 5075tc liquid handling workstation. The method does not employ a second PCR reaction for library enrichment and up to 96 samples are directly pooled without library normalization. Sequencing is conducted with Illumina MiSeq and data are analyzed using our custom designed *Altius* cloud-computing tool. We present data to associate gDNA input to sequence data output via measurements at checkpoints throughout the workflow: input DNA, post-PCR product, post-ligation library, pre-sequencing library, post-sequencing analyses with empirically derived thresholds. This approach will provide guidance for validation of NGS systems in forensic laboratories.