SEPARATION OF COMPLEX DNA MIXTURES FROM TOUCH EVIDENCE

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With rapidly improving chemistries, Next Generation Sequencing (NGS) continues to demonstrate incredible potential for forensic investigation. The amount of data generated is phenomenal and the technology could have profound effects on the law enforcement community. Specifically, The Bode Technology Group (Bode) is exploiting the potential of NGS by applying the technology to the deconvolution of complex forensic mixtures. As the molecular techniques available to forensic scientists become more sensitive, the ability to detect minor components in biological sample mixtures has increased dramatically.

To examine the efficacy and utility of NGS, Bode has designed and tested a forensic process for separating complex DNA mixtures from low copy number, degraded and touch objects and producing distinct profiles for each source using pyrosequencing based deep amplicon sequencing on the Roche 454 GS Junior. In collaboration with The Johns Hopkins University Applied Physics Laboratory, we have developed a novel forensic bioinformatic software pipeline to deconvolute mixtures of mitochondrial DNA and nuclear Y chromosome Short Tandem Repeats (Y-STR). The system offers the potential to detect all alleles in a mixture, determine inclusion and exclusion statistics, and even search against databases and generate match statistics accordingly. Minor contributors to a mixture can be detected even when present at a ratio less than 1 in 100, far exceeding the limit for forensic capillary electrophoretic instruments and currently available mixture deconvolution software applications. When applied to complex mixtures of individuals sequenced for either the mitochondrial hypervariable region or Y-STR loci, we were successfully able to separate mixtures of seven individuals using mtDNA and mixtures of five individuals using Y-STRs.

By combining the power of a the Roche 454 GS Junior pyrosequencing chemistry with the novel sequence analysis pipeline, the developed marker panels and processes will improve an investigator's ability to understand familial relations between sample donors and to better separate and interpret samples with multiple contributors. Ultimately this system will be available for use in the course of routine forensic casework and would be an important future tool in the broader forensics community.