A40 Separation of Complex DNA Mixtures From Touch Evidence

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After attending this presentation, attendees will gain an understanding of next generation sequencing and its potential application to the forensic field through deconvolution of complex DNA mixtures from evidentiary items.

This presentation will impact the forensic science community by introducing a future capability that has the potential to separate complex DNA mixtures of five to seven individuals, including low copy contributors.

DNA sequencing technology is advancing rapidly in the form of Next Generation Sequencing, and the amount of catalogued genetic human data being generated has created an explosion of possible applications. The Bode Technology Group is currently applying the use of Next Generation Sequencing to the deconvolution of complex forensic mixtures from low copy number, degraded, and touch objects. To this end, Bode has designed, developed, and tested a field functioning forensic process for separating complex deoxyribonucleic acid (DNA) mixtures of five to seven individual sources of DNA and producing distinct profiles for each source using pyrosequencing-based deep amplicon sequencing chemistry. By combining the power of a pyrosequencingbased Next Generation Sequencing platform such as the bench topsized Roche 454 GS Jr. sequencing system with a novel forensic bioinformatic software pipeline, mixtures of seven or more individuals from mock evidentiary touch samples have been successfully sequenced and separated using multiple panels of highly multiplexed forensically relevant loci.

Data will be presented from an ongoing research effort to deconvolute mixtures of both mitochondrial DNA and nuclear Y chromosome Short Tandem Repeat (Y-STR) amplicons using a bioinformatic software suite developed through a collaboration with The Johns Hopkins University Applied Physics Laboratory. Data produced by the mixture separation software suite show highly sensitive detection of low copy contributors. When applied to mixtures of between two and seven individuals sequenced at mitochondrial hypervariable and Y-STR loci, minor contributors were successfully detected below a 1:100 ratio level, far outreaching the limit for common forensic capillary electrophoretic instruments and currently available mixture deconvolution software applications. Given a maximum number of sequences per read of between 50,000 and 120,000, there is a tradeoff among several factors such as the number of multiplexed loci, the number of contributors to a mixture, and necessary sensitivity. Thus, to develop a working understanding of the limits of this system, a series of mixture samples were created changing these variables.

Several sample types were examined including: dilution series of nascent human DNA and mock touch samples created from fingerprints (handled objects for varying degrees of time), multiple biological fluids, and substrates in complex mixtures consisting of between 1-8 different contributors at different relative concentrations. Multiplexes of the mitochondrial hypervariable regions HVI/HVII and standard Y-STR loci were created, balanced, and optimized into panels compatible with the 454 GS Jr. sequencing system. The raw data files are directly imported into the software, where all of the sequence reads at each locus are detected and then separated. Each unique sequence is binned and analyzed for error. Using a ratio-driven algorithm, alleles are re-associated to the most likely contributor of origin with associated likelihood ratio values. The system also 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. Ultimately, this system will be available for use in the course of routine forensic casework and thus can be an important future tool in the broader forensic science community.

Next-Gen Sequencing, Mixture Separation, Mixture Analysis

 ** Presenting Author*