B102 Massively Parallel Sequencing — A Revolution for Complex Mixture Interpretation?

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After attending this presentation, attendees will appreciate the potential that sequence analysis of Short Tandem Repeats (STRs) via massively parallel sequencing methodologies has to alter the way forensic science deals with complex DNA mixtures.

This presentation will impact the forensic science community by demonstrating that DNA mixtures currently believed to be of little evidential value may now be usefully analyzed due to the improvements in sequencing and statistical interpretation methodologies.

Complex DNA mixtures are often encountered during forensic casework; however, interpretation of these mixtures can be problematic and contentious. Advances achieved through novel software solutions underpinned by complex statistical methodologies have shown promise to improve this analysis. Further advances with the potential to substantially aid mixture interpretation are now available due to the enhanced ability with next generation sequencing/massively parallel sequencing to analyze not only the allele length but also the allele sequence and hence increase allelic discrimination.

The ForenSeq™ kit from Illumina® was released in mid-2015 and includes 58 autosomal, Y and X chromosome STRs along with 94 identity Single Nucleotide Polymorphism (SNP) markers all analyzed in one reaction. This provides an easily accessible way to attain the extra sequence data contained within STR repeats that cannot be extrapolated from the allele size alone.

A series of DNA mixtures containing two, three, and four contributors have been prepared and run in duplicate for both a standard capillary electrophoresis-based genotyping method and with the ForenSeq™ kit utilizing the Illumina® MiSeq® massively parallel sequencing platform. Mixtures have been run with final DNA input amounts of 500pg, 250pg, and 125pg to simulate a range of scenarios that might be typically encountered during forensic casework and explore how the stochastic amplification problems associated with low-level DNA impact the efficacy of mixture analysis.

Data analysis for the ForenSeq™ results was undertaken with the provided Illumina® universal analysis software suite to determine the STR allele repeat sequence, and additionally from the raw data using a bespoke bioinformatics pipeline that allowed further analysis of any SNPs present within the Polymerase Chain Reaction (PCR) flanking regions. Freely available continuous and semi-continuous mixture interpretation models have been used to evaluate the DNA mixtures with respect to a set of suspect/victim reference profiles providing a likelihood ratio relevant to the hypothesis.

This presentation will assess what benefit the knowledge of allele sequence data brings to this mixture interpretation process and how mixture interpretation, especially for complex touch DNA samples, may evolve in the near future.

Mixtures, Massively Parallel Sequencing, Interpretation

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