TYPING AND RESOLUTION OF MIXED CONTRIBUTOR MITOCHONDRIAL DNA SAMPLES USING MASSIVELY-PARALLEL DEEP AMPLICON PYROSEQUENCING

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Resolution of mixed contributor DNA samples remains a major technical challenge in the field of human DNA forensics. Currently the deconvolution of mixtures containing more than three individuals using standard methods is not possible. Here we report the application of an emerging sequencing technology, Roche 454 pyrosequencing, to the resolution of complex mixed contributor samples of human mitochondrial DNA. The 454 process provides the capability to clonally amplify and individually sequence ~1 million (per one 454 run) of the individual DNA amplicons obtained by standard PCR methods. It thus offers the potential to detect low-copy genetic variants in mixed samples.

We performed several double-blind mixture experiments (2 and 5 contributors) by direct mixing of the mtDNA, and by recovering total mtDNA from an object after multiple (5) persons had touched it. In the direct mixing experiments, all contributor mtTypes were correctly identified against sequencing process noise (statistical confidence $p < 0.0001$). In the touch experiment, 4 of the 5 persons were identified (as one contributor did not shed sufficient mtDNA). In addition, the method revealed previously unknown, candidate low-copy heteroplasmic haplotypes for at least 2 of the persons.

This new methodology has the potential to enable higher-fidelity exclusion and identification analysis (e.g., by incorporating mtDNA heteroplasmy as forensic markers) and provide a superior forensic and investigative tool to deconvolve complex mixtures both for comparison to a reference and to link multiple incidents together based on the recovered mixed contributor profiles.