B49 Toward Developing a Forensically Relevant Single-Cell Pipeline by Incorporating Direct-to-Polymerase Chain Reaction (PCR) Extraction: Effects on Signal Quality and Allele Dropout

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Learning Overview: After attending this presentation, attendees will better understand how direct-to-PCR extraction chemistry affects the DNA Electropherogram (EPG) signal garnered from single cells.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating that four commonly used metrics of single-cell EPG's are significantly impacted by direct-to-PCR treatment employed. Along with demonstrating the impact of extraction treatment, this presentation will show the potential associated with single-cell pipelines for solving the complex DNA mixture problem.

The interpretation of mixture samples using a traditional pipeline, where the DNA of many cells are extracted together, is difficult since an unknown number of contributors of unknown concentration renders EPGs so complex that interpretation requires significant computational power to complete. An alternate to the bulk-processing pipeline is a single-cell one, where the sample is collected, and each cell is separated. The DNA is then extracted using, preferably, a direct-to-PCR treatment that is efficient and compatible with all forensically relevant downstream processes. Though single-cell pipelines have the potential to fill the gaps left by the bulk-processing pipelines, it is a necessity to confirm novel extraction and interpretation strategies meet forensic requirements.

In this study, the feasibility of implementing single-cell pipelines into the forensic process by exploring whether allele dropout rates are cell-dependent is demonstrated. Four metrics of EPG signal quality (i.e., allele detection rates, peak heights, peak height ratios, and peak height balance across low to high molecular weight Short Tandem Repeat (STR) markers) obtained with four direct-to-PCR extraction treatments: forensicGEM® Saliva; DEPArray™ LysePrep Kit; Direct PCR Lysis Reagent; and Arcturus® PicoPure™ DNA Extraction Kit were assessed. Each of the four methods was used to extract DNA from 102 single buccal cells, whereupon the amplification reagents were immediately added to the tube and the DNA was amplified/injected using post-PCR conditions (laboratory conditions: GlobalFiler™ PCR Amplification Kit, 30 PCR cycles, and 25-second injection on ABI® 3500 Genetic Analyzer) known to elicit a Limit of Detection (LoD) of one DNA molecule. The results show that 77% of cells (313 out of 408 single buccal cells), regardless of extraction treatment, rendered EPGs with at least a 50% true positive allele detection rate, and that allele drop-out was not cell-independent. Permutation tests demonstrated that extraction treatments significantly impacted all metrics of EPG quality. Notably, the Arcturus® PicoPure™ extraction method resulted in the lowest median allele drop-out rate, the highest median average peak height, the highest median average peak height ratio, and lowest median values of EPG sloping. It is, therefore, concluded that implementing single-cell pipelines into casework is feasible and demonstrated that inferential systems assuming cell independence is not ideal for the probabilistic interpretation of a collection of single-cell EPGs.

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