DEVELOPING A FORENSICALLY RELEVANT SINGLE-CELL PIPELINE FOR HUMAN IDENTIFICATION

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Interpreting mixture samples from a bulk pipeline is arduous since the signal is a cacophony of low-fidelity fluorescence from noise, artifact and incomplete allele signal from an unknown number of contributors. The resultant electropherograms (EPGs) are sometimes so complex as to require significant computational power to complete the interpretation. An alternate to the bulk-processing pipeline is a single-cell one, where the sample is collected and each cell is sequestered. The DNA from each cell is then extracted, amplified and electrophoresed. The result is *n* single-source single-cell EPGs from *n* cells. In the single-cell pipeline, an efficient direct-to-PCR treatment that is compatible with all forensically relevant downstream processes is necessary. In the first part of the study four direct-to-PCR extraction treatments (forensicGEM® Saliva (ZyGEM Corp Ltd); DEPArray™ LysePrep Kit (Menarini Silicon Biosystems); Direct PCR Lysis Reagent (Viagen Biotech, Inc.); and Arcturus® PicoPure™ DNA Extraction Kit (Arcturus Bioscience, Inc.)) were tested on 408 buccal cells procured with a low throughput single-cell sequestration method -- i.e., Pico pipetting. We explored if allele dropout rates are cell-dependent and examined whether the extraction treatment had downstream ramifications on signal quality. Signal quality was examined by interrogating four EPG metrics: allele detection rates; peak heights; peak height ratios; and peak height sloping across low to high molecular weight STR markers. The results show that, overall, 77% of the cells (313 out of 408 single-cells) rendered EPGs with at least a 50% allele detection rate, and that allele drop-out rates were cell dependent. Permutation tests demonstrated that extraction treatments significantly impacted all metrics of EPG quality. Notably, the Arcturus® PicoPure™ extraction treatment 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 EPG sloping values.

In the second part of the study we replace the manual pico-pipetting method with a semi-automated one by integrating micro-fluidics and DEPArray™ technology. Phosphate buffer saline (PBS) is an important reagent in this process, as it mitigates cell rupture. If the cells rupture prior to DNA extraction it could result in poor signal quality, negatively affecting forensic outcomes. Despite PBS's value in mitigating cell-rupture, it is a known PCR inhibitor. It, therefore, became necessary to optimize PBS concentrations to ensure pre-mature cell lysis was minimized while maximizing PCR efficiency. Thus, we use the same metrics of EPG quality to test the effects of four different PBS+Pro K extraction treatments on 241 single leukocytes: 1. High Pro K/ 1X PBS; 2. Low Pro K/1X PBS 3. Low Pro K/0.5X PBS; and Low Pro K/0.25X PBS. By decreasing the PBS concentration to 0.5X the highest overall signal quality was obtained. The results also indicated that lowering the Pro K concentration did not significantly impact signal quality. We therefore conclude that a lower than recommended Pro K concentration during PicoPure™ direct-to-PCR extraction coupled with a lower than recommended PBS concentration is a viable treatment for semi-automated single-cell pipelines.

In addition to examining effects of candidate laboratory treatments, we shall confirm whether the signal quality is unchanged between manual and semi-automated processes while exploring if signal quality is independent of cell-type. Acquiring a systems understanding of the single-cell treatment will, therefore, inform the development of a viable single-cell system having forensic relevance.