



### B10 Developing a Forensically Relevant Single-Cell Interpretation Strategy for Human Identification

Amanda J. Gonzalez, MS\*, Cherry Hill, NJ 08003; Harish Swaminathan, PhD, Boston University School of Medicine, Boston, MA 02118; Ken Duffy, PhD, Maynooth, IRELAND; Catherine M. Gricak, PhD, Rutgers University, Camden, NJ 08102

**Learning Overview:** After attending this presentation, attendees will better understand how confounding signal (i.e., allelic drop-out, stutter, and allelic drop-in) adversely affects mixture interpretation of bulk-processed samples and how the characterization of signal garnered from single cells is an important step toward the development of single-cell inference systems.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the potential of single-cell systems to improve mixture interpretation.

Biological evidence submitted to the forensic DNA laboratory contains cells from an unknown number of contributors in unknown proportions, resulting in interleaved mixture profiles, which are difficult to interpret, affecting match statistics or inclusion/exclusion decisions reported to the trier-of-fact.

Given the complexities associated with bulk-mixture interpretation, recent efforts have focused on introducing single-cell systems into the forensic laboratory in order to de-convolve the mixture signal by separating cells at the front end of processing. However, low-template and single-cell signal are often obfuscated by the presence of elevated stutter; false negative detection of alleles, known as allelic drop-out; and false positive detection of alleles, commonly referred to as allelic drop-in.

In this study, 1,845 single-cell profiles were analyzed, 556 of which were acquired from single-source, single-cell samples (laboratory conditions: Globalfiler™ amplification, 30 Polymerase Chain Reaction (PCR) cycles, 25-second injection on ABI® 3500 Genetic Analyzer). The data from all 556 samples was evaluated to determine the distributions associated with allelic drop-out, stutter, and allelic drop-in. The data was statistically assessed to determine whether confounding signal observed in the Electropherograms (EPGs) of single cells was significantly different from artifactual signal acquired from bulk-processed samples. The results demonstrate that, in contrast to bulk-processed samples, allelic drop-out is likely cell dependent. Stutter was again found to be locus dependent. In addition, the propensity of single-cell samples to exhibit elevated stutter ratios was observed. Notably, the stutter percentages for the single-cell samples ranged from 0% to 241% as predicted by the stochastic model described by Duffy et al.<sup>1</sup> Further, the frequency of allelic drop-in was consistent with that of bulk-processed samples (i.e., 0.04%) and cell independent, suggesting that its detection is mainly driven by laboratory parameters and conditions rather than sample condition or total DNA concentration. The presence of cell-dependent drop-out rates and high levels of stutter suggest that if a full evaluation of multiple single-cell signal is desired, new probabilistic constructs that do not rely on assumptions of cell-independence for drop-out are likely required. Since probabilistic inference relies heavily on models that adequately represent the system in question, this study forms the foundation from which these inference systems can be developed.

To further explore the viability of single-cell pipelines for forensic DNA casework, a protocol was developed to desorb buccal cells from cotton-tipped applicators. To measure its efficacy, hemocytometry was used to determine the percent of cells recovered. The 52% recovery of buccal cells was consistent with previous work wherein 54% of DNA was recovered from cotton swabs.<sup>2</sup> These results demonstrate that a single-cell strategy for forensic mixture interpretation is a viable alternative to bulk-mixture interpretation strategies that can lead to signal that is too low or highly masked by other contributors in the DNA mixture.

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#### Reference(s):

1. Duffy, K.R. et al. Exploring STR signal in the single- and multicopy number regimes: Deductions from an in silico model of the entire DNA laboratory process. *Electrophoresis* 38, no. 6 (2017): 855-68.
2. Adamowicz, M.S. et al. Evaluation of Methods to Improve the Extraction and Recovery of DNA from Cotton Swabs for Forensic Analysis. *PLoS ONE* 9, no. 12 (2014): e116351.

#### Single-Cell, Stutter Artifact, Allelic Drop-Out