OSAC RESEARCH NEEDS ASSESSMENT FORM

Title of research need: Characterization, development and validation of methods in single cell analysis and interpretation

Describe the need:

Subcategory: emerging technologies

Single cell analysis has grown over the last decade as a result of technological innovations and is now commonplace in the greater biological research community. Consequently, the applications and benefits of the single cell analysis technologies have crossed over into the forensic DNA analysis field. The technology and methods to make this possible have become more accessible, easier to use, more diverse, and less expensive than they were even a decade ago. The primary benefit of single cell analysis is the ability to avoid or simplify the interpretation of DNA mixtures – a single cell is by definition contributed by a single donor. This analysis is versatile, being appropriate for use with standard forensic DNA typing methods, includingDNA fragment analysis using capillary electrophoresis, and DNA sequencing technologies. The potential benefits extend beyond that of mixture analysis including the ability to identify the tissue source of the DNA profile that was generated and through characterizing the behavior and response of single cells in the forensic workflow, can reveal more about the dynamics of stochastic effects such as inter- and intra- locus peak or read balance, allele dropout, and detection thresholds. Specific needs include: development and validation of single cell isolation techniques with specific emphasis on throughput and practical implementation within a forensic laboratory, development and validation of wet bench processing, interpretation and analysis for single cells for both fragment and sequence data, artifact identification and mitigation, optimization of amplification methods, methods for identification of cell types (for example the use of probes or RNA)

Keyword(s): DNA mixture, single-cell analysis, mixture deconvolution, low-template DNA, trace DNA

Submitting subcommittee(s): Human Biology **Date Approved:** 05/16/2025

Background Information:

1. Does this research need address a gap(s) in a current or planned standard? (ex.: Field identification system for on scene opioid detection and confirmation)

No

2. Are you aware of any ongoing research that may address this research need that has not yet been published (e.g., research presented in conference proceedings, studies that you or a colleague have participated in but have yet to be published)?

Yes

3. Key bibliographic references relating to this research need: (ex.: Toll, L., Standifer, K. M., Massotte, D., eds. (2019). Current Topics in Opioid Research. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88963-180-3)

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4. Review the annual operational/research needs published by the National Institute of Justice (NIJ) at https://nij.ojp.gov/topics/articles/forensic-science-research-and-development-technology-working-group-operational#latest? Is your research need identified by NIJ?

Yes. "The ability to differentiate, physically separate, and selectively analyze DNA and/or cells from multiple donors or multiple tissue/ cell types contributing to mixtures, with minimal or no sample loss. For example, alternative methods of differential extraction with limited sample manipulation (no centrifugation step) and/or automatable sperm capture that can be utilized on existing lab equipment (EZ2, Hamilton, etc.)." "The ability to associate cell type and/or fluid with a DNA profile, to include mixed DNA profiles enabling profiles to be reported at source level within existing laboratory instrumentation., "Research that documents the various impacts of methods, reagents, and materials on the recovery, repair, and/or preservation of low-quantity and/or low-quality DNA from various cell types."

5. In what ways would the research results improve current laboratory capabilities?

Research into the use of single cells, either in isolation or in bulk analyses, will allow laboratories to have increased resolution when analyzing mixture samples and less reliance on the computer modeling of mixtures. In addition, this will permit the correlation between the DNA profile generated and the tissue source used to generate the profile.

6. In what ways would the research results improve understanding of the scientific basis for the subcommittee(s)?

Gaining knowledge on the dynamics of low template analyses using PCR-based fragment analysis and DNA sequencing including, stochastic effects and detection thresholds, will enable the committee members to make more informed recommendations for aspects of forensic analyses that involve these topics.

7. In what ways would the research results improve services to the criminal justice system?

Research into the use of single cells will permit less reliance on mixture analysis. This will lead to less complex data analysis resulting in higher confidence in the interpretations, comparisons, statistical values and statements of conclusions generated by the laboratory for the end user. This type of analysis is in its infancy, and, with proper research support, these benefits can come to fruition.

8. Status assessment (I, II, III, or IV): II

	Major gap in current knowledge	Minor gap in current knowledge
No or limited current research is being conducted	I	III

Existing current research is being conducted	II	IV

This research need has been identified by one or more subcommittees of OSAC and is being provided as an informational resource to the community.