OSAC RESEARCH NEEDS ASSESSMENT FORM

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

Describe the need: Single cell research 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 decades 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, such as DNA fragment analysis and 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. This type of analysis is in its infancy, and, with proper research support, these benefits can come to fruition.

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

Submitting subcommittee(s): Human Biology **Date Approved:** 10/05/2021

(If SAC review identifies additional subcommittees, add them to the box above.)

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, these details, however, are not available at this point.

3. Key bibliographic references relating to this research need:

1) Gill, P. et al. Genotyping and interpretation of STR-DNA: Low-template, mixtures and database matches - Twenty years of research and development. Forensic Sci. Int. Genet. (2015). doi:10.1016/j.fsigen.2015.03.014

2) Benschop, C. C. G., Haned, H., de Blaeij, T. J. P., Meulenbroek, A. J. & Sijen, T. Assessment of mock cases involving complex low template DNA mixtures: A descriptive study. Forensic Sci. Int. Genet. 6, 697–707 (2012). 9.
3) Watkins, D. R. L., Myers, D., Xavier, H. E. & Marciano, M. A. Revisiting single cell analysis in forensic science. Sci. Reports 2021 111 11, 1–12 (2021).
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5) Verdon, T. J., Mitchell, R. J., Chen, W., Xiao, K. & Van Oorschot, R. A. H. FACS separation of non-compromised forensically relevant biological mixtures. Forensic Sci. Int. Genet. 14, 194–200 (2015).
6) Vandewoestyne, M. & Deforce, D. Laser capture microdissection in forensic research: a review. Int. J. Legal Med. 124, 513–521 (2010).
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11) Harrel, M., Gangitano, D. & Hughes-Stamm, S. The effects of extra PCR cycles when amplifying skeletal samples with the GlobalFiler® PCR Amplification Kit. Int. J. Legal Med. 133, 745–750 (2019).
12) Pfeifer, C. M., Klein-Unseld, R., Klintschar, M. & Wiegand, P. Comparison of different interpretation strategies for low template DNA mixtures. Forensic Sci. Int. Genet. 6, 716–722 (2012).
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14) Weiler, N. E. C., Matai, A. S. & Sijen, T. Extended PCR conditions to reduce drop-out frequencies in low template STR typing including unequal mixtures. doi:10.1016/j.fsigen.2011.03.002
15) Duijs, F., Van De Merwe, L., Sijen, T. & Benschop, C. C. G. Low-template methods yield limited extra information for PowerPlex® Fusion 6C profiling. (2018). doi:10.1016/j.legalmed.2018.06.001
16) Gill, P., Whitaker, J., Flaxman, C., Brown, N. & Buckleton, J. An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. Forensic Science International 112, (2000).
17) Kloosterman, A. D. & Kersbergen, P. Efficacy and limits of genotyping low copy number DNA samples by multiplex PCR of STR loci.
18) Butler, J. M. & Hill, C. R. Scientific Issues with Analysis of Low Amounts of DNA. [Internet] (2010). Available at: https://www.promega.com/resources/profiles-in-dna/2010/scientific-issues-with-analysis-of-low-amounts-of-dna/ . (Accessed: 10th April 2020)
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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?

Indirectly 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"*

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

Research into the use of single cells will permit less reliance on mixture analysis. This may lead to less complex, high confidence interpretation and conclusions. 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, higher confidence interpretations conclusions. 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.