OSAC RESEARCH NEEDS ASSESSMENT FORM

Title of research need: Non-PCR based methods for DNA amplification and/or detection

Describe the need: There are problematic issues with PCR based analysis such as analysis time, stutter and stochastic amplification effects. Alternative and non-PCR based methods have the potential to change the way samples are analyzed, attempting to mitigate the use of thermal cycling in order to achieve sufficient quantities of DNA for analysis. Currently, fieldable rapid DNA analysis is not easily performed due to the large and sometimes delicate instrumentation required for PCR and the process can take upwards of 90 minutes. PCR based methods also create problematic artifacts such as stutter and allele drop in, which can complicate mixture analysis. Newer methods exist that are more portable, potentially faster and more sensitive. These include methods based on isothermal amplification, nanopore/single molecule sequencing and biosensing. The development and application of these smaller, more portable methods in the forensic DNA community could alleviate the challenges encountered when using PCR, thus leading to a less cumbersome analysis of mixture profiles.

Keyword(s): Isothermal amplification, biosensing, sequencing, nanopore, non-PCR

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)

Fieldable DNA, mixture analysis, DNA sequencing.

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)?

Not at the present time.

3. Key bibliographic references relating to this research need:

1) Li, J., Macdonald, J. and von Stetten, F. Review: a comprehensive summary of a decade development of the recombinase polymerase amplification.
2) Daunay, A., Duval, A., Baudrin, L.G., Buhard, O., Renault, V., Deleuze, J.F. and How-Kit, A. Low temperature isothermal amplification of microsatellites drastically reduces stutter artifact formation and improves microsatellite instability detection in cancer. Nucleic Acids Research, Volume 47, Issue 21, 02 December 2019, Page e141

3) Khalila, I., Yehyea, W.A., Julkaplia, N.M., Rahmatia, S.. Ibn Sinab, A.A., Basirunac, W.J., and Johana M.R. Graphene oxide and gold nanoparticle based dual platform with short DNA probe for the PCR free DNA biosensing using surface-enhanced Raman scattering, Biosensors and Bioelectronics Volume 131, 15 April 2019, Pages 214-223
4) McGoldrick, L.K. and Halámek, J. Recent Advances in Noninvasive Biosensors for Forensics, Biometrics, and Cybersecurity, Sensors 2020, 20(21), 5974.
5) Harris, T.D. et al. Single-Molecule DNA Sequencing of a Viral Genome Science 320, 106 (2008)
6) Li, N., Hao, X., Kang, B. H., Xu, Z., Shi, Y., Li, N. B., & Luo, H. Q. (2016). Enzyme-free fluorescent biosensor for the detection of DNA based on core–shell Fe3O4 polydopamine nanoparticles and hybridization chain reaction amplification. Biosensors and Bioelectronics, 77, 525-529.

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 - Better understanding of advanced approaches to removing steps from typical DNA processing workflows (e.g., extraction, quantitation, amplification); Better ways to enrich or target genomic areas of forensic DNA interest as opposed to a traditional PCR-based approach.

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

Fieldable DNA analysis would permit sample screening, methods that eliminate stutter and drop-in would simplify mixture analysis, single molecule sequencing would eliminate amplification artifacts and the process could potentially be made faster.

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

The development of alternative analysis methods would also impact understanding of existing procedures, particularly PCR bias and stochastic amplification.

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

Improve speed of results and potentially remove complex artifacts.

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

	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.