OSAC RESEARCH NEEDS ASSESSMENT FORM

Title of research need: Increasing efficiency, optimization and standardization of DNA sequencing methods

Describe the need:

The introduction of massively parallel sequencing (MPS) into the field of forensic DNA analysis has resulted in technological advances and new opportunities. Sequencing provides an increased level of resolution for STR typing, without the constraints of electrophoresis methods that limit the number of markers that can be simultaneously targeted. MPS has also facilitated the development of panels targeting other informative marker types, including SNPs, the mitochondrial genome, and microhaplotypes. Commercially-available MPS kits that target one or more marker types have been the primary focus for implementation in crime laboratories, largely due to the ease of adoption. MPS data developed using some STR and mitochondrial DNA kits is CODIS-eligible, and MPS methods have been accepted in U.S. courts. Yet, the power of dense genome-wide approaches (i.e., microarray hybridization and target hybridization capture) and whole genome sequencing for distant kinship inference has the potential to resolve many additional forensic cases. Given these advances and the potential of MPS methods, there is a need to:

1. Compare the technical performance and applied performance (e.g., distant kinship inference, ancestry inference) of SNP profiles generated from targeted sequencing, hybridization methods, and WGS. 2. Evaluate the impact of different library preparation methods and process variables (e.g., purification steps such as bead ratios and final elution volume). 3. Compare different sequencing platforms and chemistries/throughput (e.g., cartridge and flow cell types), along with the impact of procedure parameters (e.g., multiplexing level, pooling strategy). 4. Evaluate analysis software and bioinformatic pipelines for technical performance, ease of use, and data visualization capabilities. 5. Develop and establish performance metrics and means to assess these uniformly across laboratories. 6. Particularly assess the above components with low input, degraded, and mixture samples.

Keyword(s): Massive Parallel Sequencing, Whole Genome Sequencing, Microarray hybridization, SNPs, library preparation, analysis software, bioinformatic pipelines

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)

Yes, in the use of MPS for casework.

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, but not enough in detail.

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, "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?

MPS and microarray hybridization allow for the generation of many more markers simultaneously, which allows for the generation of both identification and investigative leads (e.g., ancestry, phenotypic, and kinship inference). The methods by themselves, and then potentially paired with automation, generally allow for higher throughput processing of samples. Further, dense genome-wide approaches and whole genome sequencing (WGS) make possible distant kinship inference, which expands the potential for investigative leads. Finally, a deep understanding and optimization of the technical methods will establish a firm foundation for utilizing SNPs in direct and kinship identification.

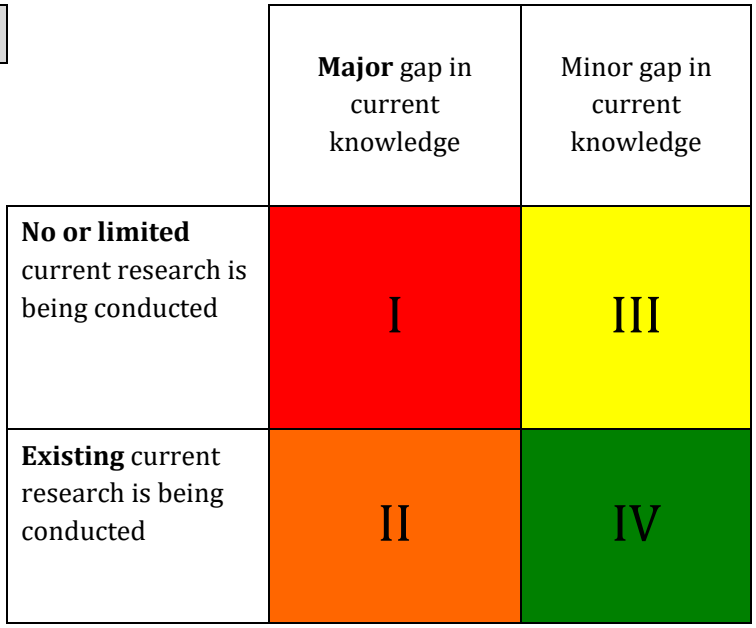
6. In what ways would the research results improve understanding of the scientific basis for the subcommittee(s)?

Better exploration of the library preparation, sequencing and analysis methods for both high quality and challenging samples will help establish an understanding of accurate genotyping calls, which is critical for downstream application in generating intelligence leads and performing direct and kinship identification.

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

It will provide an assessment of the overview of the current "climate", that includes commercial entities, types of methods, kit performance, including new approaches (i.e., hybridization vs. PCR), pre-sequencing, and more importantly a significant exploration of the algorithms utilized for variant calling, from STRs to SNPs. This step is vital for correct input of data into statistical and modeling tools/programs downstream. Sequencing alignment methodologies and thresholds for genotyping calling need exploration and comparison within commercial and non-commercial software/tools.

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