IN-BRIEF

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A program of the National Institute of Justice

Next Generation Sequencing Virtual Roundtable: Perspectives from Early Adopters and Researchers

Objectives

- Summarize discussions from a team of NGS early adopter forensic science practitioners and researchers about the enablers and limitations of procuring NGS technology in house (versus outsourcing via an NGS vendor laboratory).
- Identify opportunities, including resources and approaches, to support adoption of NGS technology in-house within the forensic community.

Overview

In 2023, the Forensic Technology Center of Excellence (FTCOE), in partnership with the National Institute of Justice (NIJ), convened a virtual roundtable of forensic science service providers (FSSPs) and forensic science researchers with experience implementing next generation sequencing (NGS) within their laboratories for practical application.

NGS represents a potentially transformative opportunity for FSSPs because the technology can provide comprehensive information from a DNA sample and can improve analysis of highly degraded or otherwise compromised samples.[1,2,3] NGS implementation may offer long-term cost, labor, or time savings compared with traditional forensic DNA sequencing and detection methods[A] (e.g., eliminating the need to perform various sequential analyses to achieve the same information yield, decreased required labor input).[4,1] In-house implementation (i.e., procuring NGS products to process samples within an FSSP) may provide additional benefits compared with outsourcing samples for analysis by NGS vendor laboratories, such as reduced turnaround times. However, initial procurement, planning, validation, protocol writing, training, and data storage for NGS requires a significant amount of technical, financial, and staffing resources to implement.[5] As a result, in-house NGS implementation across the forensic science community is low, and the benefits of bringing this technology internally may not yet be fully understood.

A. For example, polymerase chain reaction (PCR) followed by capillary electrophoresis (CE) for short tandem repeats (STRs) or CE-based Sanger sequencing for mitochondrial DNA (mtDNA).

For this virtual roundtable, FTCOE leveraged a representative group across state and local FSSPs who possess a wide array of experiences with NGS applications and products and researchers focusing on technical challenges associated with forensic-specific NGS implementation and applications (see **Exhibit 1**). During this virtual roundtable, panelists identified current drivers of NGS technology into FSSP operation, current needs related to NGS implementation, and potential paths forward. This in-brief summarizes virtual roundtable panelists' insights regarding NGS implementation and potential action items that the forensic science community (including vendors, researchers, and professional organizations) could take to lower the NGS implementation barrier for FSSPs and drive continued improvement and increased technology adoption.

These practitioners and researchers provided their thoughtful experiences, insights, and perspectives during this virtual roundtable.

Exhibit 1: Virtual Roundtable Panelists.

Name	Title	Affiliation*
Megan Foley	Visiting Assistant Professor, Forensic Molecular Biology	The George Washington University
Adam Garver	CODIS Forensic Scientist and MPS Technical Leader	Ohio Bureau of Criminal Investigation
Craig O'Connor	Deputy Director, Department of Forensic Biology	New York City Office of Chief Medical Examiner
Fabio Oldoni	Program Director/Assistant Professor	Arcadia University
Mandi Van Buren	DNA Technical Leader	Kern Regional Crime Laboratory
Jeanette Wallin	Criminalist Supervisor, Method Development	California Department of Justice Jan Bashinski DNA Laboratory
Elisa Wurmbach	City Research Scientist, Department of Forensic Biology	New York City Office of Chief Medical Examiner

*Panelist affiliation at time of virtual roundtable participation.

Enablers of NGS Implementation

Beyond time, funding, and personnel, the panelists discussed the following factors as key enablers that can contribute to successful implementation of in-house NGS capabilities:

Technical and operational benefits offered by NGS that drive interest and demand: Several factors have driven FSSPs to consider implementing NGS in their laboratories including the amount of information it yields, the ability to utilize an increased number of forensically relevant markers and exploit isoalleles, the reduced presence and impact of artifacts on resultant data, and other benefits that this technology offers. Additionally, panelists noted that if batching properly and maximizing samples on a flow cell, NGS may provide a cost-efficient alternative to running multiple sequential analyses, reduce the time associated with sample preparation, and decrease the amount of sample consumed for testing. For more information, a detailed overview of NGS forensic applications and products can be found in the 2023 FTCOE report, Landscape Study of Next Generation Sequencing Technologies for Forensic Applications.

Depending on the library preparation kit used, NGS technology enables users to obtain a breadth of identity-informative data (e.g., autosomal and Y-chromosome STRs, single nucleotide polymorphisms [SNPs]) from a single assay.[B] Furthermore, NGS technology has demonstrated capabilities for producing data from low-template/-quality DNA, enabling users to test lower quality, degraded, or otherwise compromised samples.[6,7] Some panelists indicated that communicating the benefits and similarities of NGS in comparison to PCR and CE to FSSP decision-makers (e.g., comparisons of data outputs and yields, analysis of return on investment for profile and data generation for both methods) helped drive implementation of NGS technology. Panelists found NGS helpful for all DNA markers (e.g., STRs, SNPs) and especially helpful to build technical capacity for unidentified human remains (UHRs) and missing persons cases. For example, NGS technology and the resultant data can enable FSSPs to perform familial analysis or familial searches more efficiently for UHRs or assist in the generation of investigative leads for criminal investigations, thereby expanding capabilities and value to a broad set of cases.[C]

Potential benefit of faster turnaround times: Panelists noted that at the time of their decision-making, NGS outsourcing options often had lengthy turnaround times, driving decisions to bring NGS technology in house. One panelist noted that bringing NGS online at their FSSP for mtDNA sequencing significantly reduced their turnaround times, enabling the FSSP to service not only their UHR and missing persons cases, but UHR requests from their state and neighboring jurisdictions as well. However, possible impact of in-house NGS implementation on turnaround times may vary based on an FSSP's caseload demands.

Technology and vendor developments: Vendors have responded to FSSPs' growing interest in NGS by developing commercially available products specifically for forensic science applications. In addition to the rising number of library preparation kits, vendors are offering software options tailored to forensic use cases, such as bioinformatics tools and analysis software that assist in performing kinship analysis. Forensic-specific software significantly lowers the technical barrier to analyzing data generated. Additionally, full or partial automation tools that integrate with or are made specifically for NGS workflows can streamline library preparation and sample loading, thereby reducing the hands-on work needed when preparing samples for NGS, reducing human error or variability that can occur during sample preparation, and reducing instances of needing to re-prepare a sample, all of which can help standardize testing approaches in use within and across FSSPs, leading to more repeatable and reproducible results. These NGS product offerings, including instrumentation, library preparation kits, and data analysis software, empower FSSPs to select products that best fit their needs. When considering library preparation kit options, FSSPs have flexibility in the type or robustness of assays procured, validated, and implemented for use. For example, FSSPs employing NGS for mtDNA analysis can evaluate some or all of the mitochondrial genome.[D]

Forensic science community buy-in, peer support, and resources: Support and lessons learned from early adopters further enables NGS implementation within the forensic science community. Panelists noted the value of fostering communication channels across FSSPs and sharing resources such as validation studies, implementation plans, and assessment protocols. Working closely with legal experts and other criminal justice colleagues to share experiences and advice on NGS opportunities and realities can help allay concerns and help the community collectively plan for effective implementation of the technology. For example, partnerships with research-based bioinformatics experts and programmers helped one panelist's FSSP analyze and interpret complicated NGS data outputs during validation (prior to forensic-specific bioinformatics tools being made available).

B. FSSPs can choose from several library preparation kits developed specifically for forensic applications; for example, Verogen offers the ForenSeq™ DNA Signature Prep Kit (DNA Primer Mix B), which contains primer pairs for global autosomal, X-, and Y-chromosome STR targets; and identity-, phenotypic-, and biogeographical ancestry-informative SNPs.

C. For example, Verogen offers the ForenSeq Kintelligence Kit for forensic kinship applications (see Antunes, J., Walichiewicz, P., Forouzmand, E., Barta, R., Didier, M., Han, Y., Perez, J. C., Snedecor, J., Zlatkov, C., Padmabandu, G., Devesse, L., Radecke, S., Holt, C. L., Kumar, S. A., Budowle, B., & Stephens, K. M. (2024). Developmental validation of the ForenSeq Kintelligence Kit, MiSeq FGx sequencing system and ForenSeq Universal Analysis Software. *Forensic Science International: Genetics*, 71, 103055. https://doi.org/10.1016/j.fsigen.2024.103055.

D. FSSPs can choose from several mtDNA library preparation kits developed specifically for forensic applications; for example, Thermo Fisher Scientific offers the Precision ID mtDNA Whole Genome Panel Kit for FSSPs desiring to evaluate the entire mitochondrial genome and the Precision ID mtDNA Control Region Panel Kit for FSSPs desiring to evaluate only the mtDNA control region.

FTCOE developed the following resources to help FSSPs understand NGS costs, product options, and workflows:

- Landscape Study of Next Generation Sequencing Technologies for Forensic Applications
- Implementation Strategies: Next Generation Sequencing for DNA Analysis (in partnership with NIJ's Forensic Laboratory Needs Technology Working Group)
- MPS Workflow Through Simulation Tool

Realities of NGS Implementation

Although it may provide significant value for well-resourced FSSPs, internal NGS implementation may not be appropriate for every FSSP. In-house NGS implementation requires significant up-front and ongoing investments and technical capital and staffing to bring the technology online. Because of the large investment and technical barriers to implementation (e.g., validation of NGS instrumentation and assays for particular forensic science applications), the return on investment may not be realized, or the approach may not be appropriate for some FSSPs. Third-party NGS service providers may serve as a more accessible, cost-effective, and scalable approach to implementing NGS in casework. The following discussions of opportunities that can support NGS implementation may still be helpful for FSSPs interested in outsourcing (e.g., centralizing available resources).

Opportunities to Improve NGS Implementation

NGS represents an opportunity to enhance technical capabilities, but the requirements of front-end investments in the forms of time, funding, personnel, and technical expertise are often significant implementation barriers. Beyond the resources and time needed to make NGS implementation a priority, panelists discussed some key themes from their experiences and ideas for community drivers for enhanced adoption:

Centralizing available resources: FSSPs may not be aware of existing resources that could influence their purchasing decisions and approach to assessing NGS technology. Although valuable resources exist (e.g., FTCOE, the American Society of Crime Laboratory Directors Validation & Evaluation Repository), these are often dispersed. The forensic science community could benefit from a regularly updated, impartial, and comprehensive repository that houses not only emerging products and available options (e.g., informational documents detailing available bioinformatics tools and automation solutions for forensic-specific NGS applications) but also includes NGS validation and evaluation data performed by FSSPs. Comprehensive and up-to-date information such as when new NGS-specific standards, research, guidance documents, or publicly available training opportunities become available can help FSSPs stay ahead of new, forensic-specific NGS information.

Additionally, panelists noted the forensic science community would benefit from development of a regularly updated list of FSSPs who have brought each NGS system, library preparation kit, software, and automation solution online for internal testing capabilities. This list would include a point of contact who would be willing to speak to their FSSP's experiences implementing NGS, review validation plans, or answer questions about workflows. Such a contact list would strengthen community engagement and improve accessibility to reach out to peers as knowledgeable resources.

Enabling standardization within the forensic science community through the development of consensus-based guidance: Developing consensus-based best practices that keep technology adopters in alignment, eliminate the need for FSSPs to reinvent the wheel, and reduce inter- and intra-FSSP variability is critical for the forensic science community. Some organizations, such as the Scientific Working Group on DNA Analysis

Methods, have developed helpful resources such as Interpretation Guidelines for SNP Analysis and Autosomal STR Typing. Additional needs for community-developed guidance, as noted by panelists, could include NGS-specific validation protocols and standardized nomenclature, similar to the International Society for Forensic Genetics' recommendations for STR sequence nomenclature.[8] Panelists noted that further guidance on standardized nomenclature will help FSSP use and enable comparisons of NGS data and results to legacy technologies and techniques.

Data storage is a significant operational challenge to sustainable implementation because of the volume of data (including the variety of output file types) generated from NGS and lack of guidance regarding how and where non–Combined DNA Index System (CODIS)-compatible data should be stored. Panelists discussed the need for guidance pertaining to the secure storage of human subjects' data, emphasizing the use of cloud-based technology for storing NGS data. See Tools to integrate emerging NGS technology into additional forensic applications.

Vendor-agnostic training elements and resources: Peer-led, FSSP-driven training may help increase accessibility and availability of resources to less-resourced FSSPs. Panelists noted that developing an ideal, peer-led training course would consist of a blend of practical information delivery and hands-on learning that spans training on how to use NGS technology to process high-template/-quality single-source DNA samples to low-template/-quality DNA mixture samples to mimic samples commonly encountered in casework. Ideally, this would cover the principles of NGS technology (e.g., library preparation: normalization, multiplexing, indexing; clonal amplification; sequencing), provide interactive walkthroughs of how to perform NGS sample preparation and data analysis, and prepare analysts to testify on NGS data or results in court.

Panelists also discussed the importance of access to non-vendor technical resources such as mock datasets (similar to the PROVEDIt database[9] and the National Institute of Standards and Technology's Research-Grade Test Materials).[10] Ideal mock datasets would span DNA samples from one to four contributors and include both true and false reference samples. Such mock datasets would be invaluable for FSSPs who have purchased NGS technology for internal training, workflow development (e.g., assembling a case file, defining the technical review process), building analysts' familiarity with NGS data, and research purposes. These types of mock datasets would also be helpful for FSSPs considering purchasing NGS technology to trial NGS software options prior to purchase to determine which software best fits their needs.

Evaluation of tools and return on investment: NGS is not a universal or one-size-fits-all approach; FSSPs need to understand what workflows and library preparation kits may work best depending on evidence type and other case circumstances. In addition to side-by-side evaluations of commercially available library preparation kits, panelists noted that FSSPs would like more information on the cost-benefit analyses of NGS technology and consumables, including automation solutions.

NIJ has supported several research projects to evaluate and improve NGS technology for forensic applications. For example, The New York City Office of Chief Medical Examiner, through NIJ support, has conducted evaluations and validation studies of NGS systems and kits for specific forensic applications. Research projects included validation of Illumina's MiSeq FGx NGS Platform for casework (2015-DN-BX-K005), an evaluation of NGS technology for missing persons identification (2016-DN-BX-0172), a general evaluation of NGS for routine forensic casework (2018-DU-BX-0166), and a comparative evaluation of NGS kits for mixture deconvolution using probabilistic genotyping (15PNIJ-22-GG-03560-SLFO). These studies are valuable steps toward understanding how NGS may be applied to forensic casework; however, additional steps are needed to evaluate kit performance as it relates to specific evidence and case types.

Forensic-specific technical products: Panelists noted a need for better bioinformatics tools—especially open-source tools—that allow analysts to evaluate data from single-source profiles and mixture outputs and enable them to filter locus-specific artifacts (e.g., stutter, sequence errors) in resulting NGS data. Panelists also noted a need for more automation tools to streamline NGS sample preparation. More research and technical capital are needed to address low-level mixtures, which continue to be a significant technical barrier for existing NGS solutions. Although evaluations of probabilistic genotyping software to interpret NGS mixture data have begun,[11] panelists expressed that additional research in this area is a vital need to enable FSSPs to make the most of NGS data and decrease the time associated with mixture interpretations.

Tools to integrate emerging NGS technology into additional forensic applications: Currently, there is no clear path forward for integrating (or having the ability to upload, store, and search) NGS data that could be relevant to criminal investigations (e.g., flanking region SNPs, intra-STR allele SNPs) in the National DNA Index System. Additionally, commercially available software currently lacks the ability to store and search against newer marker types such as microhaplotypes, indels, and deletion/insertion polymorphism-STRs. Panelists also discussed the need for access to programming information to help build FSSP elimination databases to house information beyond what is currently captured for FSSPs solely performing STR analysis via PCR and CE.[E]

Proficiency testing options: Panelists who have brought NGS online in their laboratories are reporting NGS results to proficiency test (PT) providers in varied ways. Some FSSPs are equipped to report solely length-based data whereas others are equipped to report both length- and sequence-based data. Although PT providers accept the length-based results for single-source profiles and mixtures, there is currently no mechanism for accepting sequence-based results for single-source profiles or mixtures. Most FSSPs report length-based single-source profiles, but others report length-based mixtures generated via NGS that are interpreted via probabilistic genotyping. Panelists discussed the need for PT providers to expand their services to include sequence-based data. In addition, panelists emphasized the need for PT providers to expand their results reporting options to better accommodate differences in FSSP-dependent reporting policies. Panelists noted this expansion will enable the field to better understand inter-laboratory variability and current FSSP accordance related to NGS.

Conclusion

Improving implementation of in-house NGS technology is a collective responsibility across the forensic science community and a task that benefits from coordination across researchers, vendors, and FSSPs. To enable more sustainable implementation by FSSPs, initial action items may look like the following:

FSSPs looking to implement this technology should continue seeking input from early adopters, who "learned as they went" through setting up and preparing to testify on NGS results in court. FSSPs could benefit from communicating technical needs and leaning on researchers and vendors to help address these needs. Staying on top of—and contributing to—research and community-based guidance may help demonstrate the return on investment for internal implementation.

Researchers and technology vendors can benefit from listening to FSSPs' needs for technical developments and advanced tools. The community can only be strengthened by these individuals providing technical support when needed and as appropriate. For example, working with practitioners who may need support designing and executing an independent validation study is an excellent way to strengthen the researcher, vendor, and practitioner nexus.

Convening organizations such as working groups and professional associations, can assist the community through aggregating and cross-promoting resources that help FSSPs bring technology in house, such as cost-benefit analyses and publicly available validation studies. Although forensic genetic genealogy is an important application of NGS technology, focusing on a wide range of applications when developing resources and training materials for the community can be valuable in building a robust knowledge base. Additionally, convening organizations are in a great position to promote dialogue as a "community of practice" for more consistent use of NGS, and collectively plan for NGS implementation as a regular part of a forensic workflow.

E. For example, see Development and Use of an NGS-Based Elimination Database.

References

1. Yang, Y, Xie, B. I., & Yan, J. (2014). Application of next-generation sequencing technology in forensic science. *Genomics, Proteomics & Bioinformatics,* 12(5), 190–197. https://doi.org/10.1016/j.gpb.2014.09.001.

2. Senst, A., Caliebe, A., Scheurer, E., & Schulz, I. (2022). Validation and beyond: Next generation sequencing of forensic casework samples including challenging tissue samples from altered human corpses using the MiSeq FGx™ system. *Journal of Forensic Science*, 67(4), 1382–1398. https://doi.org/10.1111/1556-4029.15028.

3. National Institute of Standards and Technology. (2018, July 23). *NIST builds statistical foundation for next-generation forensic DNA profiling.* https://www.nist.gov/news-events/news/2018/07/nist-builds-statistical-foundation-next-generation-forensic-dna-profiling.

4. NIJ Forensic Laboratory Needs Technology Working Group (FLN-TWG). (2021, September). *Implementation strategies: Next generation sequencing for DNA analysis*. Research Triangle Park, NC: RTI International. https://forensiccoe.org/fln-twg-next-generation-sequencing/.

5. Foley, M. & Oldoni, F. (2023). A global snapshot of current opinions of next-generation sequencing technologies usage in forensics. *Forensic Science International: Genetics*, 63, 102819. https://doi.org/10.1016/j.fsigen.2022.102819.

6. Sharma, V., van der Plaat, D., Liu, Y., & Wurmbach, E. (2020). Analyzing degraded DNA and challenging samples using the ForenSeq DNA Signature Prep Kit. *Science & Justice*, 60(3), 243–252. https://doi.org/10.1016/j.scijus.2019.11.004.

7. Cuenca, D., Battaglia, J., Halsing, M., & Sheehan, S. (2020). Mitochondrial sequencing of missing persons DNA casework by implementing Thermo Fisher's Precision ID mtDNA Whole Genome assay. *Genes, 11*(11), 1303. https://doi.org/10.3390/genes11111303.

8. Gettings, K. B., Bodner, M., Borsuk, L. A., King, J. L., Ballard, D., Parson, W., Benschop, C. C. G., Børsting, C., Budowle, B., Butler, J. M., van der Gaag, K. J., Gill, P., Gusmão, L., Hares, D. R., Hoogenboom, J., Irwin, J., Prieto, L., Schneider, P. M., Vennemann, M., & Phillips, C. (2024). Recommendations of the DNA Commission of the International Society for Forensic Genetics (ISFG) on short tandem repeat sequence nomenclature. FSI *Genetics*, 68 (102946). https://doi.org/10.1016/j.fsigen.2023.102946.

9. Alfonse, L. E., Garrett, A. D., Lun, D. S., Duffy, K. R., & Grgicak, C. M. (2018). A large-scale dataset of single and mixed-source short tandem repeat profiles to inform human identification strategies: PROVED-It. *Forensic Science International: Genetics*, 32, 62–70. https://doi.org/10.1016/j.fsigen.2017.10.006.

10. National Institute of Standards and Technology. (2023, July 14). Forensic DNA Resource Samples Guidance Document: NIST Research-Grade Test Material 10235.

11. National Institute of Justice. (2022). *Comparative evaluation of massively parallel sequencing STR kits with the emphasis on mixture deconvolution utilizing probabilistic genotyping*. https://nij.ojp.gov/funding/awards/15pnij-22-gg-03560-slfo.

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