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Potential Applications of Nanopore Sequencing for Forensic Analysis

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Potential Applications of Nanopore Sequencing for Forensic Analysis[a]

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ABSTRACT: Advancements in DNA sequencing technologies are occurring at a rapid rate. Various platforms have proven useful in all aspects of health and science research, from molecular diagnostics in cancer research to spore identification in bioterrorism. In the field of forensics, one particular single-molecule sequencing platform shows promise for becoming a viable solution for small to midsize forensic laboratories.

Oxford Nanopore Technologies (ONT) has developed a portable, nanopore-based sequencing instrument that has already been utilized for on-site identification of Zika and Ebola viruses, full genome sequencing, evaluation of DNA and RNA base modifications, and enrichment-free mitochondrial DNA analysis. The rapid development of this technology creates possibilities relevant to standard DNA sequencing, direct analysis of forensic samples, including blood, semen, and buccal swabs, mitochondrial DNA analysis, SNP and STR analysis, familial identification, and microbial identification for bioterrorism and geolocation. The small size of the platform, its low cost, and its requirement of only basic laboratory equipment makes this platform well suited for small laboratories wishing to begin developing expertise in sequence-based forensic analyses.

Herein, we outline recent developments and applications of nanopore sequencing technologies and their potential application in forensic analysis. We address current and potential techniques in mitochondrial DNA analysis, SNP and STR typing, and microbial identification. Additionally, we discuss recent developments in library preparation and data analysis tool further streamlining the sequencing process that integrate workflows in laboratories or in remote field scenarios.

KEYWORDS: Forensic DNA, microbial forensics, mtDNA, nanopore sequencing, SNP, STR.

INTRODUCTION

Forensic Genetic Marker Systems

DNA evidence has long been considered the gold standard for human identification in forensic investigations. Forensic genetic analyses may harness the information within various markers depending on both the nature of the evidence and case at hand. Most often, DNA typing exploits the high variability of short tandem repeat (STR) sequences to differentiate between individuals at the genetic level [22,48,73,92]. Comparison of STR profiles can be used for human identification in a wide range of forensic cases including homicides, sexual assaults, missing persons, and mass disaster victims [92]. In addition to autosomal short tandem repeats (auSTRs), lineage-specific genetic markers located on the Y chromosome (Y-STRs) can facilitate in the identification of male sources and may provide critical information in cases involving sexual assault evidence and unestablished paternity [22,92,93].

Typical STR typing workflow consists of amplification followed by size-based separation and detection via capillary electrophoresis (CE) [24,92]. The power of discrimination achieved by the 20 loci in the expanded core CODIS panel is often sufficient for routine forensic casework [78]. However, the information obtained using standard STR typing approaches may be inadequate for the deconvolution of mixed DNA profiles and some complex kinship analyses even when additional loci are interrogated [122,123]. The abundance of nucleotide variation observed within and around common forensic STR markers demonstrates that sequence-level data not captured by repeat length techniques are highly beneficial in human identity testing [62–64,121,122,144,146,173,174]. Detection of hidden variation at these microsatellite loci would significantly expand upon the level of resolution realized via CE by enabling differentiation between alleles with the same base composition but alternate motif organizations [145].

STR loci are the most commonly utilized marker system for forensic human identity testing due to the high genetic instability and ease of CE-based interrogation [177]. Despite these advantages, current typing techniques are limited to repeat length information and rely on the generation of longer range PCR amplicons, which may prove challenging when working with highly degraded or low template samples. Researchers have demonstrated that other genetic markers, including single nucleotide polymorphisms (SNPs) [97], mitochondrial DNA (mtDNA) [15], and even microbial species [20,74], harbor additional information capable of generating pivotal

[a]Roxanne Zascavage has participated in Oxford Nanopore Technologies-sponsored meetings over the past 3 years and received travel reimbursement for presenting at an event. Fritz Sedlazeck obtained a PacBio SMART grant in 2018 and has had multiple travels sponsored by Pacific Biosciences Inc. and Oxford Nanopore Technologies Ltd.

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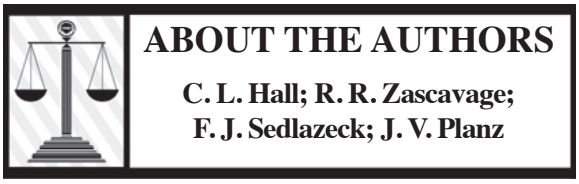

ABOUT THE AUTHORS

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Courtney L. Hall holds a B.S. degree in forensic science from St. Edward's University (Austin, TX) as well as an M.S. degree in forensic genetics from the University of North Texas Health Science Center (UNTHSC; Fort Worth, TX). She is currently a genetics doctoral student in the Department of Microbiology, Immunology, and Molecular Genetics at UNTHSC.

Ms. Hall received a predoctoral fellowship in the Neurobiology of Aging and Alzheimer's Disease Training Program. Her research interests include the role of epigenetic modifications in neurobiological and cognitive processes, and thus the progression and pathology of age-related brain disorders. Ms. Hall is a member of the American Academy of Forensic Science (AAFS) and the American Society of Human Genetics (ASHG).

Roxanne R. Zascavage earned a B.S. degree in biology from the Texas Woman's University (Denton, TX) and an M.S. degree in forensic and investigative genetics from the University of North Texas Health Science Center (UNTHSC; Fort Worth, TX), where she continued on to earn her Ph.D. in Molecular and Medical Genetics. Dr. Zascavage is now an assistant professor in the Criminology and Criminal Justice Department at the University of Texas at Arlington (UTA).

Prior to advancing her education at UNTHSC, Dr. Zascavage held a position at Baylor College of Medicine (Houston, TX) in a human genetics laboratory studying autism and genetic links to autism-like diseases. She also has a background in teaching, including high school biology, assisting with molecular methods courses for master's students, and graduate-level tutoring in microbiology and immunology. At UTA, as one of two full-time forensic experts, Dr. Zascavage teaches forensic science courses for undergraduate students, and since her arrival in 2017, has helped to develop a minor in Forensic Applications in Science and Technology. Dr. Zascavage's research focuses on utilization of the Oxford Nanopore MinION device for human identification purposes. In particular, she is passionate about the mtGenome, and also studies the interaction between the nuclear and mitochondrial genomes as they relate to energy production via oxidative phosphorylation.

Fritz Sedlazeck completed his Ph.D. in 2012 in the group of Dr. Arndt von Haeseler at the Max F. Perutz Laboratory (Vienna, Austria). After a two-year postdoc, he transitioned to the lab of Dr. Michael Schatz at Cold Spring Harbor Laboratory (Cold Spring Harbor, NY) and later to Johns Hopkins University (Baltimore, MD). Since 2017 he is an assistant professor at the Human Genome Sequencing Center at Baylor College of Medicine (Houston, TX).

Dr. Sedlazeck's research focuses on developing computational methods to detect and analyze genomic variations with a focus on structural variations. Structural variations are genomic events that manipulate multiple positions in a genome, which impact evolution, genomic disorders, and regulation as well as play an important role in explaining multiple phenotypes. The impact of these events on the variability within and between individuals is his main focus and how we can leverage novel sequencing technologies to obtain better insights into medical, research, and forensic applications.

John V. Planz holds a B.S. degree in biology and zoology (double major) from the State University of New York (Oswego, NY), an M.S. degree in behavioral ecology from Shippensburg University (Shippensburg, PA), and a Ph.D. degree in molecular evolutionary genetics and population genetics from the University of North Texas (Denton, TX). Dr. Planz was a postdoctoral fellow at the Carnegie Museum of Natural History, Section of Mammals (Pittsburgh, PA), in mammalian phylogenetic systematics. He is currently an associate professor in the Department of Microbiology, Immunology and Genetics at the University of North Texas Health Science Center (UNTHSC; Fort Worth, TX).

Dr. Planz joined the forensic community as a forensic serologist at the Southwestern Institute of Forensic Science (Dallas, TX) in 1993, where he served as a forensic analyst and implemented PCR-based DNA testing methods at the laboratory. He later served as director of identity testing at GeneScreen, Inc. (Dallas, TX) and director of the forensics unit at Biosynthesis, Inc. (Lewisville, TX). Upon joining the faculty of UNTHSC in 2001, Dr. Planz implemented the use of capillary electrophoresis and mitochondrial DNA testing for the DNA Testing Laboratory and along with the late Dr. Arthur Eisenberg established the UNT Center for Human Identification. Dr. Planz served in the role of associate director for the laboratory for 17 years, establishing its operations, workflows, and quality assurance program. At UNTHSC, Dr. Planz serves as program advisor for genetics and provides training in biostatistics, molecular genetics, and population genetics in the Graduate School of Biomedical Sciences as well as the Texas College of Osteopathic Medicine and the joint TCU-UNTHSC School of Medicine. He has developed the program and foundational courses of the Masters in Forensic Genetics Program offered by the university that provided advanced training in forensic genetics applications to over 100 students now serving in crime laboratories throughout the US.

Dr. Planz has presented workshops in forensic statistics at numerous national and international conferences and provided continuing education in this area to many crime laboratories nationwide. He currently serves as a member of the Scientific Working Group on DNA Analysis Methods on the Next Generation Sequencing Committee.