Deep-Sequencing Technologies and Potential Applications in Forensic DNA Testing

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ABSTRACT: Development of second- and third-generation DNA sequencing technologies have enabled an increasing number of applications in different areas such as molecular diagnostics, gene therapy, monitoring food and pharmaceutical products, biosecurity, and forensics. These technologies are based on different biochemical principles such as monitoring released pyrophosphate upon incorporation of a base (pyrosequencing), fluorescence detection subsequent to reversible incorporation of a fluorescently labeled terminator base, ligation based approach wherein fluorescence of cleaved nucleotide after ligation is measured, measuring the proton released after incorporation of a base (semiconductor-based sequencing), monitoring incorporation of a nucleotide by measuring the fluorescence of the fluorophore attached to the phosphate chain of the nucleotide, and by detecting the altered charge- in a protein nanopore due to released nucleotide by exonuclease cleavage of a DNA strand. Analysis of multiple DNA fragments in parallel increases the depth of coverage while decreasing labor, cost, and time, highlighting some major advantages of deep-sequencing technologies. DNA sequencing has been routinely used in the forensic laboratories for mitochondrial DNA analysis. Fragment analysis, however, is the preferred method for Short Tandem Repeat genotyping due to the cumbersome and costly nature of first-generation DNA sequencing methodologies. Deep-sequencing technologies have brought a new perspective to forensic DNA analysis. Studies include STR analysis to reveal hidden variation in the repeat regions, mtDNA sequencing, Single Nucleotide Polymorphism analysis, mixture resolution, and body fluid identification. Recent publications reveal that attempts are being made to expand the capability.

KEY WORDS: DNA, DNA sequencing, mtDNA, next-generation sequencing, RNA body fluid identification, single nucleotide polymorphisms, STR.

INTRODUCTION

Forensic DNA testing most frequently includes analysis of short tandem repeat (STR) markers. STRs are routinely utilized for comparison of autosomal and Y-chromosomal DNA for paternity testing [15,16,33,36,94, 135], criminal investigation [42,96,141,162,191], human remains identification [6,57,64,90,155,163], and population structure analysis [12,23,45,101,176]. The current method for STR analysis involves polymerase chain reaction (PCR) amplification of the target STR loci using a commercialized kit followed by fragment analysis of fluorescently labeled amplicons via capillary electrophoresis (CE) [31,32,102,190]. The data resulting from STR analysis using the CE method consists of the number of repeat motifs present for alleles at each locus relative to an allelic ladder. However, analysis of the fluorescently labeled amplicons using CE cannot identify differences in nucleotide composition of the amplified region other than those that alter fragment size, e.g., insertions/deletions.

When resolution at the nucleotide level is required for comparative analyses, DNA sequencing is performed. At present, DNA sequence analysis is limited to the investigation of portions of the mitochondrial DNA (mtDNA) Control Region. Approximately 600–1,000 nucleotides of the 16.5-kilobase genome are typically compared between evidentiary and known samples for two hypervariable regions. Although these regions are of the highest diversity in the mitochondrial genome, the investigation of variants in the remainder of the genome is often necessary to differentiate between common haplotypes. Deep-sequencing technologies will have the capability to increase discrimination power of traditional forensic loci by providing sequence-based information that can increase allelic diversity and will permit the entire mitochondrial genome of an individual to be sequenced with resolved nucleotide determination. By directly sequencing forensic markers, inferences to allelic identity can be eliminated and allow for databasing and comparison of DNA data on its most discrete level.

Traditional DNA-sequencing methods originally evolved from three approaches. The original technique developed in 1975 by Sanger and Coulson [157] was referred to as Plus-Minus Sequencing. To prepare for sequencing, the DNA region of interest was cloned to produce a single-stranded template. The template was then hybridized to a primer and incubated with DNA polymerase and a cocktail of deoxyribonucleotide triphosphates (dNTPs). The reaction created varying-length complimentary copies of

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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). She is currently a Ph.D. candidate at UNTHSC continuing her studies in forensic and investigative genetics.

Prior to advancing her education at UNTHSC, Ms. 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. Her current research interests include the study of the interaction between the nuclear and mitochondrial genomes as they relate to energy production via oxidative phosphorylation.

Ms. Zascavage is a current member of Forensic Investigation, Research, and Education (a student organization at UNTHSC), as well as the Association of Forensic DNA Analysts and Administrators.

Shantanu J. Shewale holds a B.S. degree in microbiology and a minor in chemistry from Louisiana State University (Baton Rouge, LA). Mr. Shewale is currently a doctoral student in the Department of Forensic and Investigative Genetics at the University of North Texas Health Science Center (UNTHSC; Fort Worth, TX). He is also working on his project management certification from the University of California–Berkeley Extension. He also serves as the president of Forensic Investigation, Research, and Education (a student organization at UNTHSC), where he is involved in community outreach and volunteer projects throughout the university.

Mr. Shewale has worked on a research project during his undergraduate studies within the Department of Entomology that was funded by the College of Agriculture at Louisiana State University. Before his undergraduate studies, Mr. Shewale has held an internship at ReliaGene Technologies (New Orleans, LA) where he created a program used to generate results for a genotyping assay, and has also placed first in a statewide technical math competition held within the state of Louisiana.

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 Forensic and Investigative Genetics, University of North Texas Health Science Center (UNTHSC; Fort Worth, TX). He also serves as the associate director of the UNT Center for Human Identification.

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 at the laboratory and developed the foundational courses of the Professional Masters' Degree Program in Forensic Genetics offered by the university.

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 and the Virginia Scientific Advisory Committee.