MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 36 Issue 1, Jan-June 2026, Pages: 1366-1370

An Integrating Third-Generation Allelic Research on Enhancing Biological Evidence in Criminal Investigations

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Abstract
The combination of third-generation allelic research with forensic science equals a revolutionary bent in the applicability of biological evidence in criminality investigation. This paper aims to discuss the third-generation sequencing technologies in the advancement and uses of the techniques with a focus on their high accuracy, resolution, and dependability compared to the previous generations. By breaking down the different advancements, and using case studies, we show how improvements can be made to the DNA profiling process to increase the reliability, which is beneficial when used in legal proceedings. The results presented here clearly demonstrate the future possibilities of the third-generation allelic study in changing the course of `modern forensics and providing a better and more detailed means of identifying and comprehending the outcome of the allelic distribution in question. Therefore, besides increasing the scientific basis of forensic examination, there are advantages for judicial work as a result of this integration, such as the use of more reliable and definitive biological evidence. The results highlight the necessity of incorporating third-generation allelic research into the fields of forensic science, stressing that its implementation might completely change the modern state of practices in the sphere. It also guarantees to add professionalism and accuracy of criminal investigation, as well as to improve the general credibility of the judicial system, as the new integration guarantees more certain and precise biological proof.

Keywords: Third-Generation Sequencing, Allelic Research, Forensic Science, DNA Profiling, Biological Evidence, Criminal Investigations, Long-Read Sequencing, Genetic Analysis, Forensic Methodologies, Judicial Outcomes

1. Introduction

Bodily remains have always been associated with forensic science as one of the pillars of gathering evidence that would lead to the identification of the culprit behind a crime. DNA technology has developed tremendously over the past few decades and has greatly improved the efficiency as well as the effectiveness of forensic investigations. This paper therefore seeks to discuss how Third-generation allelic research will help improve the use of biological evidence in criminal trials. In this section, future technological changes, which define third-generation sequencing, will be discussed, arranged in comparison with the previous generations, as well as the potential consequences for forensic procedures and legal/punitive repercussions (Carratto, et al. 2022). In presenting various case studies and comparative analyses, the authors shall illustrate how such technologies can change forensic science and the different issues and benefits that forensic science can accrue when implementing such technologies.

1.1 Background

The field of forensic science has experienced a significant transformation in the last few decades mainly because of the development of molecular biology especially in DNA analysis. Since the discovery of DNA profiling in the late 1980s, the assessment of a person's genetic material has become an indispensable feature in criminal cases as regards to recognition of certain persons. The progress in allelic research has also contributed to these improvements as this approach lies in the variations of specific DNA sequences. The first generation of DNA sequencing was introduced by Sanger and is regarded as the basis for modern genetic analysis. The second wave of sequencing that originated in the mid-2000s also benefited genetic analysis by raising the speed and scale of DNA sequencing a notch higher (Chakravarty, et al. 2022). Third-generation sequencing makes it possible to create significantly longer reads than second-generation sequencing, which offers a more extensive viewpoint on the sequence of the genetic code. It also allows for the correct assembling of the large complicated genomic zones which was not achievable earlier. Concisely, the incorporation of third-generation allelic research into the discipline of forensics could be regarded as a major addition to biological process usage. As a result of eliminating previous sequencing technologies' shortcomings, third-generation Sequencing provides greater sensitivity and specificity of DNA analysis, the application of which may bring significant change to the practice of forensic science and improve crime-fighting capabilities.

2. Literature Review

This section aims at presenting information about the historical background, technologies and application of the mentioned methodologies focusing on the relevance of those methodologies in the improvement of the utilization of biological traces in criminal proceedings.

The article provides a detailed history of allelic research in forensic science, starting with the discovery of DNA profiling in the 1980s. Variable number tandem repeats, discovered by Alec Jeffreyn, enabled DNA fingerprinting, making identification of individuals based on their genetic structure possible. The first-generation sequencing, represented by Sanger sequencing, allowed for the sequencing of short DNA fragments but had limitations such as slowness, high costs, and difficulty handling long DNA sequences.

Second-generation sequencing (NgS) emerged in the mid-2000s, contributing to better identification of DNA features and increasing the definition of genetic differences. Third-generation sequencing, such as nanopore sequencing, allows for real-time DNA sequencing, which can be useful in cases requiring quick results, such as forensic investigations.

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Biological evidence, particularly DNA, is increasingly used in criminal cases, with fingerprinting being used to identify culprits, unconvicted accused, and determine family tendencies. A cross-sectional comparison of sequencing generations highlights the progressive improvements in allelic research. First-generation sequencing suffers from short read lengths and low throughput, while second-generation sequencing improves productivity and detection of smaller fragments but faces issues with the complexity of some genetic regions and reduced read length capability.

The application of third-generation allelic research in forensic science has enormous potential to enhance DNA profiling efficacy, as it accurately sequences different samples, identifying minor genetic differences and intricate patterns of genetic make-up, making evidence more conclusive and perception-based. Future directions in forensic allelic research include further improvements and optimization of third-generation sequencing technologies, as well as exploring their routine use in forensic cases and their cost, time, and ease of access..

2.1 Research Gap

Although, allelic research and third-generation sequencing technologies have been advanced significantly in recent years the following knowledge and application gaps are still existing as per contexts of Elkins, & Zeller, (2021). Such gaps point to the need for future research to explore the use of third-generation allelic research in forensic science to its full capabilities.

2.2 Research Question

I. How is it possible to incorporate third-generation allelic studies into the essence of forensic science to increase its effectiveness, efficiency, and applicability to the given criminal cases?
II. What are the effects of the use of third-generation sequencing technologies in forensic investigation especially regarding cases with degraded or mixed samples?
III. What are the realistic consequences of implementing third-generation sequencing into the regular requests of forensic science, and how do such technologies affect the organization, distribution of resources, and productivity of forensic facilities?

2.3 Research Objectives

I. To identify the current gaps in the literature regarding protocols for third-generation sequencing in forensic science.
II. To verify the accuracy, analytical replicate ability and reproducibility of the third-generation sequencing approaches in the investigation of forensic cases.
III. To discover ways of linking and incorporating third-generation sequencing data to the existing databases in forensic DNA analysis based on fundamental sequencing technologies.

2.4 Research Limitations

Next-generation sequencing platforms are still under development and its uses or implementations in forensic science may have several issues such as higher levels of difficulty in data analysis and possible errors in sequencing. Applying and sustaining the 3G sequencing at the working site is expensive, and demands a lot of money for purchasing the sequencing instruments, chemicals, and training of the technicians.

3. Research Methodology

3.1. Research method and Design

The secondary research methodology involves defining the research scope, objectives, and focus of third-generation allelic research in forensic science. Significant research questions will be identified to help review and synthesize literature. The literature search strategy includes electronic searches in academic databases like PubMed, Scopus, Web of Science, and other forensic science journals, institutional repositories, and conventional websites. Major sources of studies and reviews will be included using search terms like third-generation sequencing, DNA analysis, and specific technology.

3.2. Research Approach

The qualitative research methodology involves selecting a research philosophy such as phenomenology, ethnography, grounded theory, or case study, and selecting participants with expertise in fields like forensic science, third-generation sequencing, information technology, law, and policymakers. Data collection methods include in-depth interviews with key informants, experts, legal practitioners, and stakeholders, as well as focus groups to gain multiple perspectives and convergence on relevant themes. Focus groups are organized to gain multiple perspectives and communication from participants. Document analysis is conducted through interviews with target respondents to supplement data gleaned from relevant documents, including scientific publications, policy papers, legal documents, and organizational reports. This research approach aims to understand the experiences, views, concerns, and attitudes of stakeholders in the application of forensic science.

4. Results

4.1. How is it possible to incorporate third-generation allelic studies into the essence of forensic science to increase its effectiveness, efficiency, and applicability to the given criminal cases?

Incorporating third-generation allelic studies into forensic science can significantly enhance its effectiveness, efficiency, and applicability to criminal cases through several key strategies: Incorporating third-generation allelic studies into forensic science can significantly enhance its effectiveness, efficiency, and applicability to criminal cases through several key strategies. Improved Resolution and Accuracy: Third-generation sequencing technologies provide novel features such as longer read lengths and improved accuracy in comparison with the second-generation. This capability also enables accurate identification and denomination of genetic differences; thus, improving the discriminative coefficients of DNA ways in forensic measurements. Microsatellites are more precise features to screen and portray since they allow forensic practitioners to better link the found samples with certain people or rule out suspects.

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Enhanced Detection of Complex Variants: Third-generation sequencing is best suited for whole-genome, as compared to single nucleotide, analysis and its strengths include the identification of structural variations, repetitive sequences, and epigenetic changes (Malhotra, et al. 2024). These variants are especially essential in forensic applications of DNA typing when the samples are fragmented or complicated by the presence of more than one individual's DNA. The obtainment of such broad genetic data considerably enhances the chances of obtaining incriminating forensic evidence.

Resolution of Complex DNA Mixtures: It is, however, important to realize that in most forensic cases, there are DNA mixtures from two or more people that make the interpretation using conventional approaches difficult. A clearer vision of the contributors of complex cases is made possible by Third-generation sequencing which promises to differentiate between the genetic profiles in mixed samples.

Handling Degraded DNA Samples: Environmental factors, time and conditions under which samples are stored can hamper the conventional DNA analysis techniques. These difficulties can be surmounted by third-generation sequencing which yields longer reads from the DNA fragments, unlike the first and second-generation sequencing.

Real-Time and Rapid Analysis: Technologies such as nanopore sequencing where the process of identifying the sequence of a DNA strand is done in real-time will enable fast processing of forensic cases. Accomplished swift analysis of biological evidence can enhance the rate at which cases are solved by LEAs and assist provide appropriate decision support at the right time.

Integration with Forensic Databases: The ability of third-generation sequencing data allows incorporating them into the existing forensic DNA databases while increasing the density of genetics of the database individuals. This integration increases some parameters of the database connection of criminal-related DNA with known persons or with other criminal cases, making it more effective.

Cost-Effectiveness and Scalability: The initial cost per sample might be an issue; however, constant improvements in technologies associated with third-generation sequencing cast a plan to revolutionize the costs regarding equipment, reagents, and analysis (Sobiah, et al. 2018). With higher efficiency, the application of these technologies increases and it becomes less expensive making it more applicable to forensic laboratories across the globe.

Training and Education: This means that to get proper integration of TGS into forensic science, the forensic scientists and technicians have to undergo special training. Criminal professionals' educational programs and workshops are aimed at enhancing their skills in using different sequencing methodologies and data analysis and interpretation granting the technology optimum employment in criminal investigations.

4.2. What are the effects of the use of third-generation sequencing technologies in forensic investigation especially regarding cases with degraded or mixed samples?

The use of third-generation sequencing (TGS) technologies in forensic investigations, especially in cases involving degraded or mixed DNA samples, brings several significant effects and advantages to the field: The use of third-generation sequencing (TGS) technologies in forensic investigations, especially in cases involving degraded or mixed DNA samples, brings several significant effects and advantages to the field:

Improved Resolution and Accuracy: Degraded Samples: Compared to the traditional methods, TGS is capable of handling degraded DNA samples since it generates longer sequencing reads. This capability enables more accurate identification when using partially degraded or damaged DNA samples, as would be the case if remains were spotted years after a disaster.

Mixed Samples: Compared to other older technologies, TGS can differentiate the particular genetic patterns in the mixtures with a higher level of clarity. This ability is essential in situations where several individuals are contributing and enables forensic scientists to isolate and correctly determine the individual contributor's DNA profile.

Enhanced Detection of Genetic Variants: TGS is capable of identifying numerous genetic changes such as structural variations, repeats and modifications to DNA (Simayijiang, et al. 2023). These variants are sometimes not detected or are described inadequately using older sequencing techniques, thus giving a better view of the genetic character of the evidence.

Increased Sensitivity and Coverage: TGS technologies, that have better sensitivity and coverage, allow the detection of DNA sequences with low copy numbers or traces in samples. This capability is vital, especially in other forensic circumstances where there are little DNA traces that can be analyzed or where the DNA has deteriorated.

Faster and Real-Time Analysis: Some of the TGS platforms include nanopore sequencing that has real-time analysis possibilities. This feature enables forensic scientists to acquire sequencing results quickly enough, and it may even enhance the rate at which forensic investigations are conducted thereby presenting timely information to the concerned law enforcement bodies.

Forensic Database Integration: The addition of TGS data into existing forensic DNA databases is quite possible, and as a result, the database will be complemented by more detailed genetic information (Sessa, et al. 2020). It also increases the capacity of the database to link the recovered DNA profiles from crime scenes to individuals or other forensic instances, thus increasing the efficiency and effectiveness of forensic investigations.

Challenges and Considerations:

- Cost and Complexity: Some of the challenges identified in forensic laboratories pertain to the costs, particularly the initial investment, and the difficulty in analyzing and interpreting the data gathered from TGS technologies.
- Validation and Standardization: To achieve a high level of reliability and reproducibility of TGS results in forensic practice, protocol and analytical methods have to be validated and standardized.

Ethical and Legal Considerations: Matters like privacy, consent and the legal permissibility of the use of sequencing-derived evidence in controversies and trials call for adherence to ethical and legal principles.

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Therefore, the use of third-generation sequencing technologies in forensic intervention and, especially, in the context of the analysis of severely degraded and mixed DNA samples considerably expands the opportunities for the identification and investigation of genetic information with a higher resolution and accuracy (Saiz, et al. 2020). These advancements also, help in enhancing the forensic evidence, enhance the criminal investigations, and bring better abilities to justice and safety.

4.3. What are the realistic consequences of implementing third-generation sequencing into the regular requests of forensic science, and how do such technologies affect the organization, distribution of resources, and productivity of forensic facilities?

The implementation of third-generation sequencing (TGS) into regular forensic science practices can have several realistic consequences and impacts on forensic facilities, including organization, resource distribution, and productivity:

- Enhanced Analytical Capabilities:
  - Increased Resolution and Accuracy: Technologies in TGS provide higher resolution and accuracy in the DNA profiling thus enabling the forensic scientists to get more information from the biological samples (Perera, et al. 2022). This capability improves the efficiency and the accuracy of the discrimination of people using DNA analysis in criminal investigations.
  - Resolution of Complex Cases: TGS is particularly useful in working with degraded or mixed DNA samples which sometimes may be difficult to analyze using conventional methods. This capability decreases the number of triangular cases and increases the prospects of acquiring effective forensic information.
- Resource Allocation and Management:
  - Initial Investment: Enforcement of TGS entails considerable capital investments in instruments, technology, and training of personnel on technical aspects of forensic work. TGS platforms will require resources for purchasing them as well as funds for maintenance and for hiring people that take care of these requirements in forensic facilities.
  - Operational Costs: Although through operating with TGS technologies it is possible to save a great deal of time and money by cutting down on the reagents and improving the overall efficiency, initially, the operational costs may be higher compared to comparable conventional methods (Nigam, et al. 2020). Effective ongoing operations must implement proper strategies and mechanisms of budgeting and resource utilization.
- Workflow Integration and Efficiency:
  - Integration Challenges: Implementation of TGS in current forensic practices means the modification and fine-tuning of the approaches and routines in the forensic laboratory. It may be seen that, to incorporate TGS capabilities, forensic facilities may require modifying their workflow concerning sample processing, data analysis, reporting etc.
  - Productivity Gains: When implemented, TGS can increase productivity by shortening the time to DNA analysis results, and increasing the total amount of samples that can be tested. Some TGS platforms come with real-time or rapid sequencing which has the potential advantage of enhancing the speed of forensic investigations hence faster dispositions of cases and increased efficiency.
- Quality Assurance and Standardization:
  - Validation and Accreditation: TGS methodologies have to be proven effective in forensic facilities and meet the requirements of accreditation and regulations (Mehta, 2019). Being able to report the images generated by the TGS for use in a court of law and being able to convince the presiding Judge that the images that are being presented in the court are genuine and authentic requires a structured quality assurance process that must be followed to the letter.
  - Standard Operating Procedures: The generation of standard operating procedures (SOPs) for TGS applications in forensic science is crucial as it will provide the guidelines for the proper and efficient running of the applications in different forensic laboratories.
- Impact on Forensic Database Management:
  - Database Enrichment: TGS data can enhance the forensic DNA database by enhancing a higher resolution match type and thus, an improved capacity of the database to link matches from the evidence found in different cases or forensic regions.
  - Data Handling and Storage: TGS churns out large volumes of sequencing data hence proper data management systems are very vital and secure space to store the data. Therefore, it is understood that the facilities for forensics must have various measures and norms for managing, storing and protecting data containing particular genetic information.
- Training and Human Resources:
  - Skills Development: Thus, for TGS technologies to be effectively used in the forensic context, forensic scientists, technicians and bioinformaticians need to be trained on how to use them apart from acquiring on-the-job skills updates (McNevin, 2020). It would be useful to mention that the training programs can include information about the available instruments, methods of data analysis, interpretation of complex genetic data, and other important aspects like problem-solving.
  - Workforce Capacity: TGS may require centre facilities to recruit or reallocate more staff to efficiently lead the facilities as forensic centres. A sufficient number of properly trained staff is a necessity to make the best out of the innovations applied to TGS technologies and to ensure sustainable quality of forensic work.

5. Conclusion

Therefore, third-generation allelic research enhances the application prospects of biological evidence in forensic science, thus being a giant leap forward. Since the third generation of sequencers is designed to be more efficient in delivering better quality genomic data, it also provides greater performance in identifying multiple variations in the DNA sequence and

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offering detailed nucleotide types. These enhancements advance forensic science specifically in the aspects of discrimination authors associated with the DNA. The common issues that are associated with the implementation of third-generation sequencing include standardization, validation, and bioinformatics tools. Each of these challenges must be addressed to prevent variability in results obtained in forensic casework. Possible future studies need to address the fine-tuning of the existing protocols, the achievement of lower costs, further inclusion of databases and improvement of the bioinformatics tools used in the forensic environment. All of these will help to enhance the establishment of the third-generation allelic research This will further the enhancement of the application of allelic research in the standard forensic functioning. Conclusively, there are certain difficulties and/or obstacles that have to be addressed, but the incorporation of third-generation allelic research is the key to the future of forensic DNA science, which implies increased accuracy, reliability, and informativeness of DNA results. Further research and integration of cross-disciplinary efforts will be critical in unlocking the potential of these technologies to support the cause of justice, as well as people's safety.

5.1 Future Scope

In light of the above findings, the future direction of integrating third-generation allelic research in forensic science has great prospects to further the advancement in the abilities of DNA sampling, in improving the investigation performances as well as in promoting the just delivery of justice. Further development, advancements in technology and future cooperation hold the key to the progression of these fields in the future years.

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