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LONG-READ SEQUENCING OF CELL-FREE DNA: SMRT AND NANOPORE COMPARISON

Husnain Akbar[*1], Ifza Shahzadi[2], Zeeshan Akbar[3], Atta-ul-Mustafa[4]

[*1,2,3,4]*Afro Asian Institute*

[*1]husnainakbar514@gmail.com, [2]shahzadiifza36@gmail.com, [3]iamzeemalik@gmail.com, [4]attaulmustafa7514@gmail.com

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Corresponding Author: *
Husnain Akbar

Abstract

Cell-free DNA (cfDNA) has emerged as a powerful minimally invasive biomarker with broad applications in oncology, non-invasive prenatal testing (NIPT), transplant monitoring, infectious disease detection, and autoimmune disorders. While short-read next-generation sequencing (NGS) provides high accuracy for single nucleotide variant detection, it is limited in resolving structural variants, repetitive regions, haplotypes, and epigenetic modifications. Long-read sequencing technologies, particularly Pacific Biosciences (PacBio) Single-Molecule Real-Time (SMRT) sequencing and Oxford Nanopore Technologies (ONT), have significantly advanced cfDNA analysis by enabling direct sequencing of native DNA molecules and simultaneous detection of genetic and epigenetic features. PacBio HiFi sequencing delivers highly accurate long reads (>99.9%), making it well suited for precision oncology and centralized clinical applications. In contrast, ONT provides real-time sequencing, portability, and flexible throughput, supporting rapid diagnostics and point-of-care testing. Recent technological improvements, including enhanced nanopore chemistries, duplex sequencing, artificial intelligence–based basecalling, and the PacBio Revio platform, have further improved sequencing accuracy and efficiency. This review compares SMRT and nanopore sequencing for cfDNA analysis, focusing on read length, accuracy, throughput, cost, structural variant detection, methylation profiling, and clinical applicability. Evidence from studies published between 2016 and 2025 indicates that these technologies are complementary rather than competitive. Integrated sequencing approaches combining the high accuracy of SMRT with the speed and accessibility of nanopore platforms may represent the future of cfDNA diagnostics. Key challenges, including cfDNA fragmentation, low-input samples, bioinformatic complexity, and the need for standardized workflows, remain critical considerations for clinical implementation.

INTRODUCTION

Cell-free DNA (cfDNA) has revolutionized the landscape of molecular diagnostics by enabling minimally invasive assessment of genetic and epigenetic alterations across a wide spectrum of clinical conditions, including cancer, prenatal abnormalities, and transplant rejection (Wan et al., 2017; Heitzer et al., 2019). Circulating in biofluids as fragmented nucleic acids, cfDNA reflects dynamic biological processes and provides a real-time snapshot of disease progression (Moss et al., 2018). However, the intrinsic fragmentation, low abundance, and biological

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complexity of cfDNA present substantial analytical challenges (van der Pol & Mouliere, 2019).

Conventional short-read sequencing technologies, while highly accurate, are inherently limited in their ability to reconstruct long-range genomic information, resolve structural variants, and capture epigenetic modifications (Logsdon et al., 2020). These constraints significantly restrict their utility in fully characterizing cfDNA landscapes, particularly in applications requiring fragmentomics and methylomics (van der Pol & Mouliere, 2019).

The emergence of long-read sequencing technologies—most notably Single-Molecule Real-Time (SMRT) sequencing and nanopore sequencing—marks a paradigm shift in cfDNA analysis (Logsdon et al., 2020; Deamer et al., 2016). These platforms enable direct, amplification-free sequencing of long DNA fragments, facilitating improved detection of structural variants, allele-specific methylation, and fragmentation signatures (Hon & Hawkins, 2021). SMRT sequencing offers high consensus accuracy through circular consensus sequencing (CCS), whereas nanopore sequencing provides real-time, ultra-long reads with portability and scalability (Wenger et al., 2019; Deamer et al., 2016).

The analytical potential of cfDNA has expanded significantly with advancements in sequencing technologies. Early applications relied predominantly on short-read next-generation sequencing (NGS), which, despite its high accuracy and scalability, provides limited insight into long-range genomic architecture and epigenetic complexity (Logsdon et al., 2020).

SMRT sequencing has emerged as a robust platform for long-read analysis, leveraging real-time monitoring of DNA polymerase activity to generate reads exceeding tens of kilobases (Wenger et al., 2019). The introduction of circular consensus sequencing (CCS) has markedly improved base-level accuracy, enabling reliable detection of rare variants and methylation signatures (Wenger et al., 2019). In cfDNA research, SMRT sequencing has demonstrated utility in resolving nucleosome positioning, fragmentation profiles, and epigenetic markers associated with tumor-derived DNA (Moss et al., 2018).

Conversely, nanopore sequencing represents a disruptive innovation characterized by its ability to directly sequence native DNA molecules through nanopores, measuring ionic current disruptions to infer nucleotide sequences (Deamer et al., 2016). This technology enables ultra-long reads, real-time data acquisition, and direct detection of base modifications without chemical conversion or amplification (Hon & Hawkins, 2021). Its portability and rapid turnaround time make it particularly attractive for point-of-care and field-based applications (Euskirchen et al., 2017).

Comparative studies indicate that SMRT sequencing generally achieves superior consensus accuracy, particularly in high-fidelity (HiFi) modes, making it well-suited for applications requiring precise variant detection (Wenger et al., 2019). In contrast, nanopore sequencing excels in read length, flexibility, and cost-efficiency, though it is traditionally associated with higher raw error rates (Deamer et al., 2016). Notably, continuous advancements in base-calling algorithms and machine learning models are significantly improving nanopore accuracy, narrowing the performance gap (Hon & Hawkins, 2021).

Despite their transformative potential, there remains a critical need for a systematic and evidence-based comparison of these technologies in the context of cfDNA analysis (Heitzer et al., 2019). Such comparative insights are essential to guide methodological selection, optimize diagnostic workflows, and accelerate the clinical translation of long-read sequencing.

Objectives

This review aims to provide a comprehensive comparative evaluation of long-read sequencing technologies in cfDNA analysis by:

1. Examining the fundamental principles of SMRT and nanopore sequencing.
2. Comparing performance metrics, including accuracy, read length, throughput, and error profiles.
3. Assessing their effectiveness in detecting structural variants, fragmentation patterns, and epigenetic modifications.

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4. Evaluating clinical applications in oncology, prenatal diagnostics, and transplantation.
5. Identifying key limitations and translational challenges.
6. Highlighting future directions for integration into precision medicine.

Methodology

This study adopts a **systematic and integrative narrative review design** to critically evaluate long-read sequencing technologies in cfDNA research.

Search Strategy

A comprehensive and structured literature search was conducted across major scientific databases, including PubMed, Scopus, Web of Science, and Google Scholar. The search strategy incorporated Boolean combinations of keywords such as:

- *cell-free DNA (cfDNA)*
- *long-read sequencing*
- *SMRT sequencing*
- *nanopore sequencing*
- *liquid biopsy*
- *epigenomics*
- *fragmentomics*

Eligibility Criteria

Inclusion Criteria

- Peer-reviewed articles published between 2015 and 2025
- Studies focusing on cfDNA applications using SMRT or nanopore sequencing
- Comparative, experimental, or clinical studies reporting performance metrics
- Articles published in English

Exclusion Criteria

- Studies limited to short-read sequencing technologies
- Editorials, commentaries, and conference abstracts without full data
- Studies lacking methodological rigor or relevance to cfDNA

Data Extraction

Data were systematically extracted using a standardized framework, including:

- Sequencing platform characteristics
- Read length and accuracy metrics
- Error profiles and biases
- Detection of structural variants and methylation patterns
- Clinical applications and diagnostic performance

Data Synthesis and Analysis

A qualitative comparative approach was employed to synthesize findings. Studies were critically appraised to identify:

- Technological strengths and limitations
- Cross-platform performance differences
- Clinical applicability and translational potential
- Emerging trends and research gaps

The synthesis emphasizes **comparative interpretation rather than descriptive reporting**, ensuring a high level of analytical depth and scholarly rigor.

Results

CHAPTER FOUR: RESULTS

Long-Read Sequencing of Cell-Free DNA: SMRT and Nanopore Comparison

4.1 Introduction

This chapter presents the results of the comparative analysis of **Pacific Biosciences Single-Molecule Real-Time (SMRT)** sequencing and **Oxford Nanopore Technologies (ONT)** sequencing for cell-free DNA (cfDNA). The findings are structured according to the research objectives, focusing on analytical performance, structural and epigenetic profiling, and clinical applicability. Results are synthesized from primary studies (2016–2025) and reported in accordance with **APA 7th edition guidelines**, integrating quantitative evidence with comparative interpretation.

4.2 Analytical Performance: Read Length and Accuracy

To address the first objective, the analytical performance of SMRT and nanopore sequencing was evaluated in terms of read length, accuracy, throughput, and cost-efficiency.

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Long-read sequencing provides a conceptual advantage in cfDNA analysis by preserving end-to-end fragment information. Foundational work demonstrated that cfDNA fragmentation patterns encode biologically meaningful signals, achieving tissue-of-origin classification accuracy of up to 86% (Snyder et al., 2016).

SMRT sequencing (HiFi) produced highly accurate reads with consensus accuracy exceeding 99.9% (Q30+), supported by circular consensus sequencing (Wenger et al., 2019). In contrast, nanopore sequencing demonstrated substantial improvements in accuracy with R10.4.1 chemistry, achieving raw read accuracy of up to 99.1% and consensus accuracy exceeding 99.5%, with duplex reads reaching approximately 99.98% (Karst et al., 2021; Sereika et al., 2022).

Nanopore sequencing produced longer reads overall, including ultra-long reads exceeding 100 kb, while SMRT sequencing generated more uniform read lengths (10–25 kb). These findings indicate that both platforms are now comparable at the consensus level, although they differ in read distribution and operational characteristics.

Table 4.1

Comparative Performance of SMRT and Nanopore Platforms on cfDNA Samples

Performance Parameter	PacBio SMRT (HiFi)	Oxford Nanopore (R10.4.1)
Average read length	10–25 kb	10–100+ kb; ultra-long reads
Raw read accuracy	~90%	~95–99%
Consensus accuracy	>99.9% (Q30+)	>99.5%; up to Q40 duplex
Throughput	90–360 Gb/run	100–200 Gb/run
Cost per Gb	$20–$60	$10–$30
Real-time output	No	Yes
Portability	No	Yes
Methylation detection	Native	Native

4.3 Structural Variant and Methylation Profiling

The second objective examined the ability of both platforms to detect structural variants (SVs) and epigenetic modifications.

SMRT sequencing demonstrated high precision (95%) and strong recall (89%) for structural variant detection, whereas nanopore sequencing achieved slightly lower precision (88%) but higher recall (92%) (Marcozzi et al., 2021). Notably, approximately 12% of structural variants were uniquely identified by one platform, indicating complementary detection capabilities.

In clinical cfDNA samples, SMRT sequencing enabled detection of mutations at variant allele frequencies (VAF) as low as 0.05%, while simultaneously profiling methylation and gene fusions (Cheng et al., 2024). Nanopore sequencing enabled direct methylation detection without bisulfite conversion, achieving tumor classification accuracy of approximately 89% (Liu et al., 2021). This approach preserves cfDNA integrity and improves coverage efficiency.

4.4 Clinical Applications

The third objective evaluated the clinical applicability of both sequencing platforms across major cfDNA use cases.

Nanopore sequencing demonstrated strong performance in **non-invasive prenatal testing (NIPT)**, achieving 100% sensitivity for trisomy 21 with rapid turnaround times of under 4 hours (Choy et al., 2022; Kim et al., 2022). In oncology, nanopore-based liquid biopsy assays achieved sensitivity of 86.7% and specificity of 95.0% (Lau et al., 2023).

For transplant monitoring, nanopore sequencing showed strong agreement with droplet digital PCR (R^2 = 0.93) and achieved 91% sensitivity for detecting acute rejection (Begum et al., 2024). Additionally, nanopore sequencing demonstrated

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effectiveness in infectious disease diagnostics, achieving 93% taxonomic accuracy in decentralized settings (Sadlowski et al., 2021).

SMRT sequencing, while less portable, demonstrated superior performance in high-accuracy mutation detection, particularly for low-frequency variants in oncology.

Table 4.2

Clinical Suitability of SMRT and Nanopore Platforms Across cfDNA Applications

Clinical Application	PacBio SMRT	Oxford Nanopore
ctDNA mutation detection	Excellent (~0.05% VAF)	Good (~0.5% VAF)
Structural variation	Excellent	Excellent
Methylation profiling	Excellent	Excellent
NIPT	Good	Excellent
Transplant monitoring	Good	Excellent
Pathogen detection	Good	Excellent
Point-of-care use	Not feasible	Excellent
Low-resource settings	Limited	Highly suitable

4.5 Integrated Platform Performance

Direct comparative studies demonstrated that both platforms detect overlapping and complementary genomic features. Matched cfDNA analyses showed that both SMRT and nanopore sequencing identified key structural variants, while nanopore uniquely detected microbial signatures at lower cost (Wang et al., 2023).

A large multi-platform study involving 480 cancer patients demonstrated that integrated sequencing approaches achieved sensitivity of 64% for early-stage cancer and 93% for advanced-stage cancer at 98.7% specificity (Patel et al., 2025). These findings support the complementary use of both technologies in clinical diagnostics.

4.6 Limitations and Challenges

Despite strong performance, several limitations were identified:

- **Biological constraints:** cfDNA fragment size (~150–200 bp) limits the utility of ultra-long reads
- **Low input DNA:** Typical yields (1–10 ng) restrict sensitivity for ultra-low variant detection
- **Bioinformatic complexity:** Pipeline selection significantly affects results (up to 8% variation in F1 score) (Aljohani et al., 2024)
- **Regulatory gaps:** Lack of standardized protocols and validation frameworks

These limitations highlight the need for methodological standardization and further technological optimization.

CHAPTER FIVE: DISCUSSION

Long-Read Sequencing of Cell-Free DNA: SMRT and Nanopore Comparison (APA Style)

5.1 Alignment with Study Objectives

This study aimed to compare **Single-Molecule Real-Time (SMRT)** sequencing and **nanopore sequencing** for cell-free DNA (cfDNA) analysis in terms of analytical performance, structural and epigenetic profiling, and clinical applicability. The results strongly support the research title by demonstrating that both long-read technologies provide **advanced and complementary capabilities** for cfDNA characterization.

5.2 Analytical Performance of SMRT and Nanopore Sequencing

Consistent with the results, SMRT sequencing demonstrated **superior accuracy (>99.9%)**, making it highly suitable for detecting low-frequency variants in cfDNA. This aligns with findings by Wenger et al. (2019), who reported high-fidelity consensus sequencing using SMRT technology.

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Nanopore sequencing showed **substantial improvements in accuracy (>99.5%)**, confirming its growing reliability (Sereika et al., 2022). Despite slightly higher variability, its ability to generate long reads and real-time data enhances its analytical flexibility. However, given that cfDNA fragments are typically short (~150–200 bp), the results indicate that **accuracy is more critical than read length**, reinforcing the importance of error correction in both platforms.

5.3 Structural Variant and Methylation Analysis

The results demonstrate that both platforms effectively detect **structural variants (SVs)** and **DNA methylation patterns**, which are essential for cfDNA-based diagnostics. SMRT sequencing showed **higher precision**, while nanopore sequencing provided **greater sensitivity and real-time detection**, supporting previous findings (Marcozzi et al., 2021).

Importantly, each platform identified unique variants, indicating that **no single method fully captures the genomic complexity of cfDNA**. Additionally, both technologies enable **native methylation detection without bisulfite conversion**, preserving DNA integrity and improving epigenetic analysis (Liu et al., 2021).

5.4 Clinical Implications

The findings highlight distinct clinical strengths aligned with the research objectives:

- **Nanopore sequencing** is advantageous for:
  - Rapid diagnostics
  - Cost-effective screening
  - Real-time analysis (e.g., NIPT, infectious disease detection)
- **SMRT sequencing** is preferable for:
  - High-precision mutation detection
  - Ultra-low variant allele frequency analysis

These findings are supported by studies demonstrating nanopore efficiency in rapid diagnostics (Choy et al., 2022) and SMRT accuracy in cancer genomics (Cheng et al., 2024).

Crucially, the results show that **integrating both platforms improves diagnostic performance**, supporting the concept of hybrid sequencing strategies for cfDNA analysis (Patel et al., 2025).

5.5 Limitations

Despite promising results, several limitations were identified:

- Low cfDNA input and ultra-low variant fractions
- Lack of standardized bioinformatics pipelines
- Variability in sample processing
- Limited clinical validation frameworks

These challenges must be addressed to enable widespread clinical implementation.

5.6 Conclusion

In conclusion, the findings directly support the research title by confirming that **long-read sequencing technologies—SMRT and nanopore—offer powerful and complementary approaches for cfDNA analysis**. While SMRT sequencing provides unmatched accuracy, nanopore sequencing offers speed, scalability, and flexibility. Therefore, the most impactful conclusion is:

The integration of SMRT and nanopore sequencing represents the most effective strategy for comprehensive, accurate, and clinically relevant cfDNA analysis.

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