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Original Research Article

RAPID AND ACCURATE TRACKING OF COVID-19 VARIANTS: THE ROLE OF OXFORD NANOPORE SEQUENCING IN SARS-COV-2 GENOME SURVEILLANCE

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Abstract

In this comprehensive review, we have explored the role of Oxford Nanopore Technologies (ONT) in the sequencing of the SARS-CoV-2 genome, highlighting its significant contributions, methodologies, achievements, and challenges. ONT stands out for its real-time sequencing capabilities, portability, and long-read sequencing, providing crucial insights into the virus's genetic makeup, aiding in the rapid identification of variants, and enhancing global epidemiological surveillance. Despite challenges such as higher error rates and complex data analysis requirements, ongoing advancements in nanopore technology and computational methods are steadily overcoming these hurdles. Looking forward, the integration of artificial intelligence and machine learning, along with the expansion of ONT's applications into areas like clinical diagnostics and personalized medicine, are poised to further revolutionize genomic research. This review underscores the importance of ONT in current and future genomic studies, particularly in understanding and combating infectious diseases like COVID-19, and highlights its potential in addressing global health challenges.

Keywords: Oxford Nanopore Technologies, SARS-CoV-2 Sequencing, Genomic Surveillance, Real-time Data Generation, Long-read Sequencing

1. INTRODUCTION

The COVID-19 pandemic, caused by the novel coronavirus SARS-CoV-2, emerged as a global health crisis of unprecedented scale, affecting millions worldwide and challenging the scientific community in numerous ways. Understanding the genetic makeup of the virus became a priority for researchers globally, as it was critical for developing effective diagnostics, treatments, and vaccines. This necessity catalyzed advancements in genomic sequencing technologies, among which Oxford

Nanopore Technologies (ONT) has emerged as a pivotal tool.[1,2]

Sequencing the genome of SARS-CoV-2 provides insights into its transmission dynamics, mutation rates, and evolutionary patterns, crucial for public health responses. Traditional sequencing methods, while effective, often faced limitations in terms of speed, scalability, and the need for sophisticated laboratory infrastructure. ONT, with its unique approach to sequencing, offered solutions to many of these challenges.[3,4]

Oxford Nanopore Technologies utilizes a novel method of sequencing that involves passing DNA or RNA molecules through nanopores integrated into a synthetic membrane. The resulting changes in ionic current as the nucleic acids pass through the nanopores are monitored and converted into nucleotide sequences. This innovative approach allows for longer reads compared to other sequencing technologies, providing a more comprehensive view of a genome's structure and variations. The portability of ONT devices, particularly the MinION and Flongle, revolutionized the field by enabling in-field sequencing, rapid turnaround times, and accessibility to a broader range of researchers.[4,5,6]

The utility of ONT in sequencing SARS-CoV-2 has been profound. It played a significant role in early detection and monitoring of the virus, tracking its spread and evolution, and identifying new variants. This was crucial in countries and regions where access to large, centralized sequencing facilities was limited. Researchers were able to deploy ONT devices quickly and efficiently, providing real-time data essential for guiding public health decisions.[2,3.5]

One of the landmark achievements in using ONT for SARS-CoV-2 sequencing was its ability to identify mutations and variants of the virus. This aspect was vital in understanding how the virus evolves and adapts, influencing vaccine development and the effectiveness of therapeutic interventions. The rapid identification of variants such as Alpha, Beta, and Delta through ONT sequencing provided invaluable insights into their transmissibility, virulence, and escape mechanisms from immune responses.[5,6,7]

Despite its advantages, ONT's application in SARS-CoV-2 sequencing is not without challenges. The technology is known for a higher error rate compared to more established methods like Illumina sequencing. However, continuous improvements in base-calling algorithms and data processing techniques have significantly mitigated these issues. Another challenge is the interpretation and management of the vast amounts of data generated, necessitating advanced computational tools and expertise.[3,6]

The COVID-19 pandemic has underscored the importance of genomic surveillance in managing infectious diseases. In this context, ONT stands out not just for its technical capabilities but also for its adaptability to various research and clinical settings. As the pandemic progresses, ongoing advancements in nanopore sequencing technology are expected to enhance its accuracy, throughput, and cost-effectiveness.[1,2]

The genetic structure of SARS-CoV-2, the virus causing COVID-19, follows a common pattern. Two-thirds of its genome, situated at the beginning (5′ end), is taken up by the replicase gene. This gene is divided into two extensive, overlapping sections known as open reading frames ORF1a and ORF1b. ORF1a is responsible for producing a complex protein called polyprotein 1a (pp1a), while polyprotein 1b (pp1ab) results from a process involving a shift in the ribosomal reading frame. These complex polyproteins are then broken down into 16 smaller, nonstructural proteins crucial for

the replication and transcription of the viral genome. Towards the end of the genome (3′ terminal end), there are genes encoding four structural proteins essential for forming the virus's outer shell, along with six additional accessory proteins. These accessory proteins are not as well understood and vary among different types of coronaviruses.[4,5,6,7]

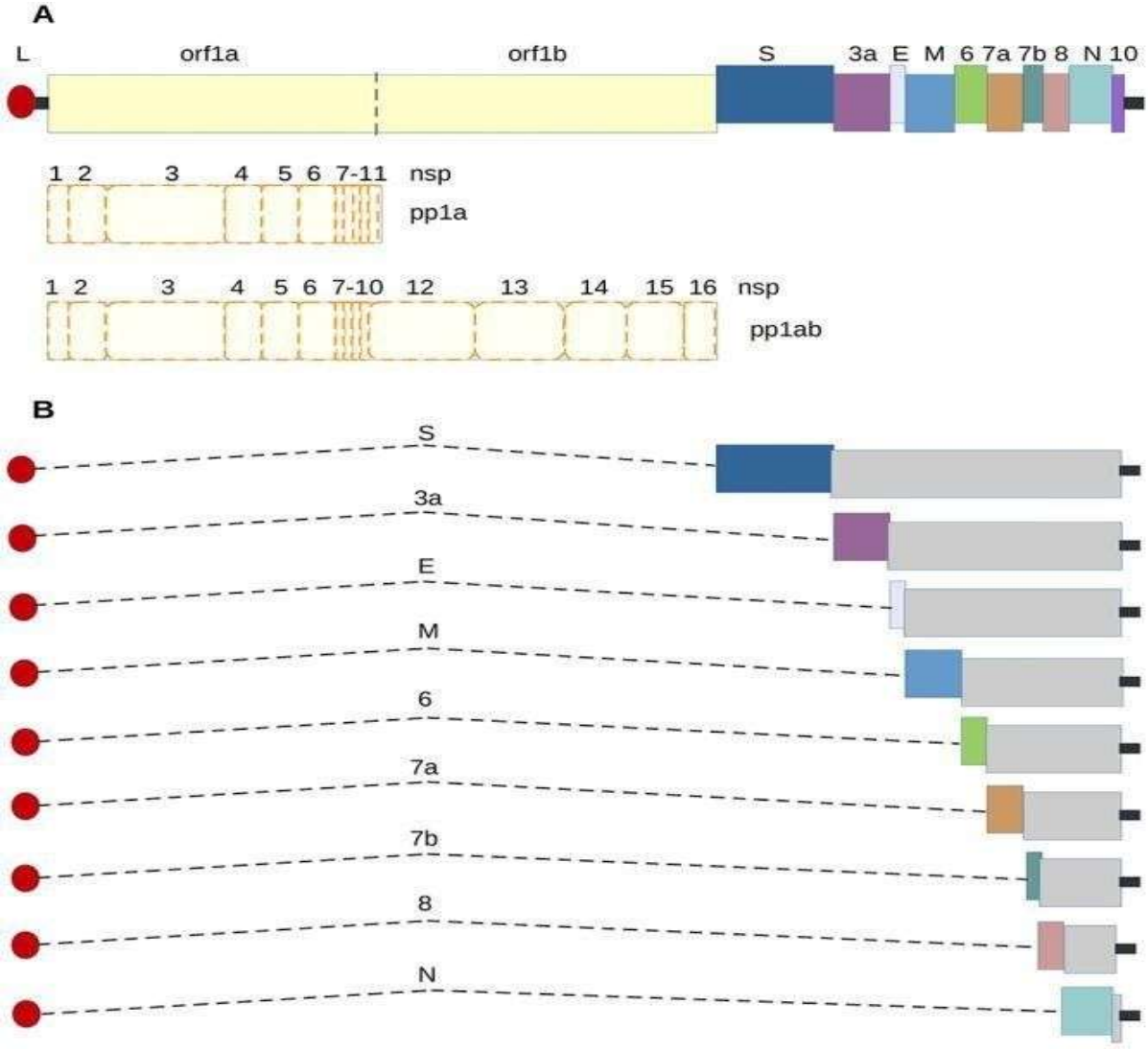

Figure 1: The genome of SARS-CoV-2 is structured in a specific way.

Part A of the description focuses on the overall genome structure of SARS-CoV-2, where different genes are marked with specific labels. A notable feature is the TRS-L, highlighted with a red circle. Below this, the diagram shows nonstructural proteins (nsps) that are produced from the processing of pp1a and pp1ab polyproteins. Part B details subgenomic mRNAs (sgmRNAs). These are represented by dotted lines connecting the TRS-L to the main part of each individual sgmRNA. For each sgmRNA, the specific gene product is identified by colored boxes and accompanying labels.(18)

In the future, ONT is poised to play an even more significant role in understanding SARS- CoV-2 and other pathogens. Innovations in the technology could lead to even faster sequencing times, lower costs, and more portable devices, broadening its application in epidemiology, outbreak response, and global health surveillance. The integration of ONT data with other biological datasets, such as

proteomics and metabolomics, could provide a more holistic understanding of viral infections and host-pathogen interactions.[4,5,7]

Oxford Nanopore Technologies has not only been instrumental in the global response to the COVID-19 pandemic but also represents a paradigm shift in genomic sequencing. Its impact extends beyond the current crisis, providing a framework for rapid, accessible, and comprehensive genomic analysis in various settings. As technology evolves, it will undoubtedly continue to play a crucial role in advancing our understanding of infectious diseases and shaping public health strategies.

OXFORD NANOPORE TECHNOLOGIES: AN OVERVIEW

Oxford Nanopore Technologies represents a revolutionary step in the field of genomic sequencing, offering a unique approach that fundamentally differs from traditional sequencing methods. This UK-based company has developed a series of devices that utilize nanopore sequencing technology, which has gained widespread attention for its versatility, portability, and ability to produce long reads in real-time. The implications of this technology for genomic research, especially in rapid response scenarios like the COVID-19 pandemic, areprofound.[7,8]

The core of ONT's technology lies in its use of nanopores – tiny biological channels embedded in an electrically resistant membrane. When a DNA or RNA molecule passes through these nanopores, it causes a disruption in the flow of an ionic current. By measuring these disruptions, the sequence of nucleotides within the DNA or RNA can be inferred. This method allows for the direct sequencing of strands of DNA or RNA without the need for amplification or conversion to a double-stranded DNA form, which is a requirement in many other sequencing technologies.[9,10]

One of the most significant advantages of ONT is its ability to produce long reads. Traditional sequencing methods, like those developed by Illumina, often generate short reads that must be pieced together like a jigsaw puzzle. This can be particularly challenging in regions of the genome with repetitive sequences. Long-read technology, on the other hand, can span these complex regions, providing a more comprehensive and contiguous genomic sequence. This feature is invaluable in studying structural variations, complex genomic regions, and whole genome sequencing.[8,9,10]

ONT's sequencing devices range from the portable MinION to the larger GridION and PromethION, each catering to different research needs. The MinION, in particular, has garnered attention for its portability and affordability, allowing researchers to conduct sequencing in the field. This has significant implications for rapid response scenarios, environmental sampling, and in resource-limited settings where traditional laboratory facilities are unavailable.[7,9]

The application of ONT in sequencing SARS-CoV-2 has demonstrated its capability in real- time surveillance and outbreak tracking. Researchers have utilized ONT to rapidly sequence viral genomes directly from patient samples, providing critical information on the virus's structure, function, and evolution. This has been essential in identifying mutations and understanding the dynamics of the virus's spread. The data generated by ONT have been crucial in guiding public health policies and strategies for controlling the pandemic.[10,11]

Despite these advantages, ONT faces several challenges. One of the primary concerns has been the higher error rate associated with nanopore sequencing compared to other methods. However,

continuous advancements in nanopore technology, base-calling algorithms, and error-correction strategies have significantly improved the accuracy of the data. Furthermore, the development of new generations of nanopores and the optimization of sequencing chemistries continue to enhance the overall performance of the technology.[6,7,8]

The potential of ONT extends beyond infectious disease surveillance. Its application in cancer research, human genetics, and environmental monitoring has demonstrated its versatility. In cancer research, for example, the ability to sequence long reads helps in identifying structural variations and complex mutational patterns that are critical for understanding tumor biology and developing targeted therapies.[7,9,10]

In addition to its scientific applications, ONT is contributing to the democratization of genomic research. By making sequencing more accessible and affordable, it allows a wider range of researchers and institutions to engage in genomic studies. This is particularly important in developing countries, where such technology can play a crucial role in addressing local health issues and biodiversity studies.[11,12]

Table 1 : Characteristics of SARS-CoV-2 sequencing approaches (18)

Sequencing Approach	Goals	Co- infection Detection	Minimum Number of Reads	Genome Coverage	Accuracy in SNV Identification	Sample Viral Load (Ct) Requested (ref
Shotgun Metatranscriptomics	SARS-CoV-2, host microbiota, and host response to infection	Yes	20–50 M	≥99%	High	<24–28
Amplicon-based	SARS-CoV-2 genome	No	5–20 M	≥95–99%	High	≥24–28
Hybrid Capture- Enrichment	SARS-CoV-2 genome	No/Yes (depending on gene panel)	5–20 M	≥95–99%	Moderate	≥24–28
Direct RNA Sequencing	SARS-CoV-2 And host transcriptome and epitranscriptome	Yes	0.5 M	≥99%	Low	<24–28

Looking towards the future, ONT is poised to continue its trajectory of innovation. Advances in nanopore technology are expected to further enhance the accuracy and speed of sequencing, making it an even more valuable tool in genomics. The integration of artificial intelligence and machine learning in data analysis will likely open new frontiers in understanding complex biological systems.[7,9,10]

In the realm of infectious diseases, particularly in the context of the COVID-19 pandemic, ONT has shown its potential in not only tracking the spread of the virus but also in identifying new variants, monitoring vaccine effectiveness, and understanding virus-host interactions. This real-time genomic data is vital for informed decision-making in public health.[6,8]

Moreover, the potential applications of ONT in personalized medicine are significant. As the technology continues to evolve, it may become possible to use nanopore sequencing for individualized disease diagnosis and treatment, tailored to the genetic makeup of each patient. This could revolutionize the field of medical genomics, offering new avenues for treatment and patient care.[11,12]

Oxford Nanopore Technologies has emerged as a pivotal player in the field of genomic sequencing. Its unique approach to DNA and RNA sequencing has opened new possibilities in various areas of

research and clinical practice. The impact of ONT is particularly evident in the ongoing fight against the COVID-19 pandemic, where its rapid, real-time sequencing capability has proven invaluable. As the technology continues to evolve, it holds the promise of further advancing our understanding of complex biological systems and enhancing our ability to respond to global health challenges.

3. SEQUENCING OF SARS-COV-2 USING ONT: METHODOLOGIES Sample Collection and Preparation

The initial step in sequencing SARS-CoV-2 using ONT is the collection of viral samples, typically obtained from nasopharyngeal swabs, saliva, or other bodily fluids from infected individuals. The quality and integrity of these samples are crucial for accurate sequencing. Once collected, the samples undergo RNA extraction, which involves isolating the viral RNA from other components in the sample. This step is critical as SARS-CoV-2 is an RNA virus, and the purity of the RNA directly impacts the sequencing quality.[1,2]

Library Preparation

The extracted RNA is then converted into a library suitable for sequencing. In the case of ONT, this involves converting the RNA into complementary DNA (cDNA) since ONT devices sequence DNA. The cDNA synthesis is followed by the addition of sequencing adapters. These adapters are crucial as they allow the DNA strands to attach to the nanopores in the ONT sequencer. The library preparation process also includes a barcoding step, especially when multiple samples are sequenced simultaneously, allowing for the identification and separation of data from individual samples during analysis.[1,2,3]

Sequencing Protocol

After library preparation, the sample is loaded into the ONT sequencing device, such as the MinION or GridION. These devices contain a flow cell with numerous nanopores. As the DNA strands pass through these nanopores, changes in the ionic current are detected, which are used to determine the nucleotide sequence of the DNA. One of the key features of ONT sequencing is its ability to produce long reads, which is particularly advantageous for identifying structural variants and understanding the full genomic architecture of the virus.[5,6,7]

Real-Time Data Generation

A significant advantage of ONT is the generation of sequencing data in real-time. This feature allows for immediate data analysis, which is critical in a fast-paced environment like a pandemic response. Researchers can start analyzing the data while the sequencing run is still ongoing, enabling rapid decision-making, such as in the identification of novel viral variants or in tracking the spread of the virus.[8,9]

Data Analysis and Interpretation

The raw data generated by ONT consist of signal changes as DNA transits through the nanopores. This data is then processed using specialized base-calling algorithms, which translate the ionic current signals into nucleotide sequences. The resulting sequences are aligned and compared to reference genomes to identify mutations and variants of the virus. Advanced bioinformatics tools are used to manage and interpret the vast amount of data generated, providing insights into the virus's structure, mutations, and evolution.[3,4,5]

Error Correction and Validation

While ONT offers many advantages, one of its challenges is a higher error rate compared to other sequencing technologies. However, advances in base-calling algorithms, error correction techniques, and improvements in nanopore technology have significantly reduced these errors. Additionally, validation of sequencing results using complementary methods or repeated runs is often conducted to ensure accuracy.[7,8]

Applications in SARS-CoV-2 Research

The application of ONT in SARS-CoV-2 sequencing has been instrumental in various research and public health areas. It has enabled rapid identification of viral variants, understanding transmission dynamics, and provided valuable information for vaccine and therapeutic development. The portability of ONT devices has also facilitated in-field sequencing, making genomic surveillance accessible in diverse geographical locations, including areas with limited laboratory infrastructure.[3,4,5]

The continuous evolution of ONT and its methodologies is expected to further enhance its accuracy and utility in viral sequencing. Integration with emerging computational methods, such as machine learning and AI, could provide more profound insights into the genomic data, potentially transforming our approach to infectious disease surveillance and response.

4. ACHIEVEMENTS AND APPLICATIONS OF ONT IN SARS-COV-2 SEQUENCING

Rapid Identification of SARS-CoV-2 and Variants

One of the most significant achievements of ONT in the context of the COVID-19 pandemic has been the rapid identification and characterization of SARS-CoV-2, including its various mutations and variants. The ability of ONT to provide real-time sequencing data enabled researchers to quickly identify the genetic makeup of the virus, which was crucial in the early stages of the pandemic. This rapid sequencing capacity also played a pivotal role in detecting and monitoring emerging variants of the virus, such as the Alpha, Beta, Delta, and Omicron variants. Understanding these variants' genetic differences was essential for assessing their transmissibility, virulence, and impact on vaccine efficacy.[13,14]

Epidemiological Surveillance and Tracking

ONT's portability and ease of use have been instrumental in conducting epidemiological surveillance and tracking the spread of SARS-CoV-2. Its application in field-based settings enabled the monitoring of viral transmission in real-time, providing valuable insights into the dynamics of the pandemic. This has been particularly beneficial in regions with limited access to traditional sequencing facilities. By analyzing the genomic data collected from various geographic locations, researchers have been able to trace the patterns of the virus's spread, identify hotspots, and inform public health interventions.[15,16]

Contributions to Vaccine Development

The detailed genomic information provided by ONT has contributed significantly to vaccine development efforts. By identifying and characterizing the spike protein mutations of SARS- CoV-2, researchers have been able to understand how these changes might affect the virus's ability to infect host cells and evade immune responses. This information has been crucial in guiding the design and modification of vaccines to ensure their effectiveness against emerging variants.[17,18]

Monitoring Viral Evolution in Real-Time

ONT's ability to generate long reads has been advantageous in studying the evolution of the SARS-CoV-2 genome. Longer reads allow for a more comprehensive view of the viral genome, helping scientists to understand the mechanisms of viral mutation and adaptation. This is critical for predicting future trends in the virus's evolution and preparing for potential challenges in managing the pandemic.[16,18]

Global Health Impact and Accessibility

The deployment of ONT has had a significant global health impact, particularly in low-resource settings. Its portability and relative affordability have made genomic sequencing more accessible worldwide, enabling countries with limited resources to participate in global genomic surveillance efforts. This democratization of sequencing technology is a crucial step in building a more comprehensive and equitable global response to infectious diseases.[14,17,18]

Advancing Diagnostic Approaches

ONT has also been explored as a tool for direct diagnostic applications. Its ability to rapidly sequence viral genomes from patient samples offers potential for developing point-of-care diagnostic tests, which could be particularly useful in outbreak settings or for rapid screening in healthcare facilities.[18,19,20]

Environmental Monitoring and Beyond

Beyond clinical and epidemiological applications, ONT has been used for environmental monitoring, such as in wastewater surveillance. This helps in detecting the presence and spread of the virus in communities, providing an early warning system for potential outbreaks. Additionally, the versatility of ONT makes it a valuable tool in other research areas, including studies on other pathogens, human genetics, and biodiversity.[18,20,21]

5. CHALLENGES AND LIMITATIONS

Addressing the challenges and limitations of Oxford Nanopore Technologies (ONT) in the context of SARS-CoV-2 sequencing is crucial for a balanced and comprehensive understanding of its capabilities and areas for improvement. This section explores various technical, logistical, and practical aspects that pose challenges or limitations to the widespread adoption and effectiveness of ONT in sequencing efforts.

Higher Error Rate

One of the primary challenges associated with ONT is its higher error rate compared to other sequencing technologies, such as those based on Illumina platforms. ONT’s sequencing errors are predominantly insertions or deletions, which can complicate the analysis and interpretation of the viral genome, particularly in regions with repetitive sequences or complex structures. While continuous improvements in base-calling algorithms and error-correction methods have significantly reduced these error rates, achieving the level of accuracy provided by other platforms remains a challenge.[18,20,21,22]

Data Analysis and Interpretation Complexity

The large volume and complexity of data generated by ONT pose challenges in data analysis and interpretation. Long-read sequencing generates vast amounts of data that require substantial computational resources and specialized bioinformatics expertise for effective analysis. This can be

a limiting factor, especially in resource-limited settings or among research groups without access to advanced computational infrastructure.[14,16,18]

Sample Quality and Quantity Requirements

ONT sequencing is sensitive to the quality and quantity of the input material. Obtaining high-quality, high-purity RNA samples, particularly from clinical specimens that may contain inhibitors or be of low viral load, can be challenging. Inconsistent sample quality can lead to variability in sequencing results, affecting the reliability and reproducibility of the data.[20,21,22]

Cost and Accessibility

While ONT devices like the MinION are more affordable and accessible compared to high-throughput sequencers, the overall costs, including consumables and data analysis, can still be a barrier for widespread adoption. Additionally, the requirement for continuous purchase of flow cells and library preparation kits can be a significant ongoing expense for some laboratories.[18,22,23]

Workflow Standardization

Standardization of workflows for ONT sequencing remains a challenge. Different protocols for library preparation, sequencing, and data analysis can lead to variability in results. Establishing standardized, validated protocols is essential for ensuring consistency and comparability of sequencing data across different laboratories and studies.[24,25]

Real-Time Sequencing vs. Throughput

While ONT offers the advantage of real-time sequencing, its throughput is generally lower compared to other high-throughput sequencing platforms. This limitation can be a drawback in scenarios where large-scale sequencing of numerous samples is required, such as in population- wide genomic surveillance programs.[18,25,26]

Technological Advancements and Training

Rapid advancements in ONT technology necessitate continuous learning and adaptation. Keeping up with the evolving protocols, software, and data analysis tools requires ongoing training and skill development for researchers and technicians. This need for continual upskilling can be a hurdle, particularly in less technologically advanced or resource- constrained settings.[17,18,19]

Dependence on External Computational Resources

For many users, especially those in field-based or remote locations, the dependence on external computational resources for data analysis can be a limitation. The requirement for high-performance computing infrastructure to handle large datasets may not be feasible in all settings, potentially restricting the utility of ONT in certain applications.[18,20,25,26]

6. FUTURE DIRECTIONS

Envisioning the future directions for Oxford Nanopore Technologies (ONT) in the context of SARS-CoV-2 sequencing, and more broadly in genomic research, involves anticipating advancements in technology, applications, and methodologies. This future outlook should consider the potential evolution of ONT in addressing current challenges, expanding its capabilities, and exploring new areas of application.

Future Directions in ONT and SARS-CoV-2 Sequencing Technological Advancements

Ongoing advancements in nanopore technology are expected to address current limitations, particularly in terms of accuracy and throughput. Future iterations of ONT devices may feature enhanced sensitivity and precision, reducing the error rates closer to those of traditional sequencing platforms. Additionally, improvements in nanopore design and sequencing chemistries could increase the throughput, enabling the processing of more samples simultaneously or in shorter time frames.[26,27,28]

Integration with Artificial Intelligence and Machine Learning

The integration of artificial intelligence (AI) and machine learning (ML) with ONT data analysis has the potential to revolutionize genomic research. AI and ML can aid in more accurate base-calling, error correction, and complex data analysis, making sense of the vast datasets generated by nanopore sequencing. These technologies could also assist in identifying patterns and mutations in the viral genome that may not be readily apparent through traditional analysis methods.[18,28]

Enhanced Data Analysis Software and Tools

The development of more user-friendly, efficient, and powerful data analysis software is anticipated. These tools will likely focus on simplifying the data analysis process, making it more accessible to researchers without specialized bioinformatics expertise. Enhanced software could automate many aspects of data processing, from base-calling to variant identification, streamlining the workflow and improving data accuracy.[16,21,29]

Expanded Applications in Clinical Diagnostics and Personalized Medicine

ONT's potential in direct clinical diagnostics and personalized medicine is a promising area of future development. As the technology becomes more refined, it could be used for rapid, point- of-care diagnostic testing, including in resource-limited settings. In personalized medicine, ONT could play a role in genotyping individual patients for tailored treatment strategies, especially in areas like oncology and infectious diseases.[24,25,26]

Wider Application in Epidemiology and Public Health

The future of ONT in epidemiology and public health looks promising, especially for real-time monitoring and management of infectious diseases beyond COVID-19. Its application could be extended to other viruses, bacteria, and pathogens, providing critical data for outbreak response and disease surveillance. Additionally, ONT could be integral in environmental monitoring, such as in tracking pathogens in wastewater or in wildlife populations, offering early warning systems for potential public health threats.[18,28,29]

Addressing Global Health Disparities

One of the future directions for ONT involves addressing global health disparities. By making the technology more affordable and accessible, ONT could enable low- and middle-income countries to participate more fully in global genomic surveillance efforts. This democratization of sequencing technology is crucial for building a more equitable global response to pandemics and other health challenges.[29,30]

Education and Training

As ONT technologies continue to evolve, there will be an increasing need for education and training programs. These programs will be essential for training the next generation of researchers, clinicians, and laboratory technicians in the use of nanopore sequencing, ensuring a wide and

effective application of this technology.[18,29,30]

Ethical and Regulatory Considerations

The expansion of ONT applications will also bring about ethical and regulatory challenges, particularly in areas like data privacy, genetic information handling, and the use of genomic data in clinical settings. Future developments in ONT will need to be accompanied by thoughtful consideration of these issues, ensuring responsible and ethical use of the technology.[29,30]

In summary, the future of Oxford Nanopore Technologies in the sequencing of SARS-CoV-2 and broader genomic research is marked by potential advancements in technology, expanded applications, and the addressing of current challenges. These developments promise to enhance the capabilities of ONT, making it an even more valuable tool in scientific research, public health, and clinical practice.

7. Conclusion

In conclusion, Oxford Nanopore Technologies (ONT) has significantly impacted genomic research, particularly in the rapid sequencing of SARS-CoV-2, offering unique advantages such as real-time data generation, portability, and long-read capabilities. Despite facing challenges like higher error rates and complex data analysis, continuous technological advancements and improved methodologies are addressing these limitations. Looking ahead,

ONT's integration with advanced computational tools, expansion into clinical diagnostics, and broader application in public health surveillance promise to further revolutionize genomic research. As we navigate the complexities of infectious diseases, ONT stands as a pivotal tool, not only in understanding pathogens like SARS-CoV-2 but also in shaping future strategies for global health challenges.

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