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Generating Enteroviruses in the Past Using a Diagnostic Nanopore Sequencing Process

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Introduction

Enteroviruses, members of the Picornaviridae family, are small, non-enveloped RNA viruses known for causing a wide range of diseases in humans, from mild respiratory illnesses to severe neurological conditions. These viruses have been studied extensively due to their impact on public health. Traditionally, enterovirus identification and characterization relied on cell culture methods and molecular techniques such as PCR and sequencing. With advancements in technology, nanopore sequencing has emerged as a powerful tool for diagnosing and studying these viruses. This article explores the generation and analysis of enteroviruses using a diagnostic nanopore sequencing process. Historically, enterovirus research has been critical due to the significant diseases caused by these viruses. Poliovirus, one of the most well-known enteroviruses, caused widespread epidemics of poliomyelitis in the 20th century. The development of the polio vaccine was a milestone in medical science, significantly reducing the incidence of the disease. Other enteroviruses, such as Coxsackieviruses and Echoviruses, have also been associated with various clinical syndromes, including myocarditis, aseptic meningitis, and hand-foot-and-mouth disease.Traditional methods for enterovirus detection included virus isolation in cell culture, which, while effective, was time-consuming and required specialized laboratory facilities. Molecular techniques like Reverse Transcription-Polymerase Chain Reaction (RT-PCR) brought significant improvements, allowing for faster and more sensitive detection of enteroviruses. However, these methods often required prior knowledge of the virus's genetic sequence for primer design, limiting their applicability to known viruses.

Description

Nanopore sequencing represents a revolutionary advance in genomic sequencing technology. Unlike traditional sequencing methods, which rely on the synthesis or fragmentation of DNA, nanopore sequencing directly reads the nucleotide sequences by passing DNA or RNA molecules through a nanopore. As each nucleotide passes through the pore, it causes a distinct change in the ionic current, which is detected and recorded. This method allows for the real-time sequencing of long DNA or RNA fragments, providing several advantages over previous technologies. The Oxford Nanopore Technologies (ONT) platform is a prominent example of nanopore sequencing technology. It offers portable, real-time, and high-throughput sequencing capabilities, making it particularly suitable for diagnostic applications in various settings, including fieldwork and point-of-care diagnostics. The application of nanopore sequencing to diagnose and study enteroviruses involves several key steps, including sample preparation, sequencing, and data analysis. Below is a detailed overview of each step in the process [1].

RNA extraction that enteroviruses are RNA viruses, the first step involves extracting viral RNA from the clinical samples. Various commercial RNA extraction kits can be used for this purpose, ensuring the removal of contaminants and inhibitors.The extracted RNA is reverse-transcribed into complementary DNA (cDNA) using reverse transcriptase enzymes. This step is crucial because the subsequent sequencing reactions typically require DNA templates.The cDNA is then prepared for nanopore sequencing. This involves adding sequencing adapters to the ends of the cDNA fragments, enabling them to be recognized and processed by the nanopore sequencer. The prepared cDNA library is loaded onto a nanopore flow cell, a device containing multiple nanopores through which the DNA strands will pass [2].

The initial step in data analysis is basecalling, where the raw ionic current data is converted into nucleotide sequences. This step is performed using algorithms that interpret the characteristic current changes associated with each nucleotide.The generated sequences, or reads, are then mapped against reference genomes of known enteroviruses. This helps in identifying the specific type of enterovirus present in the sample.If the enterovirus sequence does not exactly match any known reference, further analysis is conducted to identify any genetic variations. This can include identifying mutations or recombination events, which are common in enteroviruses.The identified sequences can be subjected to phylogenetic analysis to determine their evolutionary relationships with other enterovirus strains. This can provide insights into the origin and spread of the virus, which is particularly useful in outbreak investigations [3].

Despite its many advantages, nanopore sequencing also presents certain challenges and limitations that need to be addressed. Nanopore sequencing has historically had higher error rates compared to other sequencing technologies like Illumina. However, continuous improvements in basecalling algorithms and chemistry are gradually reducing these errors. The real-time analysis and large volume of data generated by nanopore sequencing require robust bioinformatics infrastructure and expertise. This can be a barrier in some settings, particularly where computational resources are limited [4]. The performance of nanopore flow cells can degrade over time, affecting the quality and yield of sequencing data. Careful management and quality control are required to ensure consistent performance. The quality of the starting material (RNA) is critical for successful sequencing. Degraded or contaminated samples can lead to poor sequencing outcomes, emphasizing the importance of rigorous sample preparation protocols. During an outbreak of enterovirus D68 (EV-D68) in the United States, nanopore sequencing was used to rapidly sequence and analyze clinical samples. The real-time data allowed for the identification of the outbreak strain and provided insights into its transmission dynamics. Researchers have used nanopore sequencing to identify novel enteroviruses in clinical samples from patients with unexplained viral infections. The ability to sequence without prior knowledge of the viral genome facilitated the discovery of new virus strains, expanding our understanding of enterovirus diversity [5].

Conclusion

While the development and application of microsatellite markers offer significant advantages, several challenges remain. One of the primary challenges is the need for high-quality genomic data for the target organism.

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The accuracy and reliability of microsatellite markers depend on the completeness and quality of the genome assembly. Advances in sequencing technologies and bioinformatics tools are expected to address this challenge, providing more comprehensive and accurate genomic data. Another challenge is the transferability of microsatellite markers across different species. While some markers may be conserved across closely related species, others may be species-specific. This necessitates the development of species-specific markers for each new Sporothrix species, which can be time-consuming and resource-intensive. However, the use of cross-species amplification and comparative genomics can potentially overcome this limitation, allowing for the identification of conserved markers across multiple species.

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Conflict of Interest

There are no conflicts of interest by author.

References

1. Stellrecht, K. A., I. Harding, A. M. Woron and M. L. Lepow, et al. "The impact of an enteroviral RT-PCR assay on the diagnosis of aseptic meningitis and patient management." *J Clin Virol* 25 (2002): 19-26.
2. Fischer, Thea K., Peter Simmonds and Heli Harvala. "The importance of enterovirus surveillance in a post-polio world." *Lancet Infect Dis* 22 (2022): e35-e40.
3. Gradel, C., Niranjan Reddy Ireddy, Michel Christoph Koch and Christian Baumann, et al. "Genome sequences of rare human enterovirus genotypes recovered from clinical respiratory samples in bern, Switzerland." *Microbiol Resour Announc* 11 (2022): e00276-22.
4. Majumdar, Manasi, Cristina Celma, Elaine Pegg and Krunal Polra, et al. "Detection and typing of human enteroviruses from clinical samples by entire-capsid next generation sequencing." Viruses 13 (2021): 641.
5. Wieczorek, Magdalena, Agnieszka Figas and Arleta Krzysztoszek. "Enteroviruses associated with aseptic meningitis in Poland, 2011–2014." *Pol J Microbiol* 65 (2016): 231-235.

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