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Nanopore Sequencing Technology: Working Principle, Key Issues and Applications

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Abstract. Achieving rapid and accurate DNA sequencing is of great clinical value for the detection of many infectious diseases. By determining the nucleic acid sequence of the sample, DNA sequencing can achieve rapid and accurate identification of infectious diseases. Moreover, this method has high detection flux and short time consumption, and it is increasingly used in clinical diagnosis and epidemic prevention. This Review discusses recent advancements in nanopore fabrication and sensing strategies. In this work, the speed control factors of DNA translocation across nanopores are discussed. The identification of multiple types of DNA modification are outlined. Besides, strategy for the multiplexed detection of analytes is also discussed. This technology can achieve sequencing of DNA, RNA, and proteins. It is considered to exhibit high sensitivity, selectivity and efficiency. This work will help people gain a deeper understanding of DNA sequencing technology using nanopores. This work will also promote the development of early diagnostic techniques in the future.

Keywords: Nanopore; DNA Sequencing; Multiplexed Detection; Modification.

1. Introduction

In recent years, infectious diseases have seriously affected health and safety. It is important to search for good sequencing methods to achieve early diagnosis. Nanopore sequencing technology has become a hot topic of researchers. Especially in DNA sequencing and protein sequencing, nanopore sequencing technology has developed rapidly due to its high efficiency and convenience. This technology helps doctors to delve deeper into the relationship between diseases and genetics. The outstanding performance of this technology in gene sequencing makes it a key technology in pathogen diagnosis.Compared with other traditional sequencing techniques, nanopore sequencing does not require labeling such as enzymes and fluorescent groups. This sequencing technology has high accuracy and low cost. This technology is easy to operate and has stable detection results. It has a wide range of applications and can be used for DNA, RNA, and protein sequencing. Therefore, studying nanopore sequestration technology has become a hot topic in the biomedical field.

The nanopore sequencing technology is a novel gene sequencing technology [1-3]. Researchers have conducted extensive experiments on this topic and developed sequencing technology. Besides, the equipment for nanopore sequencing has the characteristic of portability. It can meet the demand for real-time detection and can shorten the confirmation time of clinical pathogenic microorganisms. Therefore, studying nanopore sequencing is of great significance.

In this work, nanopore sequencing technology is focused. Nanoporous materials are introduced, including biological nanoporous materials and solid nanoporous materials. The working principle of nanopore sequencing technology is discussed. The key factors for improving detection efficiency through this technology have been discussed in detail.

2. Nanopore Materials

The selection of nanopore materials becomes particularly important in order to facilitate the bases which exhibit extremely small volumes go through nanopores. Nanoporous materials are divided into biological nanoporous materials and solid-state nanoporous materials.

2.1. Biological Nanopores

Biological nanopores are typically porous structures that are constructed by certain proteins [2]. This type of nanoporous material can achieve various functional properties through biochemical modification. There are various types of biological nanoporous materials. Among them, the nanopore material composed of α-hemolysin (a-HL) biological channel proteins is currently the most widely used biological nanopore. This material has almost no selectivity for the target substance. However, its internal cavity is prone to genetic mutations or chemical modifications, making it easier to achieve specific detection.

Mycobacterium smegmatis porin A (MspA) nanopore can simultaneously read information from 4-6 nucleotides. It can reduce the speed of DNA passing through nanopores. It is more conducive to the determination of single bases. In addition, it has excellent heat resistance. It can even withstand temperatures of up to 100 degrees Celsius for up to half an hour. It also has very broad requirements for the pH of the working environment. These advantages give it a broader development space.

2.2. Solid State Nanopores

Solid state nanopores are mainly prepared on solid materials through techniques such as ion beam etching [3]. These solid materials usually refer to silicon oxide or graphene. This nanoporous material has the advantage of adjustable pore size. The stability of these nanopores is very good. The raw materials are also relatively inexpensive semiconductor materials. Although currently, the detection accuracy of sensors based on solid-state nanopores needs to be improved, it still has a price advantage when compared to biological nanopores. It can also be reused, which can significantly reduce the cost of use. This solid-state nanopore has been reported for detecting HIV virus. The ion current and force of molecules passing through solid-state nanopores can be detected. Through this method, various phenomena involving DNA, RNA, and proteins can be studied.

3. Nanopore Sequencing

Nanopore sequencing is a method of detecting nucleotides in DNA using detection principles such as optical readout or electrical signature. The essence of DNA sequencing is to identify the four bases A, G, C, and T. Traditional methods correspond different bases to different pH values or fluorescence signals to achieve sequencing. Interestingly, nanopore sequencing technology converts four types of bases into electrical signals. During the detection process, different nucleotides generate different current signals. By collecting and organizing electrical signals, combined with computer software, this technology achieves the detection of base sequences.

During sequencing, the artificially synthesized polymer membrane is immersed in an ionic solution. There are a large number of nanopores on the membrane. When a voltage is applied to both sides of the membrane, the tested molecule passes through the pore in the form of a single chain molecule. Different bases can cause current signal disturbances when passing through nanopores. Software can be used to process current signals and achieve sequencing.

With the continuous development of technology, nanopore sequencing is quite important in clinical research. In the detection of SARS-CoV-2 virus, nanopore targeted sequencing (NTS) technology based on nanopore sequencing has been reported. It can achieve high sensitivity detection with a detection limit far lower than other detection technologies. It can also detect more than 10 other respiratory viruses.

Till now, nanopore sequencing technology also has limitations. The impact of fluctuations generated during molecular motion on the accuracy of the results is still unknown. Exploring ways to extend the time for DNA to pass through pores can help achieve more accurate detection.

3.1. Speed Control of DNA Translocation

When conducting DNA sequencing, finding ways to minimize the rate at which molecules pass through nanopores is considered one of the key factors affecting sequencing accuracy. In order to improve the resolution and efficiency of detection, analyte molecules must stay in the nanopores for a longer period of time. According to reports, this dwell time is too short to analyze, and it is hard to record the generated current disturbances. Researchers have conducted extensive research on this technical problem [3].

According to reports, DNA polymerase can be constructed near nanopores. This enzyme works like a motor and can be used to regulate the passage time through the pore.

Besides, a polymer network with adjustable pores can be integrated onto solid-state nanopores through electrospinning. Through experimental results, it was found that this network has binding effects on DNA. It can significantly reduce the speed of DNA. This method achieves a significant improvement in detection sensitivity and accuracy. A similar method has been reported, achieved by designing a DNA transistor. This transistor has a porous membrane structure. The pores are between 1-1.5nm. It is noted that for this type of device, the thickness of the electrode layer should not be too thick.

3.2. Identification of Multiple Types of DNA Modification

The sequencing of multiple types of DNA is of great significance for disease diagnosis in practical clinical practice. It is reported that deep learning enables identification of multiple types of DNA modification.

Researchers have developed and designed DeepMod to detect DNA modifications. DeepMod was developed to capture temporal patterns in nanopore signals for the identification of DNA alterations. A nanopore sequencer generated some data which containing event details post-basecalling. These data are used as input files for DeepMod. Through the work of DeepMod, the structural data of DNA strands is output as files [4].

Firstly, the nucleotide sequence is extracted from the input file. Subsequently, the raw data signal is collected and stored. Then, the collected data signals and base types are summarized. In addition, the DeepMod is used to predict base modifications. After calculation and prediction, the modification of DNA was inferred. Then, the modified structure of DNA is output as a separate file. This file can be used for subsequent analysis.

Furthermore, a secondary neural network is employed for specific types of modifications to account for the strong correlation between the methylation status of a CpG site and its neighboring CpG sites. It takes the predicted methylation percentage at a target genomic position and the methylation percentages of adjacent sites across both strands as input. Through this method, the methylation percentage can be adjusted. DeepMod has been reported to be able to accurately predict natural DNA modified sequences. In addition, it can also demonstrate high accuracy for artificially synthesized DNA sequences.

3.3. Strategy for the Multiplexed Detection of Analytes

Multiplexing sequencing technology has become a key strategy for effectively utilizing its sequencing capabilities. A method for detecting multiple analytes is presented which combines nanopore sequencing with DNA barcoded molecular probes [5]. The system enables accurate sorting of signals. It also can achieve quantitatively measuring at least 40 different molecules, including miRNAs, proteins, and small compounds. The identification of each analyte relies on the movement patterns of individual probes as they pass through a nanopore. In this investigation, researchers focused on 40 miRNAs and proteins associated with heart conditions [6]. The technique developed is easily adjustable and expandable, allowing for the detection of numerous biomarkers across various diseases. The test can be conducted with less than 30 μl of sample, without the need for labeling or

amplification. Meanhwhile, it can be conducted at a cost of under US$100. Additionally, the technology has the potential for multiplexing.

Moreover, the assay time on the current platform can be under 60 minutes. This represents a significant enhancement compared to alternative methods that may take hours or days to complete. The as-designed sequencing software can now recognize specific barcode sequences, enabling real-time signal sorting, which will further decrease assay time and offer a crucial advantage in detecting analytes with short lifespans. Furthermore, due to the high portability of the MinION device, assays could be conducted outside of traditional clinical settings. The rapidity and mobility of the system provide additional benefits over other approaches for biomarker detection.

4. Conclusion

Nanopore sequencing is very significant in the detection of pathogenic microorganisms. This work introduces nanopore sequencing. A summary was made around several key cutting-edge issues in this field. The speed control of DNA translocation is discussed. DeepMod which can achieve detection of DNA modifications is also outlined. Besides, strategy for the multiplexed detection of analytes is summarized.

In the future, the development of new software will become an effective means to further promote this sequencing technology. The development of nanoporous materials will also bring about an improvement in the efficiency of this sequencing technology. Especially the exploration of large-scale preparation of solid-state nanoporous materials will greatly reduce the cost of sequencing technology. As this technology becomes commercially viable, it is still needed to explore more materials and testing methods.

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