AgriCos e-Newsletter
ISSN: 2582-7049
AgriCos e-Newsletter
Open Access Multidisciplinary Monthly Online Magazine
Volume: 04 Issue: 03 March 2023 Article No: 20

Nanopore Technology and its Application in Gene Sequencing

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SUMMARY

Nanopore technology has become increasingly important in the field of life science and biomedical research. By embedding a nano-scale hole in a thin membrane and measuring the electrochemical signal, nanopore technology can be used to investigate the nucleic acids and other biomacromolecules. One of the most successful applications of nanopore technology, the Oxford Nanopore Technology, marks the beginning of the fourth generation of gene sequencing technology. These applications show that nanopore technology is promising in the field of biological and biomedical sensing.

INTRODUCTION

Nanopore technology refers to nano-scale holes embedded in a thin membrane structure to detect the potential change when charged biological molecules smaller than nanopore pass through the hole (Deamer and Akeson, 2000). Therefore, nanopore technology has the potential to sense and analyze single-molecule amino acid, DNA, RNA, etc. Nucleic acid is an important genetic material for most of the living body, and accurate sequencing of the nucleic acids is important for biomedical research, which would be useful for diagnosing human diseases and providing personalized medicine [Zhu et al., 2015]. Since the last century, gene sequencing technology has developed dramatically, and now the nanopore technology has taken a leading role in the area of gene sequencing.

Principle:

Nanopore-based technologies originated from the Coulter and ion channels (Feng et al., 2015), which could be traced back to the 1990s (Deamer and Akeson, 2000). Nanopore technology is done by applying a cathode and anode to the solution on the forward and reverse side of the membrane, respectively. Negatively charged biomolecules, such as DNA, can be placed on the forward side and these molecules can pass through pores in the membrane under the electrophoretic force with applied voltage. When different molecules translocate through the pores, the current level can be captured and be further used for calculation in computer-aided tools. The category of nanopore used in nanopore technology can be divided into two parts, solid-state nanopore and biological nanopore.

1. Solid-State Nanopore:

- Solid-state nanopore can be fabricated using different methods, such as controlled breakdown, electrochemical reactions, laser etching and laser-assisted controlled breakdown (Fried et al., 2021).
- It breaks the limit of natural occurring nanopores and has many advantages, such as very high chemical stability, control of diameter and channel length, adjustable surface properties, and the potential for integration into devices and arrays.
- Si_3N_4 and SiO_2 nanopores are one of the most widely used nanopores and their fabrication is compatible with the complementary metal oxide semiconductor industrial integrated circuit processes.

2. Biological Nanopore

- Biological nanopore comes from natural protein molecules or artificial nanopores generated by genetic engineering (Mohammad et al., 2012).
- However, the biological nanopores are frail with features such as short lifetime, intrinsic instability, and strict requirement of a specific environment, which are not able to support a biosensor's long-term operations.
- Biological nanopores are usually produced by selected bacteria, such as α-hemolysin pore protein, MspA from Mycobacterium smegmatis , and Phi29 from Bacillus subtilis.
- These biological nanopores are currently used for disease diagnosis , gene sequencing and protein sequencing .

Nanopore Sequencing Technology:

ONT is a single molecule sequencing technology based on nanopores. The first prototype, MinION, was released in 2014(Bentley , 2014). The updated platform, PromethION, was released in 2015 with improved throughput. Two versions of PromethION, naming ProtheION 24 and 48, integrate 24 and 48 flow tanks, respectively. With the booming numbers of flow tanks compared to MinION, the PromethION system could output up to 7.6 Tb data while MinION could only generate 50 Gb within 72-h operation. The process of nanopore gene sequencing can be divided into three parts, library preparation, sequencing process, and basecaller.

1. Library Preparation

The preparation of the library is crucial for the subsequent work of nanopore sequencing. The DNA fragments should be repaired whether it has been sheared or not. When repairing, the repaired connector is a DNA-protein complex with a polymerase or helicase on the complex.

2. Sequencing Process

The DNA strand to be sequenced is mixed with copies of the processive enzyme. When the DNA-protein complex approaches the nanopore, the enzyme binds to a single stranded leader at the end of the double stranded DNA template, unzips the double strand, and feeds a single strand through the nanopore. A single molecule with high specificity can interfere with the current when the unzipped DNA long strand passing through the nanopore one base at a time. These current signals can be used to determine the type of base.

3. Basecaller

In the process of base readout, owing to the difference in the charge and structure of nucleotides when they translocate through the nanopore, the measured current would cause small disturbance. These electrical signals can then be translated into DNA sequences with the deep learning algorithms. However, the readout signals are noisy and random as these signals are originated from more than one molecule in the nanopores, which is difficult for the basecaller.

Applications of Nanopore Technology

- Nanopore technology, mainly applied in clinical research which help researchers to identify and phase genetic variant, and fully characterize novel isoforms and fusion transcripts.
- Nanopore technology gives a new insight to health and disease, from cancer, immunology, to neuroscience.
- The nanopore technology can be used in detection of microbes by sequencing the DNA or RNA and it helps researchers to classify or monitor the microbes.
- It is promosing to establish microbes survrillance and response quickly during pandemic, if the nanopore technology can be applied in the area of public health.
- This technology also can overcome the short-read sequence devices meet in area the long repeated fragments.
- Nanopore sequencing technology provides a unique tool for environmental research, including biodiversity assessment, pathogen identification and animal conservation.

CONCLUSION:

Detecting the potential change when charged biological molecules smaller than nanopores pass through the hole, nanopore technology provides a new way to identify and quantify a variety of analytes. sequencing technology combines nanopore technology and biosensors, and it will have a tremendous impact in the area of gene sequencing.

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