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Abhay Kumar
Department of Agricultural Biotechnology and Molecular Biology, Dr. Rajendra Prasad Central Agricultural University, PUSA, Samastipur, Bihar, India

Vinay Kumar Sharma
Department of Agricultural Biotechnology and Molecular Biology, Dr. Rajendra Prasad Central Agricultural University, PUSA, Samastipur, Bihar, India

Prafulla Kumar
School of Biotechnology, Gautam Buddha University, Greater Noida, Uttar Pradesh, India

Correspondence
Abhay Kumar
Department of Agricultural Biotechnology and Molecular Biology, Dr. Rajendra Prasad Central Agricultural University, PUSA, Samastipur, Bihar, India

Nanopore sequencing: The fourth generation sequencing

Abhay Kumar, Vinay Kumar Sharma and Prafulla Kumar

Abstract
DNA is a molecule that makes up the genetic material of a cell, and it is responsible for carrying the information needed for survival, growth, and reproduction of an organism. DNA sequencing is the process of determining this sequence of nucleotides. Originally sequencing was an expensive process, but during the last couple of decades, the price of sequencing has drastically decreased. A significant breakthrough occurred in May 2015 with the release of MinION sequencer by Oxford Nanopore making DNA sequencing inexpensive and more available, even for small research teams. This review condensed various nanopore based sequencer utilized for DNA sequencing and currently available developed technologies in the market and under development technology for biomolecules sequencing.

Keywords: Nanopore, alpha hemolysin, Mycobacterium smegmatis porin A (MspA)

Introduction
More than thirty-five years have gone by improving the historical DNA sequence of Frederick Sanger and his associates. This progressive investigation set off the improvement of new strategies which have given extraordinary chances to minimal effort and quick DNA sequencing. Surprisingly, after the human genome project, the time between the individual sequencing innovations began to shorten, while the measurement of the logical information continued to growing exponentially. Considering the Sanger sequencing as the first generation original, new generations of DNA sequencing have been presented thus, amplification based mass parallel sequencing is the second generation, and the sequence with one molecule is the third generation. Following the three-generations, DNA sequencing technology introduced into the new period of nanoporous, single-molecule nanopore sequencing technology i.e. fourth generation sequencing [1]. Each sequencing technology has distinct advantages and disadvantages due to their methodological approach, which determine the suitability for applications. Utilizing nanopore sequencing, a solitary molecule of DNA or RNA can be sequenced without requiring PCR amplification. In any event, one of these previously mentioned advances is vital in the strategy of any recently created sequencing approach. Nanopore sequencing can possibly offer moderately minimal cost genotyping, high versatility for testing, and fast preparing of sample with the capacity to show results continuously [2]. Publications on the nanopore sequencing its usage in speedy distinctive evidence of viral pathogens, observing of antibiotic resistance, observing ebola, natural monitoring, observing of antibiotic resistance, haplotyping human genome sequencing, plant genome sequencing, surveillance ebola, sanitation checking/hygiene control, common observing, and various applications. No additional computing infrastructure is required. Not constrained to a laboratory environment, it has been used up a mountain, in a jungle, in the arctic and on the International Space Station [5].

How nanopore sequencing works?
A nanopore framework dependably contains an electrolytic arrangement when a steady electric field is connected, an electric flow can be seen in the framework. The extent of the electric flow thickness over a nanopore surface relies upon the nanopore's measurements and the synthesis of DNA or RNA that is involving the nanopore. Sequencing is made conceivable in light of the fact that, when close enough to nanopores, tests cause trademark changes in electric flow thickness crosswise over nanopore surfaces. The absolute charge coursing through a nanopore channel is equivalent to the surface fundamental of electric flow thickness transition over the nanopore unit typical surfaces between times t1 and t2.

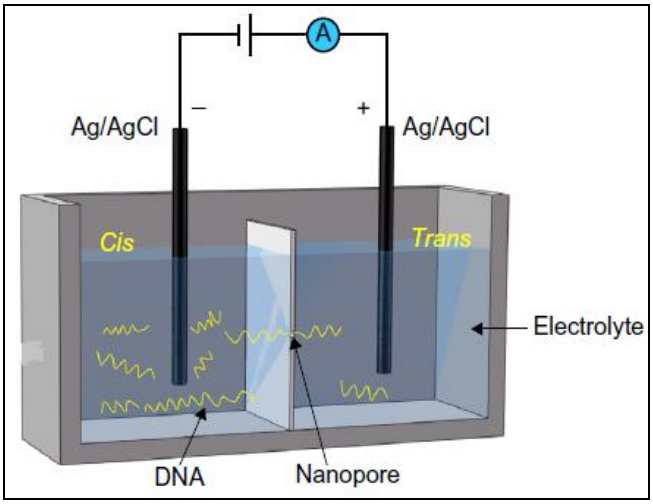

Fig 1: The basic concept of nanopore sequencing [14]

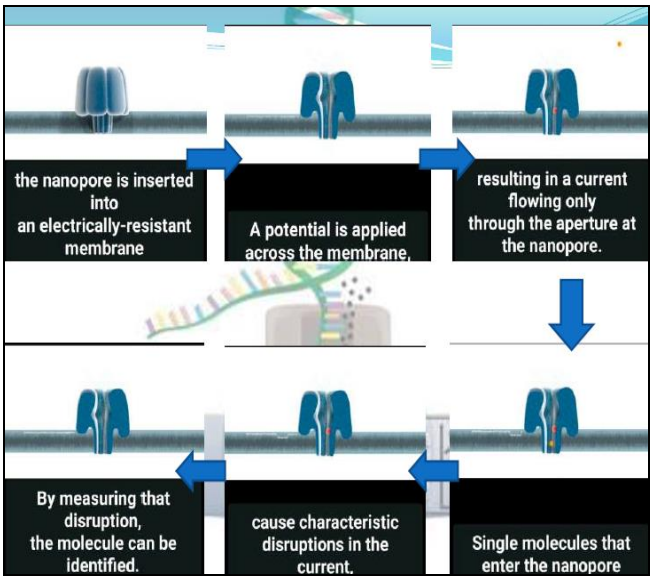

Fig 2: Explain how nanopore works [14]

Types of nanopore used in nanopore sequencing

- They are generally divided into two categories:

1. Biological nanopores – lipid layer and pore forming proteins
2. Synthetic nanopores – Si_3N_4 and SiO_2 membrane, Al_2O_3 membrane

Biological nanopores

Liposomes, planar lipid bilayers, or different polymers are used as a substrate for the insertion of nanopore protein channels. Three well-studied biological nanopores- I. Alpha - Hemolysin, II. MspA and III. Bacteriophage phi29.

α- Hemolysin

Alpha hemolysin is the first and most customarily utilized nanopore, assumed an enormous job in DNA sequencing. *Staphylococcus aureus*, microbiota of human body secrete α - HL is an exotoxin which is a mushroom-shaped 232.4-kDa heptameric transmembrane channel. Additionally, the alpha-hemolysin nanopore structure can remain stable at temperatures close to 100 ˚C inside a wide pH expand (pH 2–12) [6].

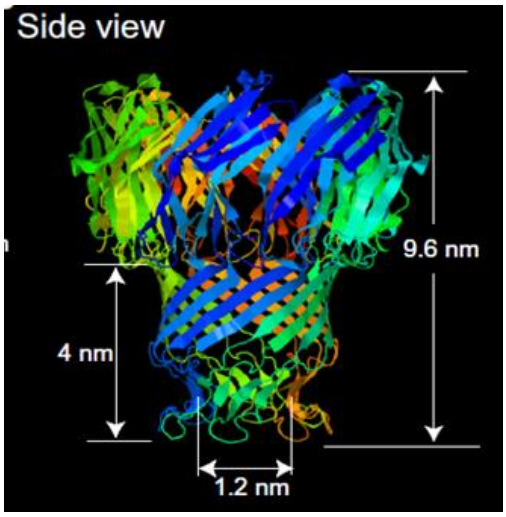

Fig 3: molecular structure of alpha hemolysin

MspA

Mycobacterium smegmatis porin A **(**MspA) is a protein secreted by Mycobacteria, enabling hydrophilic elements to enter the bacterium, with dimensions of 1.2 nm in diameter at the minimal point, this property of porin proteins is utilized for the DNA sequencing. As it moves towards the Alpha –HL MspA is Small and narrow and extremely strong and keeps the channel active under outrageous experimental conditions, for example, fluctuating the pH value from 0 to 14 and maintaining up the temperature at 100 °C for 30 min [5].

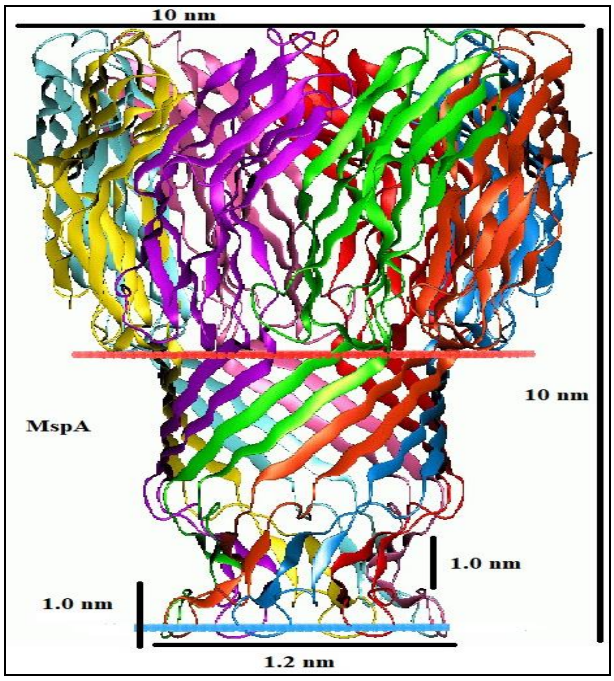

Fig 4: Molecular structure of MspA [5]

Bacteriophage phi29

The bacteriophage phi29 DNA packaging motor has a 12-subunit gp 10 connector. The phi29 connector channel shows stable occupy properties in a voltage go from - 150 mV to +150 mV, even under a broad extent of pH conditions. Appeared differently in relation to Alpha-HL and MspA, the phi29 pore has a bigger Diameter consequently dsDNA could be sequenced with the phi29 pore [8].

Solid-state nanopore

Albeit biological nanopores have seemed empowering sequencing results for ssDNA, however these nonporous proteins have a few breaking points, for example, constant pore size, lack of stability; suffer from the fragility of traditional supported lipid membranes. To change for these inadequacies, different synthetic nanopores have been created utilizing various strategies and useful for DNA and RNA sequencing.

- **Si_3N_4 and SiO_2 membrane:** Generally utilized as substrates - low mechanical stress and high chemical stability
- **Al_2O_3 membrane-** has improved electrical performance, a higher signal-to-noise ratio, and lower noise during DNA translocation.

Commercial nanopore sequencers

Hagan Bayley and Gordon Sanghera the founder of Oxford Nanopore Technologies (ONT) has been invested money for creating nanopore-based DNA sequencing platforms for business purpose [15].

1. MinION

MinION is the first commercially available nanopore based sequencing device. MinION is an inexpensive pocket-sized device just like a mobile cell phone i.e it is portable, high throughput means automation of sequencing at large scale (In hundreds of kbs). MinION is very reasonably priced as compare to conventional sequencing devices. It is begun simply paying of $1,000. It is currently industrially accessible in the market since May, 2014 [10].

Fig 5: MinION

2. GridION X5

GridION examined up to five MinION Flow Cells at a same time, it is a minimized bench top framework. It is perfect for labs with different projects that need the benefits of nanopore sequencing: simple library preparation, real-time analysis and long reads. The present chemistry and software release enables generation of upto 150 Gb of information during a GridION X5 run and the compute modele is able to analyze that data is real time. The price of industrially accessible, GridION X5 started from $49,955.00 to $158,500.00. GridION X5 is commercially available in markets since March 2017 [9].

Fig 6: GridION

3. PromethION

PromethION Offers same ongoing, real-time, direct DNA and RNA sequencing development as MinION and GridION yet at significantly greater scale. Clients can begin and quit running individual trials as required, or send different stream cells onto single investigations for more prominent speed or throughput. Every one of the 48 Flow Cells permits up to 3,000 nanopores to succession all the while, with a possibility to yield up to 12 Tb in 48 hours for the entire gadget. No capital use is required for PromethION platform. PromethION Price is begun from $160,000.00 [8].

Fig 7: PromethION

Under development

1. **VolTRAX:** This device, at the present time being created, is expected for robotized test arranging with the objective that customers needn't waste time with an examination lab to run the device. Enrollment for the early access program was opened in October 2016 [11].

Fig 8: VolTRAX

2. **SmidgION:** A cell phone sequencer declared in May 2016, right now being developed [12].

Fig 9: SmidgION

3. **Flongle:** Flongle is a connector (Just like a dongle of internet providers) for MinION or GridION, which provides the grouping of sequence information of experimentation start. Flongle is designed for the easily accessible quick experimentation and cost effective for large scale sample [13].

Fig 10: Flongle

Conclusion and future prospects

Nanopore based sequencing technologies are very fascinating and popular in researchers for the various benefits in terms of massive parallel sequencing, promising long reads, quantity and quality of large amount generated data, decreasing the cost of DNA sequencing. Rather than these advantages several other advantages also offered by the nanopore sequencers such as minimal sample preparation, no requirement of dNTPs, polymerase, ligase etc. Nanopore sequencing empowers the researchers to study plant genomes which are ordinarily huge in size, exceptionally tedious to handle due to high repetitiveness and display a wide assortment of ploidy, making them difficult to sequence and assemble utilizing conventional sequencing innovations. Nanopore based sequencing can be helpful in the Real-time results for time-critical applications such as pathogen identification. Nanopore based sequencer does not required any computing infrastructure i.e. not limited to the laboratory, nanopore based sequencer can be used in the Arctic, jungle and at the International Space Station. It is very evident that these technologies are amazingly encouraging as far as research and will quicken our understanding of life forms.

Table 1: Comparative analysis of all types of nanosequncers

Nanopore sequencers	Size	price	Current status	A progression over one another
MinION	Mobile	$1,000 to $1,0000	Commercially available in markets since 2014	just like a mobile
GridION X5	Laptop	$49,955.00 to $158,500.00	Commercially available in markets since March 2017	Five MinION flow cells
PromethION	desktop	$160,000.00 to $200,000.00	Commercially available in markets	48 MinION Flow Cells
VolTRAX	Mobile cell phone		Under development	Completely Robotized
SmidgION	Mobile		Under development	

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