

# Nanopore Sequencing: A Survey

Muhammad Zunnurain Hussain, Muhammad Zulkifl Hasan

**ABSTRACT:** A single molecule of Deoxyribonucleic acid (DNA) is being sequenced by using an innovative concept of Nanopore. The orientation of nanopore define the channel of ions through the pore comes into contact with nucleotide, the permutation and the transition kinematics of each molecule of DNA. The divergence of electrical signal observed when the four chemical bases adenine (A), guanine (G), cytosine (C), and thymine (T), present in the DNA passed through the nanopore is being determined. This led to next generation bioengineering and reduces the cost and time of mammalian genome sequencing and makes the process of DNA sequencing extremely easy and easily accessible.

**Keywords:** DNA Sequencing, Nanopore, Nanoelectrodes, Single molecule measurement, Solid-State Nanopore.

## INTRODUCTION

The Nanopore technology uses a low voltage in an aqueous electrolyte to get the ionic current using electrophysiological technique [1]. A Nanopore device which detects the flow of current due to the movement of DNA across pore [2]. Number of researches had been done in past in Nanopore which declared it as multi-layer graphene[3] and their thickness can be comparable between the bases and the silicon base which had been manufactured by electron beam[4]. The solid state Nanopores are with cheap in price, easily measurable and had constant behavior. The system has been designed to combine the charges and measure the voltage fluctuation. The sequence of four chemicals of DNA holds the genetic information, this information need to be decrypted. However, the procedures that have been followed take days and cost about \$100,000 [5].

## TECHNIQUES FOR NANOPORE SEQUENCING

### a) Nanopore DNA Sequencing using MspA

Nanopore DNA sequencing with MspA is a method that had been implemented by different researchers in various ways. Main aim of using this method according to one researcher, His views were as under; the Mycobacterium smegmatis porin A (MspA) protein has small size block region and that's why has better resolution as compared to alpha-haemolysin pore. Thus because of its diverse qualities from other approaches it can be used as pore and check the effect of molecule of single-stranded DNA (ssDNA) during the existing pore calculation time[6]. Another researcher had different point of view expressed as, Nanopore sequencing can be very fast, direct and with less cost upcoming technology to be implemented. Test had been done in past that illustrate that one nucleotides of single stranded DNA when passed through nanopore will distinctively change the flowing current through the pore, that finally permit to defer the sequencing.

He explained the process through a test in which he demonstrated, that the current passing through the Mycobacterium smegmatis porin A (MspA), had the capability to discriminate all four DNA nucleotides and agreed on single nucleotides in single-stranded DNA when double-stranded DNA momentarily grasp the nucleotides in the pore limitation condition[7]. There are common challenges that had been faced by the researchers were, DNA translocation is rapid in different cases. While considering MspA, it can offer different current partition for single nucleotides. The process finally tells us that the protein pore MspA has immense possibility to advance the nanopore DNA sequencing. MspA's small and narrow limitation can be utilized to differentiate the individual nucleotides of ssDNA while duplex DNA segment suspend the translocation [6]. One more researcher's team had presented the process in different way. They demonstrated that to judge the capabilities of MspA's in resolving nucleotides, they hold ssDNA within the pore using a Neutravidin complex. They did different test during the process to show that the single nucleotides within random DNA can be identified. Finally, they came up with conclusion that the signal-to-noise ratio and the single nucleotides sensitivity in MspA are high [8].

### b) Single Molecule Sequencing

Single molecule DNA was difficult to handle due to its Brownian motion that shows that the position and shape is ever changing. It was difficult sometimes to control the single molecule DNA, procedures used to handle the molecule can be fix by changing the shape of molecule i.e. in tense or stretched shape, use of laser beam for its reshaping or cutting[9]. So according to the author he used Opto-electrostatic micro manipulation to handle it. In the process of stretching single DNA molecules on a mica surface, it is followed by other steps like dehydration of the sample and evaporation of a thin layer on the molecules[10]. The Single-Molecule can be detected by using force spectroscopy techniques which is based on optical tweezers and atomic force microscopy. In this process, the molecules will be entered through a small hole with less than 5 nm. Through this process the author has calculated values [11]. Another scheme used for single molecule DNA sequencing will be tunneling current based identification. This process involves deep study of single nucleotides when passed through the tunnel without any chemical and amplification use [12]. As the molecules are moving, they change their position and style. Accuracy problem in the calculation will come across. The author has used pixilated detector (fluorescence microscopy) to

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- Muhammad Zunnurain Hussain, Department of Computer Science, SCET Lahore [engrhusain@gmail.com](mailto:engrhusain@gmail.com), Member, IAENG
  - Muhammad Zulkifl Hasan, Computer Science Dept NUST, NIPCONS, [engrhasan@gmail.com](mailto:engrhasan@gmail.com), Member, IAENG

estimate the attributes of the molecules. The molecular motion led to localization error and from that noise will inherent the results very badly. The author came across various previous work in that area but he himself created a bench mark i.e. Fisher information matrix which according to him will help him in the accurate results and calculations [13].

### c) Nanopore - Embedded Bi – layer - graphene Nanoelectrodes

The usage of Bi-layer graphene as a nanopore electrode for DNA Sequencing is being proposed in this method. It also shows the advantages of bi-layer graphene Nano electrode like it increases the conductance by 1 ~ 2 orders and increase the accuracy and speed up the information analysis and measurement process over single layer graphene Nano electrode. In graphene, the carbon atoms are organized like one atom thick planar sheet which shows the maximum limit of thinness of nanoelectrodes [14]. In this design the single strand DNA sequencing is passed through the graphene nanoelectrodes electrophoretically by applying the electrical field longitudinally. This translational motion stimulates the transverse conductance which is being recorded for sequencing. The arrangement showed in the diagram that to measure translation tunnel conductance, the diameter between the nanoelectrodes required to be adequately narrow and the thickness of nanoelectrodes need to be greater than the critical value to give the satisfactory interaction to the object nucleotide[15]. In order to achieve single base simplification the Nano electrode could be thick up to a certain limit otherwise we will be observing the signals from more than one nucleotide simultaneously. Here is the trade-off between conductance measurability and precision into the single base resolution. Nanopore should be constructed by keeping in mind that it should not be too thick or thin [14].

### d) Nanopore sequencing using converted targets and optical readout

An innovative mode in which we can experience Nano pore sequencing is developed that changes DNA information into two colors that we could read optically. Attaching of luminous probes to every nucleotide is not an easy task, approaches had been presented to thoroughly convert and replace every nucleotide present in the genome with a precise variation of two dissimilar 12-mer oligos (A and B), linked in a particular fashion (AB, BA, AA, BB) that replicates and converts the nucleotide arrangement of the unidentified DNA29[1][16]. This changes the DNA codes of A, T, G, and C into a binary code in which each base is represented by a pair of 12-mer oligos (A and B)[16][17]. At the moment, needs an average of 24 hours to transform a whole humanoid genome into a DNA amalgam comprising of fragments, every conforming to a 24-bp section of the native genome. Researchers are nowadays investigating ways to develop low-cost fault proof transformation of extended sections of the native genome and to significantly decrease the transformation time. The transformation method does acquaint with an additional biochemical step, which is not ultimate, but it side lined some of the challenges confronted by other methods and simplifying the following sequencing readout. For the process of readout, the transformed DNA amalgam is then mixed with another

mixture comprising two dissimilar “Molecular Beacons” both are designed to complement any of A or B. Due to phenomenon of self-quenching the molecular. The color of photon burst shows the original DNA sequence and the base is recognized by every pair of two consecutive burst. In order to probe thousands of Nanopores instantaneously we need a very high resolution electron enhancing CCD camera. In this technology, the main challenge is still is the manufacturing of a high-density array of about 1.7 – 2nm diameter [1].

### e) Double-functionalized Nanopore-Embedded gold electrodes

Through this approach, we are addressing two important aspect of efficient DNA sequencing i.e. the single base resolution problem and other one is controlling the DNA velocity while moving in between the Nano pore which is indeed one of the biggest challenges in DNA Sequencing [18]. By combining two different approaches in this process we are getting productive outcomes. The both nanopore embedded gold electrodes are designed considering two different approaches. One electrode is prone to interact with base part of DNA nucleotide. In the other approach cytosine is used to make interaction between gold electrode and weak acid bases through weak hydrogen bonds. The advantage of weak hydrogen bonding leads to control the movement of ssDNA. The second technique also gives an advantage of reducing the fluctuation in the traverse current observed. In order to probe to the DNA phosphate group, we need chemical probe which have the tendency to interact with gold so the thiol functional group is required as well interact with DNA phosphate so the amine group is required[19]. After investigating a number of chemical groups, HSC(NH<sub>2</sub>)<sub>2</sub> (thiourea or thiocarbamide) is found to be most appropriate pairing. Now we have thiocarbamide as a probe which binds the DNA phosphate group with hydrogen bonding. Broadly speaking, this approach could be divided into three main parts. Left electrode, right electrode and scattering region between these two electrodes. Each electrode comprises of six layers of Au (111) where the first three outer layers are kept rigid as in bulk for the calculations. The central scattering region consists of three inner layers of gold (111) from each electrode, the attached chemical probes, and the target nucleic acid base of the ssDNA. For the purpose of reducing computation cost we used single-zeta with polarization (SZP) orbitals for Au and double-zeta polarization (DZP) for H, C, N, O, P, and S atoms [18]. The applied biased current at both is 0.1V and 0.25V for calculating the resulting current. Hydrogen bonding interaction between the cytosine chemical probe and the target nucleic bases was found to be maximal for purine baseband minimal for pyrimidine bases [20]. The current calculated at 0.1V bias was found to be in principle capable of distinguishing between the four DNA nucleotides [18].

## CONCLUSION

This paper emphasis to elaborate some of the cutting-edge tools, methodologies and their comparisons to highlight the importance of this research area. Solid-State membrane in nanopore sequencing is proposed as an alternative because of its fabrication process with ability of modification and simple possible incorporation into a sequencing

system, leads this technology to commercial deployment. If this technology reached its maturity, the advantages of nanopore sequencing is very high in number. Including the reduction in cost and could be possible in \$1000 per mammalian genome goal set by NIH [1]. The major decrease in instrument cost and require only one day for the complete process. Capacitor or probes are being tested to find out its capability to detect DNA strand.

## FUTURE WORK

The controlling of DNA's motion through a nanopore is still a challenging part of this innovation, researches signifying that it could be achieved by using DNA processing enzymes. Solid State Nanopores are proved to be more promising in long term future run. While moving in transit the DNA molecule produces more noise in the signal of the sensors decreasing the potential of single base resolution. However, it is being considered as this technology will reach its maturity very soon because still there is no major limitation being noticed which could hinder the growth of this emerging technology.

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