http://babrone.edu.in/blog/index.php/2019/02/04/role-of-ngs-next-generation-sequencing-in-disease-diagnosis/

BioNE
E-ZINE OF BIOLOGICAL SCIENCES
ISSN: 2456-7264 | Issue – 9| Published On 04/02/2019

Role of NGS (Next Generation Sequencing) in Disease Diagnosis

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Overview

The Next Generation Sequencing (NGS), a decade old technology has revolutionized the area of genomics with substantial impact. This highly advanced sequencing technology can produce a huge amount of data in a very short period which is possible because of parallelized sequencing enabling to sequence millions of fragment simultaneously. The basic principle behind the sequencing technique is the same as that of capillary electrophoresis (Shendure and Ji, 2008) and in many occasions, the Sanger sequencing is still considered as the 'Gold-standard'. The critical difference is that, here instead of sequencing a single DNA fragment, NGS can perform in a massively parallel manner (Chaitankar *et al*., 2016). As a result, it can produce sequencing data at an exponential pace. This remarkable transformation is highly appreciable as now-a-days sequencing of the human genome of 3.2 billion bases needs merely a week, whereas it took thirteen year time to complete the Human genome project. Currently, NGS has evolved as a molecular scanner and virtually has imprinted its importance in every aspect of biomedical research.

In the last few years, the shift has been transferred in the direction of the molecular approach for the treatment of diseases especially in the field of medicine. In recent times, NGS has shown its potential in the diagnosis of numerous diseases, including infectious diseases, monogenic diseases and cancer (Johansen *et al*., 2014; Naccache *et al*., 2014; Lecuit and Eloit, 2015). A different feature of NGS has been developed in the last half decade depending upon the hypothesis along with the gradual advancement of technology. The targeted re-

sequencing approach can sequence panel of multiple genes in a single test run which is advantageous in finding many rare variants. However, the whole exome sequencing can explore genetic diversity in the exonic region of the genome and it is even possible to identify a single nucleotide variant in a large population. The genome-wide association studies based on SNP (single nucleotide polymorphism) have suggested diseases like Behçet's uveitis to have a link with several complex loci unveiled in various genetic diseases (Kim *et al*., 2013). So, overall the technology has slowly stepped up its reliability in the clinical biotechnology and modern medicine heading towards the development of a new era of disease diagnosis.

Second generation Sequencing Platform insight

The commercially available second-generation sequencing technology is performing with a different approach in various platforms developed by various companies. In NGS, the accuracy is achieved by sequencing a particular region multiple times. However, it produces a smaller read length than Sanger sequencing because of increasing signal to noise ratio with the increase in reading length that enhances the possibility of error (Sims *et al.*, 2014). Each platform has some similarities and dissimilarities and therefore advantages and disadvantages based on their performance established on varied chemistry. The Ion torrent platform developed by Life Technologies is based on the principle of detection of H+ ions released during DNA polymerization and uses sequencing by synthesis and emulsion PCR method. The Ion PI Chip is capable of generating 100 GB data, the number of reads is up to 80 million and has a read length of 200 bases with 2–4 h run time (Buermans and Den Dunnen, 2014). It is widely used in the sequencing of genes, human genomes, *de novo* sequencing, chromatin, transcriptomes, methylation analysis, whole exomes, gene expression by sequencing, small genomes, and small RNAs (Meldrum et al., 2011; Mardis, 2013). Illumina uses a fluorescence-based model for reading the incoming bases in a nucleotide sequence that run on a chip named *flowcell* (Buermans and Den Dunnen, 2014), which is currently most popular platform of NGS sequencing (Solexa, MiSeq and HiSeq). The Illumina technology is based on pair-end sequencing that can generate data up to 1 TB with more accuracy. In Illumina, all the data from a single run generates almost a similar read

length while read length of Ion Torrent platform varies. However, recent research by Nicholas F. Lahens *et al.* (2017) found both this platform equally compatible while compared following the careful selection of alignment software (Lahens *et al*., 2017). Although many other platforms are available for NGS, Illumina and Ion Torrent are most popular in the recent time. Roche's 454 were also on the list but currently is an obsolete technology. Other notable competitors of NGS are Pacific Biosciences, Beijing Genomics Institute, GATC Biotech AG., Oxford Nanopore Technologies Inc., Agilent Technologies Inc., Biomatters Ltd., DNAStar Inc., Macrogen Inc., Helicos BioSciences, CLC Bio (Qiagen), and Knome Inc.

Application of NGS in disease diagnosis and medicine

The conventional approaches to infectious disease diagnosis with cell culture and enzyme immunoassay are still in use. But at the same time, these are time-consuming and have issues with its sensitivity which can produce a false positive result. Whereas, NGS can provide an accurate result in a short period that can distinguish different pathogen strains, detect co-infection and reveal new pathogens by Whole Genome Sequencing (WGS). NGS has developed genome-wide single cell analysis technique. An individual cell outlined with a mixture of genomic and transcriptomic heterogeneity in normal and disease state is a common phenomenon (Ellsworth *et al*., 2017). Thus, deep sequencing of the whole or single cell cancer genome/ transcriptome can provide valuable information to identify "driver mutation". Liquid biopsy (based on cell-free DNA; cfDNA) based diagnosis is getting wide acceptance across the globe especially in the early detection of cancer (Malapelle et al., 2016; Heitzer *et al*., 2017). Application of NGS additionally signifies as a major boost in detecting a mutation in cfDNA formed in body fluid and monitoring in response to treatment. In clinical biotechnology, insight about how bacterial populations respond to drug treatment, by determining bacterial virulence or antibiotic resistance/associated genetic variation biomarkers can easily be exposed with help of NGS. The emergence of drug-resistant pathogens and their sequence information can also be uncovered by NGS with ease. In a broad aspect, it helps in the quick management of the disease spread by patient isolation and thereby helps prevention of outbreak.

The concept of personalized medicine has rapidly evolved in the healthcare sector, where a person's genetic profile is used to diagnose, treat and prevent genetic diseases. NGS has prompted advancements in the area of cause of genetic diseases, pathways, and identification of the novel mutation that has widely extended to the genotype-phenotype relationship (Lohmann and Klein, 2014). The information in this regard is useful to understand the genetic pattern of inheritance of the disease and probable gene-based therapy. Although the approaches are in developing state, it will be highly beneficial for molecular diagnosis and therapeutic interventions in the near future. A recent notable development of NGS is its assessment in liquid biopsies for cancer diagnosis.

Conclusion

The next-generation sequencing global market is likely to reach USD 12.45 Billion by 2022 from USD 4.62 billion in 2015. The rising inclination of scientific approach towards the field of biomedical research especially in drug discovery with the concept of personalized medicine is the driving force for the overwhelming demand of NGS in the recent time. Moreover, the gradual reduction of cost per sample and cost per amount of data generated is also another influential factor to be considered. At the same time, accuracy and standardization in diagnostic testing, simplified algorithm and composite bioinformatics software, and lack of expertise manpower are key limitations in this field. In conclusion, with the advent of such technology, it is possible to understand deep and in details about many rare diseases in relation to their genomic complexity and perhaps a simplified approach can be developed for its diagnosis and public health surveillance in the near future. Indeed, the expert bioinformatician can have a bright prospect of career to lead in the post-Sanger sequencing era in the coming years.

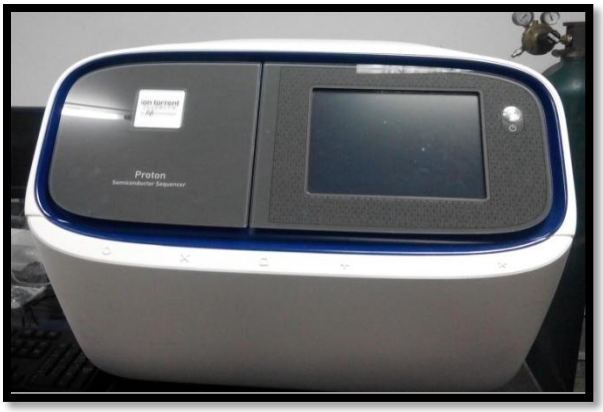

Fig 1. Ion Torrent (Proton) platform

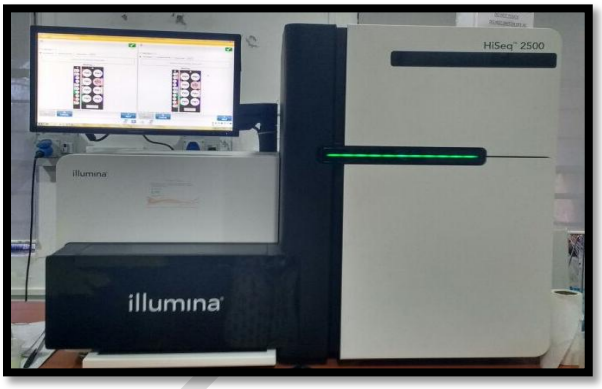

Fig 2. Illumina platform

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