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A New Generation in Genome Sequencing: Next Generation Sequencing Techniques

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Abstract:

A new scientific discipline 'Genomics' has the DNA sequencing which is used as the core of it. This technology has low cost with the improved scale of genome characterization compare to the history of 40 years in the field of genome. Before the use of NGS, Sanger sequencing was used that had many drawback such as time consuming, less accuracy and so on. Now, in the DNA sequencing, Next generation Sequencing (NGS) provides rapid ways in the characterization of genome, profiling of mRNAs, small RNAs, transcription factor regions, structure of chromatin and DNA methylation patterns, microbiology and metagenomics. These NGS technologies have many advantages in the analysis of genome such as of cost-effectiveness, unprecedented sequencing speed, high resolution and accuracy. This paper represent the applications of Next generation sequencing by the use of different platforms or software by which the disease can be detected with the high accuracy and it is also use for the medicinal purpose as it treats the disease. With the use of many platforms, NGS technologies also have the Con i.e., storage capacity (large genome data).

Keywords: *Genomics, Platforms, Storage Capacity, Diseases*

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Introduction

New opportunities has been opened in the biomedicine by the use of next generation DNA sequencing techniques. In biomedicine this technique was selected by Nature methods in 2007. Before the development of this technique, Sanger enzymatic dideoxy technique was used that was developed in 1977. Sanger method was used for sequencing of a genome region by automated fluorescent project.

The development of techniques that allows the higher sequencing is came into begin after the decision of the determination of whole human genome sequence by International Community. These high-throughput sequencing (HTS), also called Next Generation Sequencing (NGS) has a platforms that are based on cyclic array sequencing implementations. The Sequencing of dense array of DNA features by iterative cycles of enzymatic manipulation and imaging-based data collection define the concept of cyclic-array sequencing. There are many commercial product which are based on this sequencing technology such as Roche's 454, Illumina's Genome Analyzer, ABI's SOLiD and the Heliscope from Helicos. All these platforms give slightly different sequencing as well as the generation of array from each other but their flow of work is very similar according to concept. The sequencing of millions of short sequences is allowed by all of these products by which they are capable of producing a sequence of full human genome per week with the cost 200 fold that is very less compared to other previous methods **(Ansorge, 2009; Magi *et al,* 2010).**

There are three major improvements in Next Generation Sequencing compared to first generation sequencing (Sanger Sequencing).

1. It does not require a procedure of bacterial cloning and prepare libraries for sequencing in a cell free system.
2. It is able to process millions of sequencing reactions in parallel at same time.
3. The detection of nucleotides bases can be performed in both cyclically and parallel **(Park and Kim, 2016)**.

Next Generation Sequencing Platform

Genome sequencing projects provide high speed and throughputs. These platforms have an advantage that they can determine the sequence data from amplified single DNA fragments by avoiding the need of cloning of DNA fragments. The disadvantage of this technique is high cost for generating the sequence with very high throughput. There are some techniques that is defined as follows:

The Illumina Genome Analyzer

It is also called Solexa Sequencer originated by Turcatti and their colleagues during their work in 2006. This High throughout sequencing technology is widely available. The mechanism of this technology is as: PCR generate the amplified sequencing features. Then, the immobilization occurs in the array. The sequencing of all molecules are done in parallel by the synthesis.

Each nucleotide is recorded by the imaging techniques and is converted into the base cells during the process of sequencing. The sequence reading is possible up to 100 bp with relatively low error rates in the Illumina Sequencer. This read length is limited due to many factors cause the signal decay and dephasing. In this technology, the major sequencing error are substitution errors and insertion or deletion is considered as much less common. New Illumina Genome Analyzer IIe enable to generate 200 million 100 bp with total 20 Gb of data in which 2 Gb per day is used. Solexa website has the all information about this version **(Magi et al, 2010)**.

Roche 454 Genome Sequencer

This Genome sequencer instrument was developed by 454 life sciences in 2005 as the first next generation system in the market. The specific adapters are used to ligate DNA fragments due to which one fragment binds to a bead. Then, fragment is amplified by emulsion PCR in which bead is in the water droplets and PCR reagents in oil. The sufficient light signal intensity is obtained by the amplification for reliable detection in the sequencing by the use of synthesis reaction steps. After denaturation in the PCR, One amplified fragment with bead is placed at the top of an etched fiber creates in an optical fiber chip that creates from the glass fiber bundles. The individual glass fibers are good light guides and in the other end facing a sensitive CCD camera that make possible emitted light detection. The polymerase enzyme incorporate the base in the growing chain that release a pyrophosphate group, detected as an emitted light. The presence of light signal indicates the next base that is incorporated into the sequence of the growing DNA strand by which the present nucleotide is identified **(Ansorge, 2009).**

SOLiD

Another Next Generation Sequencing platform is SOLiD was developed by Life Technologies in 2006. After the development, it is purchased by Applied

Biosystems. The basic principle of SOLiD platform is 'sequencing by oligonucleotide ligation'. The amplification is done of the sequenced DNA fragment form the prepared library sample and then it is fixed on the surface of each magnetic bead. The pouring of DNA-bound bead copies into the microreactors with the all necessary reagents for PCR is take place and then poured again on the glass slide. Then, the set is transferred on the sequencer that generates 60 gb OF DNA data per run and give the result with 99.94% accuracy **(Tripathi, 2016).**

Helicos Single-molecule sequencing device-Heliscope

It is initiated by the life science that is used to perform the genetic analysis in the drug discovery and diagnosis. This analysis is perform by the use of imaging of individual DNA molecule through the Helicos Genetic Analysis System platform. The output of 1 Gb per day run is done in the sequencing by the HeliScope. To remove the PCR problems such as bias and artifacts, single-molecule sequencing has been developed. ChIP-sequencing, methyl-sequencing, RNA sequencing, small RNA-sequencing and so on are perform with the broad range of applications **(Tripathi, 2016).**

Applications of Next Generation Sequencing

NGS technology and other new methods have many application. Here, the application of NGS is classified according to the experimental purpose.

1. It can built a new genome from unknown organism.
2. The genetic variation of an organism can be measured from the existing reference genome. For example, In case of DNA sequencing, whole genome, whole exome and targeted sequencing are done which are available by the Next Generation Sequencing technologies. These data is compared with the reference genomes.
3. The researcher synthesize complementary DNA from RNA by which sequencing is done. This sequencing help in the analysis of transcriptome results. RNA-Seq is a type of Next Generation Sequencing.
4. The researcher use DNA methylation sequencing and chromatin immunoprecipitation with the ChIP Sequencing for the study of epigenome and regulatory mechanisms of the genome.
5. Microbial ecology scientists investigate the genetic material present in the environment by the help of NGS technologies. The extracted DNA from the environmental samples can be used by scientists without cloning **(Park and Kim, 2016)**.

Review of Literature

Ansorge (2009) stated that the next generation sequencing techniques are very easy and reliable for the genome wide characterization and profiling of mRNAs, small RNA transcription factor regions, Structure of chromatin and DNA methylation patterns. They also discussed about the application of next generation sequencing techniques in the field of bio-medical fields.

According to Lee *et al* (2013) Next Generation Sequencing (NGS) technologies are very effective with high sequencing speed, high resolution and accuracy for the analysis of genomes. These technologies are very helpful in the understanding of unknown species and diseases. According to the features, advantages and no of read length, different platforms are used. They also discussed about the future use of these technologies as there should be improvement in the methods that are used by NGS. In the brief, NGS can disclose the genomic information of human and their function that can give the therapeutic treatments for modified medicine.

Koboldt *et al* (2013) studied the impact of Next Generation Sequencing Technologies on human genetic diseases and concluded that these techniques have an incredible impact on the human genetic disease knowledge. These NGS technologies have many issues as sixe of whole human genome data is very large but the storage in the software is a challenge. They also discussed a future perspective of NGS's applications, will be beneficial into the clinical diagnostic setting.

Kulski (2015) stated that DNA, RNA and methylation sequencing are used by the Next-Generation Technologies that have an impact on life sciences. By the use of NGS, the understanding of structural and functional genomics have been broadened through the concept of 'omics' that have a range from basic genomics to integrated systeomics that define a new meaning of genetic conservation and diversity of living things. Now these days, the science of biological information system and big data are gained by Next Generation System. In spite of these advantages, NGS technologies face many challenges such as NGS data acquisition, storage, analysis, integration, and interpretation.

Park and Kim (2016) discussed the application of NGS technologies in the genomic field. For NGS, many bioinformatics softwares have been developed

that provide more opportunities for researcher in the field of genomes. By the use of NGS technologies, new era of whole genome sequencing has been started that build a genomes databases and give appropriate human reference genomes. These reference genomes are the necessary components of personalized and precision medicine.

According to Cao *et al* (2017) Next generation sequencing (NGS) techniques are able to understand the activities and structure of the microorganisms in the food. In their paper, they discussed about the first generation, second generation and third generation sequencing of bacteria and give the advantages of next generation sequencing over these three generations. NGS can provides genomic data of microorganism with high efficiency and accuracy.

Besser *et al* (2018) discussed about the application of next generation technologies and give a future perspective about the sequencing techniques. The sequencing techniques, bioinformatics and informatics infrastructure are evolving speedily in these days. Due to these evolution can bring a changes in the technologies field by which an effective implementation of sequencing would possible. In these technologies or platforms, there are number of advantages and disadvantages. But these technologies are more efficient in the implementation of metagenomics sequencing of specimens.

Conclusion: Next Generation Sequencing Technologies are the high throughput sequencing technique that have a high speed, efficiency and accuracy compare to other generation (First, Second and Third). This paper shows the conclusion on the basis of review studies that through the use of NGS technologies, whole genome Sequence can be determined in a very less time (within a week). These NGS technologies/ platforms are helpful in the detection of any disease and the preparation of medicine. In spite of many advantages of NGS platforms, the main issue is the storage capacity. The human genome has a very large sequence that create a problem in the downloading and saving. So, there is a need of implementation in the technologies by which the problem of storage can be reduced.

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