Austin Journal of Veterinary Science & Animal Husbandry

Open Access

Austin Publishing Group

Mini Review

Review on Illumina Sequencing Technology

Emiyu K and Lelisa K*
Animal Health Institute, Sebeta, Ethiopia

***Corresponding author:** Kumela Lelisa, Animal Health Institute, PO Box 04, Sebeta, Ethiopia

Received: January 06, 2022; **Accepted:** February 02, 2022; **Published:** February 09, 2022

Abstract

Illumina sequencing process utilizes biochemical methods to determine the correct order of nucleotide bases in a deoxyribonucleic acid. Macromolecule using sequencing-by-synthesis and reversible dye-terminators that enable the identification of single bases are introduced into DNA strands and used to determine the series of base pairs in DNA. It has a very wide range of applications that can be used for whole-genome sequencing, targeted region sequencing, transcriptome analysis, metagenomics, small Ribonucleic acid discovery, methylation profiling, and genome-wide protein-nucleic acid interaction analysis, helping people unlocking the power of the gene.

Keywords: Deoxyribonucleic acid; First generation sequencing technology; Illumina sequencing; Next generation sequencing

Introduction

The application of modern technology in sciences always significant to understand the real nature of nucleic acids and a great innovation was brought by Sanger sequencing [1]. This technology considered as the gold standard nowadays in molecular diagnostics, even though it is expensive and time-consuming [2].

Deoxyribonucleic acid sequencing gradually evolved from low throughput deoxyribonucleic acid fragment sequencing to high throughput next generation (NGS) and third generation sequencing techniques [3].A group of techniques revolutionizing the standard concept of nucleic acids sequencing and great success of NGS technology due to the capability of sequencing millions of DNA reads, with the possibility to perform, at least, multi-gene analysis, by using very low amount of nucleic acids [4].

Next generation sequencing technology is suitable for rapid and efficient sequencing of complex genomes too, with consequent time and cost reduction and also counts on a flexibility which has been reported to be successful in different research fields such as molecular diagnostics of genetic diseases, infectious diseases, cancer and pharmacogenomics [5].

Next generation sequencing technologies continue to improve and the number of sequencers increases and divides These technologies into two types The first is second generation sequencing technologies which refer to the newest sequencing technologies developed in the NGS environment after the first generation and characterized by the need to prepare amplified sequencing banks before starting the sequencing of amplified DNA clones [6].

The second one is third generation sequencing technologies that are sequencing technologies recently appeared [7]. In contrast to the second generation, these technologies are classified as Single Molecule Sequencing Technology because they can make sequencing a single molecule without the necessity to create the amplification libraries and capable of generating longer reads at much lower costs and in a shorter time [8]. Illumina sequencing technology (SOLEXA) is among NGS and described recently [9]. The deoxyribonucleic acid library preparation involves random fragmentation of template DNA and the ligation of oligonucleotide adaptors [10].

The deoxyribonucleic acid amplification strategy involved is referred to as Bridge PCR and the systems have both forward and reverse primers, with complementarity to the adaptor, are attached to a glass surface by a flexible linker where adaptor flanked DNA fragments are hybridized on to the forward and reverse primers attached to the glass surface. Bridge PCR then amplifies the DNA fragment using formamide based denaturation and DNA polymerase, resulting in a “cluster” of clonal amplicons [11].

Amplicons produced from a single DNA fragment will cluster in a single physical location on the array, Following cluster generation, the sequencing primer hybridizes to the universal sequence flanking the region of interest. Sequencing then proceeds in cycles with a modified DNA polymerase and four nucleotides and Nucleotides is labeled with a chemically cleavable fluorescent reporter group at the 3′-OH end thereby allowing only a single base incorporation in each cycle. Each cycle extends a single base followed by the chemical cleavage of the fluorescent reporter that will identify the incorporated nucleotide. The basic challenges of Illumina technology are signal decay and dephasing caused by incomplete fluorescent label cleavage or terminating moieties [12].

First generation sequencing technology like Sanger and Gilbert were drawback. Therefore, with the decreasing cost, time and efficiency of sequencing, illumina sequencing technology is rapidly introduced. Review of illumine sequencing technology is therefore needed for the following objectives:

- The standard concept of nucleic acids sequencing by Illumina sequencing technology.
- The capability of sequencing millions of DNA reads by using very low amount of nucleic by Illumina sequencing technology.

Literature Review

Illumina dye sequencing is a molecular technique used to determine the series of base pairs in DNA and developed by Shankar Balasubramanian and David Klenerman of Cambridge University, who subsequently founded Solexa, a company later acquired by

Emiyu et al. © All rights reserved

Citation: Emiyu K and Lelisa K. Review on Illumina Sequencing Technology. Austin J Vet Sci & Anim Husb. 2022; 9(1): 1088.

Illumina. This sequencing method is based on reversible dye-terminators that enable the identification of single bases as they are introduced into DNA strands. The Illumina next-generation sequencing (NGS) method is based on sequencing-by-synthesis (SBS), and reversible dye-terminators that enable the identification of single bases as they are introduced into DNA strands [13,14].

Workflow of illumina sequencing

Library Preparation: Through ultrasonic fragmentation, the genomic DNA becomes DNA fragment with 200-500base pair in length and the 5' and 3' adapter are added to the two ends of these small segments, "tagmentation" combines the fragmentation and ligation reactions into single step that greatly increases the efficiency of the library preparation process as well as Adapter-ligated fragments are then PCR amplified and gel purified and the sequencing library is constructed [15].

Cluster generation: Flow cell is a channel for adsorbing mobile DNA fragments, and it's also a core sequencing reactor vessel and the DNA fragments in the sequencing library will randomly attach to the lanes on the surface of the flow cell when they pass through it and Each flow cell has 8 Lanes and has a number of adapters attached to the surface, which can match the adapters added at the ends of the DNA fragment in the building process, which is why flow cell can absorb the DNA after the building, and can support the amplification of the bridge PCR on the surface of the DNA and bridge PCR was performed using the adapters on flow cell surface as template, after continuous amplification and mutation cycles, each DNA fragment will eventually be clustered in bundles at their respective locations, each containing many copies of a single DNA template. The purpose of this process is to amplify the signal intensity of the base to meet the signal requirements for sequencing and cluster generation is complete, those templates are ready for sequencing [16].

Sequencing: The sequencing method is based on sequencing-by-synthesis (SBS) in which DNA polymerase, connector primers and 4 dNTP with base-specific fluorescent markers were added to the reaction system and The 3′-OH of these dNTP is protected by chemical methods, which ensures that only one base will be added at a time during the sequencing process as well as All unused free dNTP and DNA polymerase are eluted after the synthesis reaction finished. Then buffer solution needed for fluorescence excitation is added, the fluorescence signal is excited by laser, and fluorescence signal is recorded by optical equipment. Finally, the optical signal is converted into sequencing base by computer analysis when the fluorescence signal is recorded, a chemical reagent is added to quench the fluorescence signal and remove the dNTP 3′-OH protective group, so that the next round of sequencing reactantis performed [17].

Alignment & data analysis: The newly identified sequence reads are aligned to a reference genome, and then many variations of bioinformatics analysis are possible such as SNP/InDel/SV/CNV calling, annotation and statistics, pathway enrichment analysis, population genetics analyzed [18,19].

Characteristics of illumina

Illumina sequencing technology has four basic characteristics as it is second generation sequencing technology [20].

- The generation of many millions of short reads in parallel;
- The speed up of sequencing the process compared to the first generation;
- The low cost of sequencing;
- The sequencing output is directly detected without the need for electrophoresis.

Advantages of illumina sequencing

Sequencing systems deliver the right level of speed, capacity, and cost for various laboratories or sequencing requirements with user-friendly bioinformatics tools that are easily accessible through the web, on instrument, or through on-site servers [21].

Disadvantages of illumina sequencing

One of the main drawbacks of the Illumina/Solexa platform is the high requirement for sample loading control because overloading can result in overlapping clusters and poor sequencing quality which results the overall error rate of this sequencing technology is about 1% [22,23].

Application of illumina sequencing

This sequencing method is based on reversible dye-terminators that enable the identification of single bases as they are introduced into DNA strands and can be used for whole-genome and region sequencing transcriptome analysis, sRNA discovery, methylation profiling, and genome-wide protein–nucleic acid interaction analysis, the innovative and flexible sequencing system enables a broad array of applications in genomics, transcriptomics, and epigenomics [24].

Conclusion

Sequencing technologies have continued to evolve after the appearance of the first sequencers from NGS (illumine sequencing) technologies. These technologies are characterized by their high throughput which gives the opportunity to produce millions of reads with inexpensive sequencing and the technologies are now the starting point for several areas of research based on the study and analysis of biological sequences.

Illumina sequencing technology can be considered as the future of high-throughput data analysis and genomic sequencing, providing a method to obtain high-throughput data with sensitivity and specificity

Therefore, the field of illumina sequencing technology and application development is a fast-moving area of biomedical research and Novel generations of sequencing technologies, such as single-molecule sequencing which holds greater promise to achieve ever-faster, cheaper, more accurate and reliable ways to produce sequence data as well as Shortcomings of today's next-generation sequencing platforms. For instance, short-read and less base accuracy will be overcome with the development of new technologies and indeed, makes this an exciting area for genomic studies.

Challenges in illumine sequencing technologies, the difficulty of storing and analysing the data generated by these technologies. This is mainly due to the production of a high number of reads. So new sequencing platforms will appear producing a larger amount of data which requires the development of new approaches and applications capable of analysing this large amount of data should be needed.

References

1. Sanger F. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA. 1983; 80: 2432-2436.
2. Schadt EE, Turner S, Kasarskis A. A window into third-generation sequencing. Human molecular genetics. 2010; 19: R227-R240.
3. Loman NJ, Pallen MJ. Twenty years of bacterial genome sequencing. Nature Reviews Microbiology. 2015; 13: 787-794.
4. Luo H, Sun C, Sun Y, Wu Q, Li Y, Song J, et al. Analysis of the transcriptome of Panax notoginseng root uncovers putative triterpene saponin-biosynthetic genes and genetic markers. BMC genomics. 2011; 12: S5.
5. Ozreti-ç L, Heukamp LC, Odenthal M, Buettner R. The role of molecular diagnostics in cancer diagnosis and treatment. Oncology Research and Treatment. 2012; 35: 8-12.
6. Thudi M, Li Y, Jackson SA, May GD, Varshney RK. Current state-of-art of sequencing technologies for plant genomics research. Briefings in Functional Genomics. 2012; 11: 3-11.
7. Pareek CS, Smoczynski R, Tretyn A. Sequencing technologies and genome sequencing. Journal of applied genetics. 2011; 52: 413-435.
8. Vezzi F. Next generation sequencing revolution challenges: Search, assemble, and validate genomes. 2012.
9. Turcatti G, Romieu A, Fedurco M, Tairi AP. A new class of cleavable fluorescent nucleotides: synthesis and optimization as reversible terminators for DNA sequencing by synthesis. Nucleic acids research. 2008; 36: e25.
10. Adessi C, Matton G, Ayala G, Turcatti G, Mermod JJ, Mayer P, et al. Solid phase DNA amplification: characterisation of primer attachment and amplification mechanisms. Nucleic acids research. 2000; 28: e87.
11. Fedurco M, Romieu A, Williams S, Lawrence I, Turcatti G. BTA, a novel reagent for DNA attachment on glass and efficient generation of solid-phase amplified DNA colonies. Nucleic acids research. 2006; 34: e22.
12. Jaratlerdsiri W, Chan EK, Petersen DC, Yang C, Croucher PI, Bornman MR, et al. Next generation mapping reveals novel large genomic rearrangements in prostate cancer. Oncotarget. 2017; 8: 23588.
13. Kamalakaran S, Varadan V, Janevski A, Banerjee N, Tuck D, McCombie WR, et al. Translating next generation sequencing to practice: opportunities and necessary steps. Molecular Oncology. 2013; 7: 743-755.
14. Rizzo JM, Buck MJ. Key principles and clinical applications of GÇ£ next-generation GÇ¥ DNA sequencing. Cancer prevention research. 2012; 5: 887-900.
15. Grada A, Weinbrecht K. Next-generation sequencing: methodology and application. The Journal of investigative dermatology. 2013; 133: e11.
16. Mardis ER. Next-generation DNA sequencing methods. Annu Rev Genomics Hum Genet. 2008; 9: 387-402.
17. Kulski JK. Next-generation sequencing: overview of the history, tools, and applications. Next Generation Sequencing GÇô Advances, Applications and Challenges. 2016: 3-60.
18. Claesson MJ, Wang Q, O'Sullivan O, Greene-Diniz R, Cole JR, Ross RP, et al. Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. Nucleic acids research. 2010; 38: e200.
19. Hui P. Next generation sequencing: chemistry, technology and applications. Chemical Diagnostics. Springer. 2012: 1-18.
20. Goodwin S, McPherson JD, McCombie WR. Coming of age: ten years of next-generation sequencing technologies. Nature Reviews Genetics. 2016; 17: 333.
21. Frey KG, Herrera-Galeano JE, Redden CL, Luu TV, Servetas SL, Mateczun AJ, et al. Comparison of three next-generation sequencing platforms for metagenomic sequencing and identification of pathogens in blood. BMC genomics. 2014; 15: 96.
22. Dohm JC, Lottaz C, Borodina T, Himmelbauer H. Substantial biases in ultra-short read data sets from high-throughput DNA sequencing. Nucleic acids research. 2008; 36: e105.
23. Nakamura K, Oshima T, Morimoto T, Ikeda S, Yoshikawa H, Shiwa Y, et al. Sequence-specific error profile of Illumina sequencers. Nucleic acids research. 2011; 39: e90.
24. Meyerson M, Gabriel S, Getz G. Advances in understanding cancer genomes through second-generation sequencing. Nature Reviews Genetics. 2010; 11: 685-696.