Global Veterinaria 22 (1): 09-18, 2020
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2020.09.18

Review on Different Types of Sequencing Technologies And Their Application; Merit and Demerit

Sultan Aman and Aman Yusuf

Jimma University, School of Veterinary Medicine, Jimma, Ethiopia

Abstract: DNA sequencing is the process of determining the nucleic acid sequence to identify the order of nucleotide in DNA. The development of rapid DNA sequencing methods has greatly accelerated biological and medical research and discovery. DNA sequencing technologies could help biologists and health care providers in a broad range of applications such as molecular cloning, breeding, finding pathogenic genes and comparative and evolution studies. Advancements in sequencing were aided by the concurrent development recombinant DNA technology, which allow DNA samples can be taken from different sources other than viruses. Sanger methods were developed by Frederick Sanger and colleagues in 1977; it was the most widely used sequencing method for approximately 40 years. Recently higher volume Sanger sequencing has been replaced by next generation sequencing methods, especially for large-scale, automated genome analyses. NGS technology refers to non-Sanger-based high-throughput DNA sequencing technology and millions or billions of DNA molecules can be sequenced in parallel, thereby increasing the throughput sequencing. Currently, pyrosequencing method is broadly being used in many applications such as Single Nucleotide Polymorphism (SNP) genotyping and identification of bacteria. The development of new methods 'third generation sequencing reduce well known limitations of the second generation sequencing platforms such as high costs, biases resulting from library amplification step and time consuming protocols. Fourth generation sequencing used for multiplex gene expression profiling and analyses of point mutations in breast cancer tissue sections use in situ sequencing. Moreover, although new technologies that will be decreasing of costs continuously in coming years an increase data production levels, an obvious bottleneck of sequencing accuracy for all NGS technologies remains to be solved during this period.

Key words: Sequencing · DNA · Nucleotide and Genome

NTRODUCTION

DNA sequencing is the process of determining the nucleic acid sequence to identify the order of nucleotide in DNA. It includes any technology that is used to determine the order of the four bases: adenine, guanine, cytosine and thymine. The development of rapid DNA sequencing methods has greatly accelerated biological and medical research and discovery. DNA sequencing technologies could help biologists and health care providers in a broad range of applications such as molecular cloning, breeding, finding pathogenic genes and comparative and evolution studies of-reading activity limits accuracy of this method [1]. Today's life sciences based on the interpretation of the relationship between the genome and phenotype from the cellular level to complex biological process like as development or diseases. After the discovery of "Central dogma'' (The relationship between gene and protein), now it's possible to assess the impact of genomic technologies on description of the complexity of a functioning living cell. Before the genomic revolution, use of genome-mapping approaches required probability statistics to identify the gene positions, followed by positional cloning to identify the underlying genes [2].

The first DNA sequences were obtained in the early 1970s by academic researchers using laborious methods based on two-dimensional chromatography. DNA sequencing can be applied to determine the sequence of individual, genes, full chromosomes, or entire genomes of any organism. The completion of full human genome reference sequence allowed next generation

Corresponding Author: Aman Yusuf, Jimma University, School of Veterinary Medicine, Jimma, Ethiopia.

sequencing development (NGS). The discovery of next generation sequencing since 2005, substantially reduced DNA sequencing time and costand remarkably increased data-production capacity, have been introduced by commercial manufacturers [3]. Therefore, genomic technologies were used mainly in the first instance for genome sequencing and increasing efforts involved improvements in molecular genetic technology such as gene cloning, genomic library construction and DNA sequencing. It also used for diagnosis of mutated DNA sequences and different diseases including various cancers, characterize antibody repertoire as well as to guide patient treatment [4].

Maxam-gilbert Sequenceing: Walter Gilbert and Allan Maxam at Harvard developed sequencing methods of "DNA sequencing by chemical degradation. In 1973, Gilbert and Maxam reported the sequence of 24 base pairs using a method known as wandering-spot analysis. Advancements in sequencing were aided by the concurrent development recombinant DNA technology, which allow DNA samples can be taken from different sources other than viruses. They developed a method for sequencing single-stranded DNA by taking advantage of a two-step catalytic process involving piperidine and two chemicals that selectively attack purines and pyrimidines [1]. Moreover, dimethyl sulfate and piperidine alone will selectively cleave guanine nucleotides but dimethyl sulfate and piperidine in formic acid will cleave both guanine and use of these selective reactions to DNA sequencing then involved creating a single stranded DNA substrate carrying a radioactive label on the 5' end. In this case the labeled substrate would be subjected to four separate cleavage reactions, each of which would create different types of labeled cleavage products ending in known nucleotides. The reactions loaded on high percentage polyacrylamide gels and the fragments resolved by electrophoresis and electrophoresis, are whether in an adenine nucleotides. The process of base calling would involve interpreting the banding pattern relative to the four chemical reactions [5].

Sanger Sequencing: Developed by Frederick and colleagues in 1977, it was the most widely used sequencing method for approximately 40 years. Rather than using chemical cleavage reactions, Sanger choose for a method involving a third form of the ribose sugars. This method was based on the activity of DNA polymerase I which able to produce a complementary strand starting from a primer sequencing in presence of deoxynucleotide triphosphates (dNTPs) using 32P labeling for one of them [6]. Under normal condition ribose has a hydroxyl group on both the 2' and the 3' carbons whereas deoxyribose has only the one hydroxyl group on the 3' carbon. Other form of ribose the third form of ribose in which the hydroxyl group is missing from both the 2' and the 3' carbons. In this reaction whenever a dideoxynucleotide was incorporated into a polynucleotide, the chain would irreversibly stop, or terminate. The procedure have four separate reactions, each incorporating a different dideoxynucleotide along with the four deoxynucleotides, would produce a population of fragments which all ending with the same dideoxynucleotide in the presence of a DNA polymerase if the ratio of the dideoxynucleotide and the corresponding deoxynucleotide was properly set [7]. Recently higher volume Sanger sequencing has been replaced by next generation sequencing methods, especially for large-scale, automated genome analyses. The Sanger chain-termination method requires a single-stranded DNA template, DNA prime, a DNA polymerase, normal deoxynucleotide triphosphates (dNTPs) and modified dideoxynucleotidetriphosphates (ddNTPs), the latter of which terminate DNA strand elongation. These chain termination nucleotides lack a3'-OH group required for the formation of a phosphodiester bond between two nucleotides, causing DNA polymerase to cease extension of DNA when a modified ddNTP is incorporated. The ddNTPs are labeled radioactively or fluorescently for detection in automated sequencing machines [8]. The DNA sample is divided into four separate sequencing reactions, containing all four of the standard deoxynucleotides (dATP, dGTP, dCTP and dTTP) and the DNA polymerase and then each reaction is added only one of the four dideoxynucleotides, the other reaction added with normal nucleotides. The band formation is frequently performed using a denaturing polyacrylamide gel -urea with each of the four reactions run in one of four individual lanes (Lanes A, T, Gand C). The DNA bands may then be visualized by autoradiography or UV light and the DNA sequence can be directly read off the X-ray film or gel image. Although it was more efficient compared to other methods, original Sanger method was also time consuming and carrying a risk by cause of the radioisotopes used for labeling [5].

Chemiluminescent Methods of Sequencing: A decade after development of Sanger method, chemiluminescent labeling was developed as a new method instead of radioactive isotopes bringing the advantage of

sequencing PCR products. In this method, an oligonucleotide sequence bound with alkaline phosphates in 5'end with a streptavidine conjugate and starts a chemical reaction that causes emission of photons which are detected on a photographic film. Other advantages of this approach include no requirement of cloning before sequencing, capability of conducting multiple reactions in one lane and performing sequence reading by using specific primers for each reaction [9]. Hence, all reactions could be loaded to one lane as a post-sequencing process and checked through gel. Development of polymerase chain reaction can be mentioned as a significant advance to simplify sample preparation. These automated DNA sequencers were successfully employed in the human genome project and reduced both costs and time need to complete the project. Total sequencing costs have decreased continuously in correlation with new advances and applications to the original method [10].

Dye-terminator Sequencing: Dye-terminator sequencing utilizes labelling of the chain terminator ddNTPs, which permits sequencing in a single reaction, rather than four reactions as in the labelled-primer method. In these methods, each of the four dideoxynucleotide chain terminators is labelled with fluorescent dyes, each of which emits light at different Wavelengths. According to the information of ddNTP which is employed to terminate the reaction, sequencing products are discriminated on a gel and results are analyzed to determine the complete DNA sequence. Its limitations include dye effects due to differences in the incorporation of the dye-labelled chain terminators into the DNA fragment, resulting in unequal peak heights and shapes in the electronic DNA sequence trace chromatogram after capillary electrophoresis [11].

Automated Fluorescence Sequencing: The most important and advance in sequencing and the one that carried DNA sequencing into a high throughput environment was the introduction of automated sequencing using fluorescence-labeled dideoxy-terminators. In this method, the primer was labeled with one of four different fluorescent dyes and each was placed in a separate sequencing reaction with one of the four dideoxynucleotides with all four deoxynucleotides. After the reactions were complete, the four reactions were pooled and run together in one lane of a polyacrylamide sequencing gel. Four-color laser induced fluorescence detector scanned the gel as the reaction fragments migrated past. The fluorescence signature of each fragment was then sent to a computer where the software was trained to perform base calling [12]. Fragment resolution was improved by substituting deoxyInosine triphosphate (dITP) for dGTP and deoxyUridine triphosphate (dUTP) for dTTP. The former helped eliminate band compression on the gels and the latter helped with ddTP incorporation in the sequencing reactions [9].

Next-Generation Sequencing Technique: NGS technology refers to non-Sanger-based high-throughput DNA sequencing technology. Millions or billions of DNA molecules can be sequenced in parallel, thereby increasing the throughput sequencing. Consequently; the entire genome could be sequenced in less than 1 day. These have drastically increased the number of bases obtained per sequencing run while at the same time decreasing the costs per base. This advance was possible due to a concomitant development of software that allows the de novo assembly of draft genomes from large numbers of short reads [13]. Furthermore, NGS technologies have quickly been adopted for other high-throughput studies that were previously performed mostly by hybridization-based methods like microarrays. All commercially available NGS technologies differ from automated Sanger sequencing in that they do not require cloning of template DNA into bacterial vectors. Many NGS platforms have been developed with low-cost and high-throughput sequencing [14]. NGS technology includes second-generation sequencing technology as well as third-generation sequencing technology. The first NGS technology that became available was the Roche/454 genome sequencer (454 Life Sciences, Branford, CT). The amplification is necessary to obtain sufficient light signal intensity for reliable detection in the sequencing-by-synthesis reaction steps [12].

Second Generation Sequencing: These technologies provide a significant time saving because of highly streamlined sample preparation before sequencing, with a minimal need for associated equipment in comparison with the highly automated and multistep pipelines necessary for clone-based sequencing. This is because the presence of adapter sequences that help the molecules to be selectively amplified by polymerase chain reaction [15]. All next generation sequencing systems, without any exception, have risen on revolutionary idea of shotgun sequencing which perfectly fit with the goals of Human Genome Project (HGP), development of cheaper

and faster sequencing technologies. So far proposed second generation sequencing systems are based on either "Sequencing by synthesis" or "Sequencing by ligation" [16].

Pyrosequencing: Pyrosequencing technology is a novel DNA sequencing technology, developed at the Royal Institute of Technology (KTH)and is the first alternative to the conventional Sanger method for de novo DNA sequencing. This method relies on the luminometric detection of pyrophosphate that is released during primer-directed DNA polymerase catalyzed nucleotide incorporation. It is important for DNA sequencing of up to one hundred bases and it offers a number of unique advantages. Poyrosequencing is a widely applicable, alternative approach for the detailed characterization of nucleic acids [17].

Pyrosequencing Chemistry: Pyrosequencing technique is based on sequencing by-synthesis principle [16, 18] and on the detection of released pyrophosphate (PPi) during DNA synthesis [19]. It involves a series of four enzymes to accurately detect nucleic acid sequences during the synthesis. In Pyrosequencing [17] the sequencing primer is hybridized to a single-stranded DNA biotin-labeled template and mixed with the enzymes; DNA polymerase, ATP sulfurylase, luciferase and apyrase and the substrates adenosine 5' phosphosulfate (APS) and luciferin. Cycles of four deoxynucleotide triphosphates (dNTPs) are separately added to the reaction mixture alternatively. The cycle starts with a nucleic acid polymerization reaction and each nucleotide incorporation event is followed by release of inorganic pyrophosphate (PPi) in a quantity equimolar to the amount of incorporated nucleotide. Subsequently the released PPi is quantitatively converted to ATP by ATP sulfurylase in the presence of APS [20]. The generated ATP drives the luciferase-mediated conversion of luciferin to oxyluciferin, producing visible light in amounts that are proportional to the amount of ATPs and the light produced then detected by a photon detection device such as a charge coupled device (CCD) camera or photomultiplier. Apyrase is a nucleotide-degrading enzyme, which continuously degrades ATP and non-incorporated dNTPs in the reaction mixture. Because the added nucleotide is known, the sequence of the template can be determined. During this synthesis process, the DNA strand is extended by complementary nucleotides and the DNA sequence is demonstrated by the pyrogram on a screen [21].

Applications of Pyrosequencing: Pyrosequencing has opened up new possibilities for performing sequence-based DNA analysis [21]. Pyrosequencing is well suited for de novo sequencing and resequencing. Currently, pyrosequencing method is broadly being used in many applications such as Single Nucleotide Polymorphism (SNP) genotyping and identification of bacteria [18, 20, 22]. Moreover, the method has demonstrated the ability to determine difficult secondary structures of nucleic acids and perform mutation detection [21, 23]. DNA methylation analysis, multiplex sequencing, tag sequencing of cDNA library and clone checking [24]. Another highly significant application is whole genome sequencing [21].

Advantages of Pyrosequencing: Pyrosequencing has emerged as an alternative method of sequencing. Although it has read-length limitations compared with di-deoxy sequencing, it is a fast method with real-time read-out that is highly suitable for sequencing short stretches of DNA. Pyrosequencing employs many jointly working enzymes to monitor DNA synthesis. Parameters such as stability, fidelity, specificity and sensitivity are mandatory for the optimal performance of the enzymes used in the sequencing reaction [22]. Unlike Sanger sequencing, which lays a reading gap of roughly 20-30 bases from the sequencing primer, pyrosequencing can generate sequence signals immediately downstream of the primer. Due to sequencing starts with the first base next to the annealed primer, making primer design becomes more flexible in this method. Sample and single-strand DNA preparation process is also relatively rapid (About 15 min). Pyrosequencing has potential advantages of accuracy, flexibility, parallel processingand can be easily automated. Furthermore, the technique avoids the need for labeled primers, labeled nucleotides and gel electrophoresis. Pyrosequencing has been successful for both confirmatory sequencing and de novo sequencing [21].

Disadvantage of Pyrosequecing: Currently, a limitation of this method is that the lengths of individual reads of DNA sequence are in the neighborhood of 300-500 nucleotides, shorter than the 800-1000 obtainable with chain termination methods in Sanger sequencing. This can make the process of genome assembly more difficult, particularly for sequences containing a large amount of repetitive DNA. Other disadvantage of pyrosequencing is lack of proof-reading activity limits accuracy of this method [25].

Applications of NGS Technologies: NGS technologies are currently used for whole genome sequencing, investigation of genome diversity, metagenomics, epigenetics, discovery of non-coding RNAs and protein-binding sites and gene-expression profiling by RNA sequencing [26]. It allows the rapid identification of causal mutations at single-nucleotide resolution even in complex cases [27]. It could provide a deeper understanding of microbial basic biology, taxonomy, evolution and their roles in environmental ecosystems and human health [28]. It opened the door in front of new areas of biology, including the ancient genomes investigation, identification of unknown etiological agent and ecological diversity characterization. It could help to detect genome-wide patterns of methylation "Epigenomic variation" and how these patterns change through the course of an organism's development, in the context of disease and other influences [29].

The most needed application of NGS is the ability to rapidly read out the results with potential of making a combination with other experiment results such as in correlative analyses of genome-wide methylation, histone binding patterns and gene expression. Deep sequencing by NGS techniques is being increasingly applicable in the clinical practice to detect low abundance drug resistant HIV variants and, with the recent availability of new drugs active against hepatitis C virus (HCV), also for the detection of HCV minor variants [30].

Third Generation Sequencing: The development of new methods 'third generation sequencing reduce well known limitations of the second generation sequencing platforms such as high costs, biases resulting from library amplification step and time consuming protocols. Third-generation sequencing has two main characteristics. The first on is PCR is not needed before sequencing, which shortens DNA preparation time for sequencing. Second, the signal is captured in real time, which means that the signal, no matter whether it is fluorescent (Pacbio) or electric current (Nanopore), is monitored during the enzymatic reaction of adding nucleotide in the complementary strand. The main difference between second and third generation sequencing technologies is that third generation sequencing systems are mostly based on direct detection of nucleotide composition of target DNA molecules without any amplification [31].

Single Molecule Fluorescent Sequencing: The virtual terminators for third generation sequencing technologies have been introduced [31]. The general features of virtual terminators are consisted of 3' free hydroxyl group which makes interaction with DNA polymerase possible and a fluorescent dye bounded to the removable linker group. Virtual terminators are employed in HeliScope sequencing platform developed by Helicos Bioscience Company in single molecule fluorescent sequencing [32]. In this sequencing technique the first step is the fragmentation of DNA sequences to suitable lengths for sequencing platform. This step make the generation of a poly A tail at the 3' end of each fragment by an enzyme called terminal transferase, prepared libraries are bound to the primer sequences containing only dTTP nucleotides and attached to a solid surface. Every nucleotide analogue added to template DNA by polymerase enzyme blocks the sequencing reaction and imaging process is conducted using CCD cameras and a confocal microscope after removal of fluorescent dye and blocking group. Thus, it is an advantageous alternative for especially RNA Sequencing applications which tend to be affected by PCR biases. Disadvantage of this platform is relative shortness of sequencing reads [33].

Single Molecule Real Time Sequencing: Single molecule real time sequencing (SMRT) relies on sequencing by synthesis approach and real time detection of incorporated fluorescently labeled nucleotides [34]. Single molecule real time sequencing technology does not require any library preparation step since single DNA molecules are read in this system [35]. At first, DNA samples are fragmented and tagged with adapter and tagged DNA molecules are directly loaded to the sequencing surface and then immobilized DNA polymerase adds appropriate nucleotide to template DNA. Fluorescent dye linked to phosphate group is removed naturally and imaging process of single molecules can be performed by use of a technology called zero mode waveguide (ZMW), a light focusing dense array reducing signal noise background [36]. SMRTS are important to increase in read lengths caused notable enhancements in de novo sequencing studies since short reads bring mistake in the assembly of DNA regions including repeats and GC rich regions [37]. One of the main advantages of SMRT technology is that base modification status and RNA based researches can be performed by using this method to get unbiased, higher quality results. Also, SMRT technology solves drawbacks of assembly process of de novo sequenced genomes by providing much longer reads which make it possible to create scaffolds in repeat regions. On the other hand, high error rate is an undeniable limitation for SMRT technology. SMRT technology has 5% error rate which especially

includes insertion and deletion mistakes thus causing errors in resequencing and de novo assembly processes [38].

Semiconductor Sequencing: Semiconductor sequencing is based on measurement of proton release during nucleotide incorporation by sequencing by synthesis. Direct measurement of pH changes in the microenvironment eliminates the time consuming imaging step by a special camera. Although it includes amplification step before sequencing, due to its unique and new sequencing methodology this technology is classified as third generation sequencing technology [39]. Sample prepared by fragmentation of DNA molecules, blunt ended and tagged with adapter sequences. Ion Torrent technology uses unmodified nucleotides then, detection of specific nucleotide is performed by adding nucleotides in a certain order. Accordingly in this approach, the sequencing and "Base calling" processes are finished in shorter time but specific errors in homopolymeric regions are common due to saturation of pH detector which causes misinterpretation of signals produced by >4 base homopolymers [33]. Currently Ion Torrent platforms can be used mainly for targeted, exome, transcriptome, de novo, small RNA sequencing, viral and bacterial typing studies. The increased output of these systems makes them convenient for applications like exome or whole genome sequencing. However, high error rates for specific regions are still an important obstacle for Ion Torrent technology [40].

Nanopore Sequencing: Nanopore sequencing is a new technology that basically depends on DNA sequence translocation through nanometer size pores by applying an electric field and measuring physical changes [41]. The basic idea of this technology was that translocation of DNA molecules through α-hemolysin nanopore was accomplished and the possibility of sequencing DNA or RNA molecules due to characteristic changes during this process indicated [42]. Moreover, the stability and geometry of the pore, the speed of procedure and the features of the signal detection system determine the efficiency of nanopore sequencing based platforms [43]. In nanopore sequencing, the speed of translocation of molecules through nanopores and detection of signals during this movement has been optimized by various modifications and the following modification are made; exonuclease assisted nanopore sequencing, hybridization based sequencing, sequencing by expansion, Nano-Tag SBS sequencing can be mentioned as improved methods mostly to address drawbacks of signal detection. The specific modifications in the analyses of data produced by this platform could increase performance of sequencing [44].

Application of Third Generation Sequencing: With the advent of high-throughput DNA sequencing platforms, there has been a reduction in the cost and time of sequencing. TGSTs provide to increase throughput and read lengths, decrease costs, run times and error rates, eliminate biases inherent in SGST approaches, require minimal input material and offer capabilities beyond nucleic acid sequencing. Such changes will have positive impact in all applications of sequencing technology to drug discovery [43]. Epigenetic markers are stable and potentially heritable modifications to the DNA molecule that are not in its sequence. DNA methylation at CpG sites, which has been found to influence gene expression, is one of modification. The current generation of sequencing technologies relies on laboratory techniques such as ChIP sequencing for the detection of epigenetic markers. Third generation sequencing may enable direct detection of these markers due to their distinctive signal from the other four nucleotide bases [45]. With their single molecule sequencing capabilities and low input quantity requirements, TGSTs may provide a direct, unbiased and economical solution for single cell measurements of DNA and RNA molecules and enable the studies of rare cell populations of therapeutic value, such as circulating tumor cells [46].

TGSTs may also be useful in oligonucleotide -based drug discovery efforts. Certain repeat regions in the genomes cannot be analyzed with SGSTs due to read length and sequencing bias limitations. Repeat element in genome role in cause disease and their value as biomarkers increases, TGSTs can be the much needed tool to studies focusing on the role of repeat elements in normal physiology and disease. TGSTs may also be important for the study of mechanism of action of antibiotic, antibiotic effects to patients and antibiotic resistance through microbial whole genome sequencing studies [46].

Advantages of Third Generation Sequencing: By making long reads lengths possible, third generation sequencing technologies have clear advantages. The main advantage for third-generation sequencing technologies in mutagenomics is their speed of sequencing in comparison to second generation techniques. Speed of sequencing is important for the disease diagnosis to allow for efficient and timely clinical actions. The relatively long reads

allowed for sequencing of a near-complete viral genome to high accuracy directly from a primary clinical sample [47]. In addition, since the sequencing process is not parallelized across regions of the genome, data could be collected and analyzed in real time. These advantages of third generation sequencing may be well-suited in hospital settings where quick and on-site data collection and analysis is demanded. Considering its potential and distinct advantages, third generation sequencing technology which can be used for DNA, RNA or protein analyses, is expected to be more widely used and have broad application spectrum [48].

Disadvantage of Third Generation Sequencing: Third generation sequencing, as it currently stands, faces important challenges mainly surrounding accurate identification of nucleotide bases; error rates are still much higher compared to second generation sequencing. This disadvantage is generally due to instability of the molecular machinery involved and because of the process happens quickly, the signals given off by individual bases may be blurred by signals from neighboring bases [46]. The high error rates involved with third generation sequencing are unavoidable problem for the purpose of characterizing individual differences that exist between members of the same species. Furthermore, performing accurate species identification for bacteria, fungi and parasite is very difficult, as they share a larger portion of the genome and some only differ by <5%. Even though the use of proteins in biological nanopore sequencing systems, have the various benefits, also brings with it some negative characteristics [49].

Fourth Generation Sequencing: Fourth generation sequencing systems has made in situ sequencing possible in fixed tissue and cells by use of second generation sequencing technologies [33]. Fourth generation sequencing used for multiplex gene expression profiling and analyses of point mutations in breast cancer tissue sections use in situ sequencing. In this method which can be used in many different cell types for production of the amplicons which bind cellular proteins through covalent bonds, target mRNA molecules were sequenced accurately by solid technology based sequencing method. In these methods, the amount of RNA in the cell can cause intensity problems for sequencing process since it depends on differentiation of two different spots on a layer. This important point determines the physical limitation for this application [46]. Fourth generation sequencing approach provided up to 400 reads per reading cell which makes it possible to determine expression of thousands of genes in the cell simultaneously for different types of RNA molecules including mRNA, non-coding RNA, rRNA and anti-sense RNA Particularly, the methodology carries advantages for the applications by which used for analysis of cell populations with single cell resolution and can provide important benefits. When compared with single cell sequencing, in situ sequencing makes screening whole cell population with single cell resolution possible and provides more detailed results owing to this profiling approach. This method also improve, the problems about standardization, cost effectiveness, practicality and full integration to current sequencing systems and this increase efficiency of these methods usage [49]. Moreover many applications of fourth generation are developed recently. Quantum Biosystems uses an approach that combines nanopore sequencing with tunneling electron detector. On the other hand, Base4 employs a chemical cascade reaction for detection of single nucleotides cleaved and separated by water-oil emulsion. Moreover, although new technologies that will be decreasing of costs continuously in coming years and increase data production levels, an obvious bottleneck of sequencing accuracy for all NGS technologies remains to be solved during this period [48].

CONCLUSION AND RECOMMENDATIONS

DNA sequencing field has been witnessed many revolutionary advances in last 40 years. The dideoxy method developed by Sanger and his collequaes can be seen as a beacon for incredible change in genomics field. Then, the announcement of completion of human genome project opened an era of high-throughput and fast sequencing platforms which are called next generation sequencing technologies. The Following third generation sequencing methods, single molecule fluorescent sequencing, single molecule real time sequencing, semiconductor sequencing have provided new opportunities to the users.

After few time of third generation sequencing, the fourth generation sequencing has become known as a new and very specific application field which depends on in situ sequencing in fixed cells and tissues and is expected to provide major contributions in key areas. Even though currently second generation sequencing technologies highly used in genomics field, third and fourth generation platforms carry a potential for accurate and practical solutions with a broader application spectrum.

Today, DNA sequencing field is very close to the gold standards stated by (National human genome research institute (NHGRI) 10 years ago but there are still important issues waiting to be solved. Moreover, although new technologies that will be decreasing of costs continuously in coming years and increase data production levels, an obvious bottleneck of sequencing accuracy for all NGS technologies remains to be solved during this period.

Based on the above conclusion the following recommendation was forwarded

- ✓ Even though there is increasing of development of different methods of DNA sequencing still now there are some errors and accuracy problem, so to eliminate this limitation improvement on the DNA sequencing methods will be done and other methods of sequencing will be develop with full laboratory equipment.
- ✓ The DNA sequencing laboratory will be well equipped.
- ✓ In Ethiopia there is no DNA sequencing facility and laboratory, therefore the government will be construct DNA sequencing laboratory with full equipment.

REFERENCES

1. Maxam, A.M. and W. Gilbert, 1977. A new method for sequencing DNA. Proceedings of the National Academy of Sciences, 74(2): 560-564.
2. Breathnach, R., C.K.O.H. Benoist, K. O'hare, F. Gannon and P. Chambon, 1978. Ovalbumin gene: evidence for a leader sequence in mRNA and DNA sequences at the exon-intron boundaries. Proceedings of the National Academy of Sciences, 75(10): 4853-4857.
3. Zhang, J.Z., Y. Fang, Y. Joan, Hong, Ji. RenRong. Jiang, Pieter Roos, Norman and J. Dovichi, 1995. Use of non-cross-linked polyacrylamide for four-color DNA sequencing by capillary electrophoresis separation of fragments up to 640 bases in length in two hours. Analytical Chemistry, 67(24): 4589-4593.
4. Jeffreys, A.J. and R.A. Flavell, 1977. The rabbit β-globin gene contains a large insert in the coding sequence. Cell, 12(4): 1097-1108.
5. Breathnach, R., J.L. Mandel and P. Chambon, 1977. Ovalbumin gene is split in chicken DNA. Nature, 270(5635): 314-319.
6. Sanger, F., S. Nicklen and A.R. Coulson, 1977. DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences, 74(12): 5463-5467.
7. Dekker, C., 2007. Solid-state nanopores. Nature Nanotechnology, 2(4): 209.
8. Schloss, P.D., M.L. Jenior, C.C. Koumpouras, S.L. Westcott and S.K. Highlander, 2016. Sequencing 16S rRNA gene fragments using the PacBio SMRT DNA sequencing System. Peer J, 4: e1869.
9. Smith, L.M., J.Z. Sanders, R.J. Kaiser, P. Hughes, C. Dodd, C.R. Connell, C. Heiner, S.B. Kent and L.E. Hood, 1986. Fluorescence detection in automated DNA sequence analysis. Nature, 321(6071): 674-679.
10. MacConaill, L. and M. Meyerson, 2008. Adding pathogens by genomic subtraction. Nature Genetics, 40(4): 380-382.
11. Metzker, M.L., 2005. Emerging technologies in DNA sequencing. Genome Research, 15(12): 1767-1776.
12. Dovichi, N.J. and J. Zhang, 2000. How capillary electrophoresis sequenced the human genome. Angewandte Chemie International Edition, 39(24): 4463-4468.
13. Tang, P. and C. Chiu, 2010. Metagenomics for the discovery of novel human viruses. Future Microbiology, 5(2): 177-189.
14. Mardis, E.R., 2008. Next-generation DNA sequencing methods. Annu. Rev. Genomics Hum. Genet, 9: 387-402.
15. Nyren, P. and A.B. Biotage, 2001. Method of sequencing DNA based on the detection of the release of pyrophosphate and enzymatic nucleotide degradation. U.S. Patent, 6: 258-568.
16. Nordström, T., M. Ronaghi, L. Forsberg, U. De Faire, R. Morgenstern and P. Nyrén, 2000. Direct analysis of single-nucleotide polymorphism on double-stranded DNA by pyrosequencing. Biotechnology and Applied Biochemistry, 31(2): 107-112.
17. Shendure, J. and H. Ji, 2008. Next-generation DNA sequencing. Nature Biotechnology, 26(10): 1135.
18. Gharizadeh, B., M. Ghaderi, D. Donnelly, B. Amini, K.L. Wallin and P. Nyrén, 2003. Multiple-primer DNA sequencing method. Electrophoresis, 24(7-8): 1145-1151.
19. Hyman, E.D., 1988. A new method of sequencing DNA. Analytical Biochemistry, 174(2): 423-436.
20. Swerdlow, H., S. Wu, H. Harke and N.J. Dovichi, 1990. Capillary gel electrophoresis for DNA sequencing: laser-induced fluorescence detection with the sheath flow cuvette. Journal of Chromatography A, 516(1): 61-67.

21. Ronaghi, M., 2001. Pyrosequencing sheds light on DNA sequencing. Genome Research, 11(1): 3-11.
22. Gharizadeh, B., M. Ghaderi and P. Nyrén, 2007. Pyrosequencing technology for short DNA sequencing and Whole Genome Sequencing, 47(2): 129-132.
23. Swerdlow, H., J.Z. Zhang, D.Y. Chen, H.R. Harke, R. Grey, S. Wu, N.J. Dovichi and C. Fuller, 1991. Three DNA sequencing methods using capillary gel electrophoresis and laser-induced fluorescence. Analytical Chemistry, 63(24): 2835-2841.
24. Ahmadian, A., J. Lundeberg, P. Nyrén, M. Uhlén and M. Ronaghi, 2000. Analysis of the pp: 53 tumor suppressor gene by pyrosequencing. Biotechniques, 28(1): 140-147.
25. Margulies, M., M. Egholm, W.E. Altman, S. Attiya, J.S. Bader, L.A. Bemben, J. Berka, M.S. Braverman, Y.J. Chen, Z. Chen and S.B. Dewell, 2005. Genome sequencing in microfabricated high-density picolitre reactors. Nature, 437(7057): 376-380.
26. Nordström, T., B. Gharizadeh, N. Pourmand, P. Nyren and M. Ronaghi, 2001. Method enabling fast partial sequencing of cDNA clones. Analytical Biochemistry, 292(2): 266-271.
27. Ansorge, W.J., 2009. Next-generation DNA sequencing techniques. New Biotechnology, 25(4): 195-203.
28. Schneeberger, K., 2014. Using next-generation sequencing to isolate mutant genes from forward genetic screens. Nature Reviews Genetics, 15(10): 662-676.
29. MacLean, D., J.D. Jones and D.J. Studholme, 2009. Application of'next-generation'sequencing technologies to microbial genetics. Nature Reviews Microbiology, 7(4): 96-97.
30. Mardis, E.R., 2013. Next-generation sequencing platforms. Annual review of Analytical Chemistry, 6: 287-303.
31. Palacios, G., J. Druce, L. Du, T. Tran, C. Birch, T. Briese, S. Conlan, P.L. Quan, J. Hui, J. Marshall and J.F. Simons, 2008. A new arena virus in a cluster of fatal transplant-associated diseases. New England journal of medicine, 358(10): 991-998. Sequencers. BMC genomics, 13(1): 341.
32. Bowers, J., J. Mitchell, E. Beer, P.R. Buzby, M. Causey, J.W. Efcavitch, M. Jarosz, E. Krzymanska-Olejnik, L. Kung, D. Lipson and G.M. Lowman, 2009. Virtual terminator nucleotides for next-generation DNA sequencing. Nature methods, 6(8): 593.
33. Kumar, S., A. Sood, J. Wegener, P.J. Finn, S. Nampalli, J.R. Nelson, A. Sekher, P. Mitsis, J. Macklin and C.W. Fuller, 2005. Terminal phosphate labeled nucleotides: synthesis, applications and linker effect on incorporation by DNA polymerases. Nucleosides, Nucleotides and Nucleic Acids, 24(5-7): 401-408.
34. Korlach, J., K.P. Bjornson, B.P. Chaudhuri, R.L. Cicero, B.A. Flusberg, J.J. Grey, D. Holden, R. Saxena, J. Wegener and S.W. Turner, 2010. Real- time DNA sequencing from single polymerase molecules. Methods Enzymol, 472: 431-455.
35. Thompson, J.F. and K.E. Steinmann, 2010. Single molecule sequencing with a HeliScope genetic analysis system. Current Protocols in Molecular Biology, 92(1): 7-10.
36. Yang, Y., B. Xie and J. Yan, 2014. Application of next-generation sequencing technology in forensic science. Genomics Proteomics Bioinformatics, 12: 190-197.
37. Hui, P., 2012. Next generation sequencing: chemistry, technology and applications. In Chemical Diagnostics (pp. 1-18). Springer, Berlin, Heidelberg.
38. Bahassi, E.M. and P.J.Stambrook, 2014. Next-generation sequencing technologies: breaking the Sound Barrier of Human Genetics. Mutagenesis, 29(5): 303-310.
39. Roberts, R.J., M.O. Carneiro and M.C. Schatz, 2013. The advantages of SMRT sequencing. Genome Biology, 14(7): 405.
40. Srinivasan, S. and J. Batra, 2014. Four generations of sequencing: Is it ready for the clinic yet? Journal of Next Generation Sequencing & Applications, 1(107).
41. Morey, M., A. Fernández-Marmiesse, D. Castineiras, J.M. Fraga, M.L. Couce and J.A. Cocho, 2013. A glimpse into past, present and future DNA sequencing. Molecular Genetics and Metabolism, 110(1-2): 3-24.
42. Rusk, N., 2014. Genomics: nanopores read long genomic DNA. Nature Methods, 11(9): 887.
43. Wang, Y., Q. Yang and Z. Wang, 2015. The evolution of nanopore sequencing. Frontiers in Genetics, 5: 449.
44. Jain, M., I.T. Fiddes, K.H. Miga, H.E. Olsen, B. Paten and M. Akeson, 2015. Improved data analysis for the MinIONnanopore sequencer. Nature Methods, 12(4): 351-356.
45. Hall, J., P. Dennler, S. Haller, A. Pratsinis, K. Sauberli, H. Towbin, K. Walthe and J. Woytschak, 2010. Genomics drugs in clinical trials. Nature Reviews Drug Discovery, 9(12).

46. Lee, J.H., E.R. Daugharthy, J. Scheiman, R. Kalhor, J.L. Yang, T.C. Ferrante, R. Terry, S.S. Jeanty, C. Li, R. Amamoto and D.T. Peters, 2014. Highly multiplexed subcellular RNA sequencing in situ. Science, 343(6177): 1360-1363.
47. Chin, C.S., D.H. Alexander, P. Marks and A. Klammer, 2013a, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J: Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods, 10: 563-9.
48. Mignardi, M. and M. Nilsson, 2014. Fourth-generation sequencing in the cell and the clinic. Genome Medicine, 6(4): 31.
49. Ke, R., M. Mignardi, A. Pacureanu, J. Svedlund, J. Botling, C. Wählby and M. Nilsson, 2013. In situ sequencing for RNA analysis in preserved tissue and cells. Nature Methods, 10(9): 857.