ISSN 2218-6263 (online)

World Journal of *Stomatology*

World J Stomatol 2018 November 26; 6(2): 6-18

Published by Baishideng Publishing Group Inc

World Journal of Stomatology

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World Journal of Stomatology

Volume 6 Number 2 November 26, 2018

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World Journal of Stomatology is now abstracted and indexed in China National Knowledge Infrastructure (CNKI), and Superstar Journals Database.

NAME OF JOURNAL
World Journal of Stomatology

ISSN
ISSN 2218-6263 (online)

LAUNCH DATE
December 31, 2011

FREQUENCY
Continuous

EDITORIAL OFFICE
Ya-Juan Ma, Director

PUBLICATION DATE
November 26, 2018

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World J Stomatol 2018 November 26; 6(2): 6-10

DOI: 10.5321/wjs.v6.i2.6

ISSN 2218-6263 (online)

EDITORIAL

Next generation sequencing in oral disease diagnostics

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Author contributions: Gokul S formulated the idea of writing the editorial; Sapna G and Gokul S together performed literature review, collected relevant data and prepared the manuscript; Sapna G also contributed in proof reading the manuscript.

Conflict-of-interest statement: The authors have no conflict of interest to declare.

Manuscript source: Invited manuscript

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Received: July 23, 2018
Peer-review started: July 23, 2018

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Abstract

DNA sequencing is the method of identifying the precise order of DNA nucleotides within a molecule. The information of DNA sequencing is of prime requisite for basic biological research as well as in various clinical specialties. They can be used to determine the individual genetic sequence, larger genetic regions, chromosomes as well as to sequence RNA and proteins. Since the first DNA sequencing in 1970s, there has been tremendous advancements in the technologies aimed to determine the entire human genome. The need for rapid and accurate sequencing of human genome has resulted in the introduction of next generation sequencing (NGS) technology. NGS refers to the second-generation DNA sequencing technologies where millions of DNA can be sequenced simultaneously. Some of the next gen sequencing methods employed are Roche/454 life science, Illumina/Solexa, SOLiD system and HeliScope. Application of NGS in decoding the genomic database of various oral diseases may possess therapeutic and prognostic value. This presentation provides an overview of the basics of NGS and their potential applications in oral disease diagnostics.

Key words: Molecular diagnostics; Next generation sequencing; Illumina; Oral diseases; Oral cancer

Core tip: Advancements in molecular biology has progressed exponentially in the past decade enabling the diagnosis and treatment of various oral and maxillofacial diseases including cancer. Next generation sequencing is one such tool which is used to determine the genetic make up of an individual as well as in identification of various genetic imbalances that occur in human diseases. Knowledge of the various sequencing methods and the genetic abnormalities may aid in its clinical application for overall improvement in disease diagnosis and prognosis.

Sapna G, Gokul S. Next generation sequencing in oral disease diagnostics. *World J Stomatol* 2018; 6(2): 6-10 Available from: URL: http://www.wjgnet.com/2218-6263/full/v6/i2/6.htm DOI:

First decision: August 20, 2018
Revised: August 31, 2018
Accepted: November 13, 2018
Article in press: November 14, 2018
Published online: November 26, 2018

http://dx.doi.org/10.5321/wjs.v6.i2.6

INTRODUCTION

Molecular diagnostics aimed at determining the alterations occurring at the genetic levels is an important factor in disease diagnosis as well as in the treatment planning or various human diseases including cancer. The availability of advanced technologies has enabled the identification and characterization of human genome, epigenome, proteome, transcriptome and metabolome. Whole genome sequencing determines the entire genomic DNA of an individual at a single time[1]. Whole genome data is considered the unbiased gold standard for obtaining sequencing data because intra- and inter-genic regions are revealed entirely[2]. The first accomplishment in DNA sequencing was obtained in 1977 with the introduction of Sanger sequencing wherein Sanger *et al*[3] and colleagues described the use of chain-terminating dideoxynucleotide analogs that caused base-specific termination of primed DNA synthesis. This was the first DNA sequencing method used to sequence human and microbial DNA based on the chain elongation using modified nucleotides and DNA polymerase[4]. Based on the sanger sequencing methods the first human genome project was completed in 2003. However, this method was disadvantageous in that it was time consuming, difficult to perform and error-prone owing to its manual laboratory-based methods and data entry. Additionally, traditional Sanger sequencing was mainly used to discover DNA substitutions, insertions and deletions [5]. The year 2005, witnessed a shift in whole genome sequencing technology with the arrival of second and third generation sequencing methods. The newer technologies employed for DNA sequencing were together referred to as second generation or next generation sequencing (NGS) methods.

The NGS methods is advantageous in that it helps in routinely extracting DNA, perform large-scale sequence data acquisition within a day[6]. They provide a valuable insight regarding the genomic pathways and thus may contribute to our understanding on disease development and subsequent progression. Various NGS techniques for DNA sequencing, characterization of the coding genome, the whole genome, copy number alterations, assessment of mRNA abundance and translocation detection exist for practical use.

Several NGS platforms are commercially available for DNA sequencing which includes the Roche/454 FLX, the Illumina/Solexa Genome Analyzer, the Applied Biosystems SOLiDTM System, the Helicos HeliscopeTM, complete Genomics, Pacific Biosciences PacBio and Life Technologies Ion Torrent[7]. Recently, single molecule real-time system from Oxford Nanopore is being developed as a third generation sequencing platform[8].

The feasibility of utilizing NGS in DNA sequencing has resulted in its application in various clinical diseases[8]. In addition to DNA sequencing, NGS is also useful in transcriptomics by detecting mRNA expression, discovering non-coding RNAs, microRNAs and metagenomics[7]. This article aims to highlight the various aspects of NGS platforms and its utility in oral disease diagnostics with emphasis on oral cancer.

NGS

The NGS was introduced in the year 2005 consisting of four main technologies. Each of these technologies is characterized by a interaction of high-resolution optics, hardware, and software engineering which allows streamlined sample preparation steps before DNA sequencing[7]. The various NGS platforms are similar in that the massively parallel sequencing of single DNA molecules are separated in a flow cell and the sequencing is performed by repeated cycles of nucleotide extensions or oligonucleotide ligation. This is different from that of Sanger sequencing, which is based on the electrophoretic separation of chain-termination products produced in individual sequencing reactions[3].

The advantages of NGS is that to obtain a higher and accurate sequence yield in shorter time while also being cost effective[8]. In general, these platforms have a high throughput, long read with short time and high coverage[4]. The main disadvantage of NGS is the requirement of infrastructure and personnel expertise to analyze and interpret the data. Also, the enormous volume of data generated by NGS should be skillfully used to extract clinically relevant information[5].

The NGS system of DNA sequencing has been used in various ways. Some of the important methods of sequencing performed with NGS include whole genome sequencing, exome sequencing, targeted sequencing, transcriptomics for total and mRNA sequencing, epigenomics and metagenomics[9,10]. One of the important utility of NGS is in the identification of non-coding and microRNAs in different organisms. The assessment of mutation in non-coding and microRNAs has helped in understanding and treatment of various diseases including cancer[11].

The different NGS platforms include Roche 454, Illumina, SOLiD systems and Ion personal Genome Machine (PGM™). The first commercially available NGS was the Roche 454 system which had a long hand on time and high error rate. Nevertheless, this platform is employed for *de novo* whole sequencing of microbes and exome sequencing[4]. The most commonly used NGS platform is the Illumina system (Illumina Hi Seq™) which are based on a sequencing by synthesis approach and is applicable for human whole genome sequencing, exome sequencing, RNA-seq and methylation[12]. The SOLiD System 2.0 platform, which is distributed by Applied Biosystems is a short-read sequencing technology based on ligation[3]. This platform is useful for human whole genome sequencing, exome sequencing, RNA-seq and methylation[4].

The method of DNA sequencing is similar among various NGS platforms in that the procedure is common for template preparation, nucleic acid sequencing, imaging and data analysis. The first step in NGS involves the preparation of the template or library. It involves preparation of library of nucleic acid by fragmentation of the DNA or cDNA sample and 5′ and 3′ adapter ligation[13]. Once constructed the library is amplified for sequencing. Nucleic acid sequencing from the amplified library is obtained through sequencing by synthesis[14]. The next step involves the data analysis of the sequences. The raw sequence data are then aliged to a known reference sequence by *de novo* assembly[15]. Following the alignment, many forms of analyses are possible using various online tools and software packages. The next crucial step is the visualization of the data obtained by the sequencing procedure. Considering the enormous amount of data obtained, it is mandatory to use a bioinformatics tool to simplify the datasets for genome resolution. While there are many commercially available software packages for enabling data visualization, constant effort in improvising the bioinformatics tool is required to support the NGS applications and help in decoding the obtained data.

CLINICAL APPLICATIONS OF NGS

Whole genome sequencing platforms have wide applications in human pathology. The application of NGS has been attempted in identifying the genomic alterations in various types of cancers including oral cancer, to determine the genetic abnormalities in hereditary conditions like cleft lip and palate as well as in various microbial infections.

The role of NGS in general microbiology is to obtain a genomic definition of pathogens which may harbor information about drug sensitivity and the inter-relationship of the various pathogens which can be used to detect infection outbreaks[5]. The oral microflora is composed of numerous microorganisms which are normal commensals of the oral cavity. While some of them are harmless, certain microorganisms are known to be pathogenic and responsible for commonly occurring oral infections. Usually, the focus of dental research was restricted to a small fraction of oral microbes especially the opportunistic pathogens. The advent of sequencing methods like next-generation sequencing has enabled newer avenues in microbiome studies thereby providing information on the broad diversity of microbial taxa regardless of their cultivability[16].

The use of NGS has made tremendous progress in identification of genetic variation in diseases with underlying genetic disorder. Prior to the use of NGS, it was not possible to identify the entire sequence of genetic alterations. NGS has enabled new the identification of the complete complement of DNA variants, *de novo* mutations and the genes underlying Mendelian forms of disease and characterization of important structural variation that may contribute to diseases like cleft lip and palate[17].

Oral squamous cell carcinoma is a common epithelial malignancy known for its heterogenous nature. The complexity of the lesion has result in the inability to accurately diagnose and manage them thereby resulting in poor prognosis. The use of NGS has enabled researchers to identify the genomic alterations evident in oral squamous cell carcinoma (OSCC). Whole exome sequencing studies have identification alterations with TP53, CDKN2A, PIK3CA and HRAS genes[18]. Another

important alteration which was identified using NGS is the NOTCH1 gene which is involved in regulating squamous differentiation[19]. Other observed alterations include EGFR, STAT3, JAK kinases, transforming growth factor-α and FBKW7 among others[20]. Evaluation of miRNA in oral squamous cell carcinoma has revealed a differential expression of miR-204-5p, miR-370, miR-1307, miR-193b-3p, and miR-144-5p, miR-30a-5p and miR-769-5p[21]. In another study three miRNAs (miR-222-3p, miR-150-5p, and miR-423-5p) were altered in oral leukoplakia and oral squamous cell carcinoma thereby suggesting their utility in early detection and to monitor the progression of oral leukoplakia to OSCC[22]. The role of miRNA in metastasis of oral squamous cell carcinoma has also been analyzed. Literature data has reported significant upregulation of 45 miRNAs in OSCC tissues than the normal controls. Further analysis of miR-21-3p suggests that they may have a potential role in cell metastasis in OSCC progression. Thus targeted therapy aimed at inhibiting the action of miR-21-3p may possess clinical utility and improve prognosis[23]. Other potential biomarkers that were analyzed in OSCC using NGS includes TP53, MDM2, CDKN2A/p16 and TNF- alpha. TP53 mutations were found to be the most frequent alteration in OSCC and hence could be used as a diagnostic marker[24]. Alteration in CDKN2A/p16, a tumor suppressor gene, aids in several molecular events responsible for the malignant transformation as well as in disease progression[25]. MDM2 amplification can promote tumorigenesis and possess increasing clinical relevance because inhibitors are under evaluation in clinical trials. Assessment of MDM2 regulation in various cancers has found that a majority of tumor type has a subset of patients with MDM2 amplification thereby suggesting its role in tumor progression[26]. The role of inflammation in OSCC has garnered renewed interest owing to the advent of NGS. Analysis of tumor necrosis factor-α in OSCC tissues has shown that they promote pro-inflammatory and pro-invasive phenotype and increased expression of TNF-α leads to tumor invasion and thereby associated with poor prognosis. Targeted therapy aimed at nullifying the effect of this gene may aid in the treatment of oral cancer[27].

CONCLUSION

NGS is being developed as an important research means in assessment of genomic alterations in various human diseases. The advantage is that most of the available NGS platforms share a common parallel sequencing process of clonally amplified DNA molecules. With ever improving knowledge regarding its utility, NGS can have a wider role in clinical practice provided some of the limitations are addressed. The need of the hour is to educate the current and future clinicians regarding its applications, the availability of accurate bioinformatics tool to assess the enormous data generated; and to improvise the technical skill and expertise of the laboratory operators. Overall, NGS is a significant discovery to help in disease diagnosis and implementation of appropriate therapy with minimal complications.

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P- Reviewer: Chen YK, Munhoz EA, Vieyra JP
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