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## Next-generation sequencing techniques

Omar Raheem Khalaf AL-Obaidi<sup>1</sup>, Mohammed Ahmed Mustafa<sup>2,3</sup>, Rasha Abdul Adheem Yaseen<sup>1</sup>

<sup>1</sup>College of Education, Biology Department, University of Samarra, Iraq

<sup>2</sup>Department of Pathological Analysis, College of Applied Sciences, University of Samarra, Iraq

<sup>3</sup>Department of Medical Laboratory Techniques, College of Technology, University of Imam Jafar Al-Sadiq Dujail

\*Corresponding author: mohammed.alsad3@gmail.com

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### Abstract

Next-generation sequencing (NGS) is a sort of DNA sequencing innovation that utilizes equal sequencing of numerous little sections of DNA to decide succession. As opposed to Sanger sequencing, the speed of sequencing and measures of DNA grouping information created with NGS, which is viewed as a "high-throughput innovation", are dramatically more prominent, and are delivered at sLJnLficDntl\ diminished expenses. NGS opens the whole range of genomic adjustments for the hereditary examination of complex characteristics. The primary test in the quickly developing HT-NGS advances is to adapt to the investigation of the huge creation of sequencing data set through cutting-edge bioinformatics apparatuses.

**Keywords** Next-generation sequencing, PCR, DNA sequencing

### Introduction

The science is developing quick and cutting-edge sequencing innovations are at present the most sweltering study in human field and creature's genomics explores (Chandra et al., 2011). Early efforts at sequencing qualities were careful, tedious, and work concentrated. HLs circumstances started to change during the mid-1970s when scientist Frederick Sanger built up a few quicker, more easy methods to grouping DNA. Without a doubt, Sanger's work here was so momentous that it prompted his receipt of the Nobel Prize in Chemistry in 1980 (Adams, 2008).

Next-generation sequencing (NGS) is a sort of DNA sequencing innovation that utilizes equal sequencing of numerous little sections of DNA to decide succession (Rizzo & Buck, 2012). As opposed to Sanger sequencing, the speed of sequencing and measures of DNA grouping information created with NGS, which is viewed as a "high-throughput innovation", are dramatically more prominent, and are delivered at sLJnLficDntl\ diminished expenses (Voelkerding et al., 2009). HLrd-JenerDtLon sequencing utilizes equal sequencing like NGS, however dissimilar to NGS, third-age sequencing utilizes single DNA atoms as opposed to DmplL find DNA as a format. Hus, third era sequencing possibly

disposes of blunders in DNA arrangement presented in the study methodology regarding the DNA DmplLficDtLon measure (Schadt et al., 2010). The beginning material that gives a layout to clinical NGS is twofold abandoned atomic DNA. HLs can be gotten from an assortment of cell types. Also, the DNA is further modified in the research facilities to eliminate non-coding locales when exome sequencing is performed (Mardis, 2008; Venter et al., 2001).

NGS opens the whole range of genomic adjustments for the hereditary examination of complex characteristics. The primary test in the quickly developing HT-NGS advances is to adapt to the investigation of the huge creation of sequencing data set through cutting edge bioinformatics apparatuses (Chandra et al., 2011).

### Sequencing techniques applied to characterize food-related microbiomes: 16S rDNA sequencing

This is perhaps the main culture-free technique utilized for ordinary microbiome examination. Most microbes contain 16S rDNA quality which is comprised of nine hypervariable locales flanked by preserved successions (Neefs et al., 1993).

This offers an extraordinary chance to plan conventional PCR groundworks to enhance and grouping these hypervariable loci to distinguish the comparing bacterial scientific categorization of the species related with the food framework. Essentially, the 18S rDNA quality can be utilized to recognize parasites. Because of the nucleotide arrangement similitude, these groupings are bunched into Operational Taxonomic Units (OTU). OTUs are then contrasted against data sets to distinguish the microorganisms present in the microbiome. The principal endeavor to describe a microbiome using this approach is the ID of the microbial populace from Sargasso Sea picoplankton (Giovannoni et al., 1990).

Customary Sanger sequencing permits just a more modest extent of amplicons to be sequenced. These outcomes in less bountiful individuals from the microbiome populace being missed, in this manner bargaining the extensive depiction of the microbial local area. The ensuing consideration of NGS stages in 16S rDNA sequencing expanded the limit concerning a more exhaustive distinguishing proof of the bacterial individuals in the local areas by a few significant degrees and at a lower cost. Since just a short amplicon was sequenced, a lot higher inclusion for each example was gotten. Moreover, pyrosequencing permitted singular examples to be filed and encouraged multiplexing during each instrument run. This last advance gave a forward leap in the manner natural prokaryotes could be dissected. From that point forward 16S rDNA sequencing has become the most common method to distinguish the microbiome individuals related to food grids. One of the principal points of interest in utilizing the 16S rDNA sequencing approach is the accessibility of numerous bioinformatic instruments intended for sequencing information investigation which are free and simple to work. Generally utilized programming to investigate 16S rDNA information from food/natural examples incorporates QIIME (Quantitative Insights Into Microbial Ecology), mothur, and USEARCH (super quick grouping examination), (Schloss et al., 2009; Edgar, 2010).

Because of more limited peruses acquired from NGS conventions, particularly from Illumina stages, bacterial characterization utilizing 16S rDNA sequencing frequently can't be recognized past the class level. Besides, 16S rDNA sequencing was found to belittle the commitment of Gram-negative microorganisms when contrasted with bacterial tallies noticed utilizing transmission electron microscopy and Gram staining. A further test to this methodology is identified with the decision of nine hypervariable districts (V1–V9) contained inside the 16S rDNA quality. The choice of the hypervariable locale for 16S rDNA sequencing for the most part has not been subjected to the example climate yet rather on distributed or in-house planned conventions. Numerous creators have supported diverse hypervariable areas, for example, V1/V2/V4, V2/V3/V4, V2/V4, and V2/V3 (Liu et al., 2008; Wang et al., 2007; Chakravorty et al., 2007; Mustafa et al., 2020). A precise overview of the effectiveness of various hypervariable districts distinguished that PCR groundwork sets focusing on V4/V5 was ideal to recognize the microbiome with diminished intensification predisposition contrasted with the standard V3/V4 (Claesson et al., 2009).

PCR amplicon parts as short as 82 bp focusing on the 16S rDNA V5 variable area have ended up being of adequate length for bacterial order at the phylum level, and a more drawn out amplicon piece of 100 bp joined with appropriate groundwork plan and downstream examination was equipped for showing similar bunching data as longer 16S rDNA grouping (Liu et al., 2008; Mustafa & AL-Samarraie, 2020). Indeed, even with longer factor areas, pyrosequencing and higher inclusion, the enhancement of various polymorphic districts brought about a predisposition in surveying the microbiome. Additionally, the emphasis on one marker quality, disregarding other genomic biomarkers makes this strategy unsatisfactory for disconnect explicit distinguishing proof. By the by, considering the ease per test and the prerequisite of low info layout DNA fixations, 16S rDNA sequencing stays quite possibly the most famous high-throughput sequencing strategies.

### Application of NGS techniques in some microbiome studies

Identifying the appropriate sequencing method suitable for the analysis of various food products depending on the specificity of the sample to be studied and the extent of bacterial taxonomic knowledge needed. A practice spread of the microbial population within a food sample will include an original 16S rDNA sequencing dependent profile.

Even so, this approach lacks the requisite resolution needed to provide recognition of species-level/strain level. Furthermore, an evaluation of the functional potential of these species, found within the study, will not be given. Accordingly, for the in-depth level of the genus or the classification of the strain level or the thorough functional characterization of the various members of the microbiome, metagenomics, and metatranscriptomics could be useful as well.

### Application to raw materials and processing environment monitoring of NGS-based techniques

Cleanliness is a fundamental advance in the homestead-to-fork continuum and all creation/handling lines are made to keep up sterile guidelines at all phases of food production. Upgrades in the support of clean norms have improved food handling by diminishing the number of pathogenic microbes colonizing these conditions and at last cross polluting the last food products. The nature of the last food product does not just rely on the cleanliness of the handling climate yet besides on the nature of the crude materials utilized. For instance, a 16S rDNA sequencing-based methodology detailed that milk with higher substantial cell checks was related to a higher wealth of certain bacterial types such as *Acinetobacter*, *Enterobacteriaceae*, *Corynebacterium*, and *Streptococcus* species (Rodrigues et al., 2017), alongside *Thermo anaerobacterium*, a variety recognized without precedent for the center microbiome of a milk test. The presence of waste creatures, (for example, *Acinetobacter*, *Thermo anaerobacterium* among others) alongside pathogenic microorganisms (counting *Enterobacteriaceae*, *Corynebacterium* species, and *Streptococcus*) in the crude

material can defile the occupant microbiome of mass milk tank in which the crude material is put away speaking to a danger to the whole downstream creation line. The effect of biological handling conditions on food quality appeared in a more detailed examination including mass milk big haulers (Kable et al., 2016).

Utilizing a 16S rDNA sequencing-based methodology, it was demonstrated that the microbiome of milk containing storehouses was impacted by that of the mass milk big haulers taking care of them, and that unmistakable bacterial networks were developed inside various milk storehouses. Besides, the investigation reveals that the piece of the milk microbiome changed occasionally, as the higher bacterial variety was related to the spring season. These perceptions, nonetheless, were not restricted to dairy offices. In cheddar creation locales, the in-house natural greenery ruled the cheddar microbiome (Bokulich et al., 2015).

### Sequencing by hybridization

In the 1980s, this technique was originally formulated using arrayed DNA oligonucleotides of known sequence on filters which were hybridized to sequence labeled fragments of the DNA. It was possible to establish whether the hybridizing labeled fragments suited the sequence of the DNA probes on the filter by frequently hybridizing and washing away the unwanted non-hybridized DNA. Based on overlapping information from the probe hybridization spots, it was thus possible to create larger contiguous sequence information. Hybridization sequencing has been primarily restricted to technologies that rely on the use of simple probes to interrogate genomes, such as in clinical diagnostics for detecting single nucleotide polymorphisms (SNPS) associated with disease in specific genes. abnormalities (rearrangements, deletions, duplications, copy number variants, CNVs) (Hanna et al., 2000; Qin et al., 2012).

### Electron microscopy DNA sequencing

Another single-molecule sequencing technology is electron microscopy DNA sequencing, first considered in the 1960s and 70s. DNA must be labeled with heavy atoms to be visualized since the electron microscope is unable to image individual nucleotides comprising the normal isotopes of carbon, hydrogen, nitrogen, oxygen, and phosphorus in DNA. The DNA must be denatured, labeled, and spread out on an electron microscope grid for these procedures to operate, using a "hypophase" procedure to keep the DNA denatured and linear. In principle, DNA sequencing transmission electron microscopy could have incredibly long read distances, but the problem of disruption to the electron beam has not been addressed and the technique has not yet been commercially advanced.

### Advancements on the "Fourth" Generation Cusp

It is conceivable to pass long DNA atoms through little breadth "openings" and measure varying flows as every

nucleotide passes by a connected indicator In principle, more than a hundred kb of DNA could be strung through the nanopore, and with numerous channels, tens to many Gb of succession could be accomplished at generally minimal effort. Two kinds of nanopore frameworks for DNA sequencing have been created, natural layer frameworks and strong state sensor innovation. Organic nanopore sequencing depends on the utilization of transmembrane proteins inserted in a lipid layer to create the pores.

Two types of proteins that have been used to create pores have been broadly examined: alpha-hemolysin and Mycobacterium smegmatis porin A (MspA). The pace of DNA entry through the pores is organized by the expansion of engine proteins, for example, a profoundly processive DNA polymerase (phi29), that ratchets DNA through nucleotide expansion. Other extra proteins, for example, a DNA helicase, exonuclease I or oligonucleotides to tie DNA strands, empower loosening up and "tightening" of the DNA nucleotides through the nanopore for identification. DNA would be traveling through the pores at a continuous rate for several thousands of nucleotides. Strong state sensor innovation utilizes different metal or metal compound substrates with nanometer estimated pores that permit DNA or RNA to go through (Benner et al., 2007; Branton et al., 2008; Cherf et al., 2012; Liu et al., 2016).

### Conclusion

Next-generation sequencing (NGS) is a sort of DNA sequencing innovation that utilizes equal sequencing of numerous little sections of DNA to decide succession (Rizzo & Buck, 2012). As opposed to Sanger sequencing, the speed of sequencing and measures of DNA grouping information created with NGS, which is viewed as a "high-throughput innovation", are dramatically more prominent, and are delivered at sLJnLficDntl\ diminished expenses. HLrd-JenerDtLon sequencing utilizes equal sequencing like NGS, however dissimilar to NGS, third-age sequencing utilizes single DNA atoms as opposed to DmplLfied DNA as a format. Thus, third-era sequencing possibly disposes of blunders in DNA arrangement presented in the study methodology regarding the DNA DmplLficDtLon measure.

### Conflict of Interest

The author hereby declares no conflict of interest.

### Consent for publication

The author declares that the work has consent for publication.

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