Introduction

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LEARNING OBJECTIVES

1. Discuss the history of DNA sequencing.
2. Describe the components of DNA and DNA synthesis.
3. Compare and contrast dNTPs and ddNTPs.
4. List several next generation sequencing platforms.

ABBREVIATIONS: A - adenine; ATP - adenosine triphosphate; C - cytosine; dATP - deoxyadenosine triphosphate; dCTP - deoxycytidine triphosphate; ddNTP - dideoxynucleotide triphosphate ; dGTP - deoxyguanosine triphosphate; DNA - deoxyribonucleic acid; dNTP - deoxynucleotide triphosphate; dTTP - deoxythymidine triphosphate; G - guanine; mRNA - messenger ribonucleic acid; NGS – next generation sequencing; OH - hydroxyl; SMRT - single molecule real time; T - thymine.

INDEX TERMS: Sequencing; DNA Sequencing; Sanger Method; Dideoxynucleotide Triphosphates; Next Generation Sequencing

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In 1953, James Watson and Francis Crick were credited with discovering the structure of the DNA molecule: a three dimensional, double helical complex which dictates the genetic code for all forms of life.[1] Although much controversy exists over this discovery notably that the model Watson and Crick proposed was the compilation of the work of several pioneers before them,[2] they were awarded the Noble Prize in Physiology or Medicine in 1962 along with Maurice Hugh Frederick Wilkins "for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material."[3]

The DNA molecule consists of a sugar (deoxyribose) and phosphate backbone along with four chemical base pairs: adenine (A), thymine (T), cytosine (C), and guanine (G). Each base pair is covalently linked to the sugar phosphate backbone. The double helical structure forms once each base pair links to the complementary base on the corresponding strand of deoxyribonucleic acid (DNA) through hydrogen bonding. Due to the chemical make-up of each base, adenine always binds with thymine while cytosine always binds with guanine. The base, sugar and phosphate together constitute a nucleotide.[4] A key property of the DNA molecule is that it can replicate itself and synthesize a new copy of DNA from the preexisting strand during cell division.

DNA synthesis requires several components: DNA template; primer; deoxyribonucleotide triphosphates (dNTPs): deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP) and deoxythymidine triphosphate (dTTP); various enzymes; and an energy source to fuel the reaction adenosine triphosphate (ATP).[5] Once the strand is replicated, the segment of DNA containing the gene is then transcribed into messenger ribonucleic acid (mRNA) which ultimately is translated into protein. Hence, the classic view of the central dogma in biology: DNA (transcription) to mRNA (translation) to protein.[6]

In 2003, the entire human genome was sequenced which mapped out the location of every gene (approximately 20,500 genes) in the human body.[7] This feat was accomplished by the international,

collaborative efforts of scientists working together on the Human Genome Project.[7] The significance of identifying the location of every gene in the human genome has enabled scientists and researchers to investigate individual genes by testing for a particular mutation such as those leading to cancer, metabolic disorders, congenital disorders, etc. DNA sequencing and next generation sequencing (NGS) techniques are at the forefront of this diagnostic testing.

Sequencing techniques have been around since the 1940s. A pioneer in the development of protein sequencing and DNA sequencing techniques was Dr. Frederick Sanger. In 1943 Dr. Sanger developed a tool that enabled him to sequence the very first protein molecule - insulin.[8] This discovery earned him the Nobel Prize in Chemistry in 1958.[8] In 1960 he then focused his studies on working with nucleic acids where he later developed the "Sanger Method of DNA Sequencing" utilizing dideoxynucleotides resulting in chain termination.[8] Dideoxynucleotides (ddNTPs) differ from dNTPs in that they lack a 3′ hydroxyl (OH) group essential for chain elongation. Once a dideoxynucleotide is incorporated into the DNA strand, chain termination occurs.[9] This technique results in the production of many DNA fragments which are analyzed and interpreted revealing the actual DNA sequence being investigated. The principle of the Sanger method of DNA Sequencing will be explained in greater detail in the article entitled "Next Generation Sequencing - Platforms." The principle of this technique is the bases for several DNA sequencing and NGS assays utilized in both clinical and research laboratories today. This discovery earned Dr. Sanger his second Nobel Prize in Chemistry in 1980.[8]

This continuing education series introduces clinical laboratory scientists to the principles of DNA sequencing and next generation sequencing. The first article entitled "Next Generation Sequencing - Platforms" describes in detail the Sanger method of DNA sequencing and the modifications made to this method utilized in NGS platforms which are currently used in both clinical and research laboratories. Several platforms available for use include but are not limited to the Ion Torrent™ by Life Technologies,[10] Illumnia MiSeq[11] and the SMRT (single molecule, real time) Technology by Pacific Biosciences.[12] These platforms are discussed with regard to work flow, sample preparation, turnaround time and cost. The second article entitled "Next Generation Sequencing - Applications" highlights the various applications the above platforms offer including discussion of the various cancer, metabolic, congenital and infectious disease, and pediatric panels used in diagnosis. Applications also include using sequencing to identify infectious disease mutations in surveillance of hospital outbreaks, public health epidemics as well as in pandemics caused by cholera,[13] influenza[14] and measles[15] to name a few. The third article in the series entitled "Next Generation Sequencing - Personalized Medicine and Ethics" discusses the benefits of using NGS in personalized medicine but also describes the controversy and ethical issues that arise from this type of accessible patient information.

In summary, many NGS platforms are available for research and clinical use resulting in affordable costs, higher output and increased accuracy. The use of sequencing in diagnosis will prove to be instrumental in the clinical laboratory. Once an individual's DNA sequence is known and mutations are identified, the idea of personalized medicine in the future becomes a reality. However, this knowledge does not go without controversy and ethical consequences. With rapidly advancing technologies, the way a physician treats a patient will be based on the results obtained from the patient's own genetic make-up, which ultimately will result in a better prognosis and overall improved patient care.

NOTE: The author is not endorsing any particular company or product and has no financial gain or otherwise interest in the products presented.

REFERENCES

1. Watson JD, Crick FHC. A structure for deoxyribose nucleic acid. Nature 1953;171:737–8.
2. Pray LA. Discovery of DNA structure and function: Watson and Crick. Nature Education. 2008;1(1):100.
3. The Nobel Prize in Physiology or Medicine 1962 Francis Crick, James Watson, Maurice Wilkins. Available from http://www.nobelprize.org/nobel_prizes/medicine/laureates/1962/. Accessed 2014 March 19.
4. Genetics Homepage: What is DNA. Available from http://ghr.nlm.nih.gov/handbook/basics/dna. Accessed 2014 March 19.
5. DNA Structure & DNA Replication. Available from http://www.biology-online.org/1/5_DNA.htm. Accessed 2014 March 19.

6. Central Dogma of Biology: Classic View. Available from http://www.ncbi.nlm.nih.gov/Class/MLACourse/Modules/MolBioReview/central_dogma.html. Accessed 2014 March 19.
7. An Overview of the Human Genome Project. Available from http://www.genome.gov/12011238. Accessed 2014 March 20.
8. Frederick Sanger – Biographical. Available from http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1980/sanger-bio.html. Accessed 2014 March 19.
9. Mahon CR, Lehman DC, Manuselis G, editors. Textbook of Diagnostic Microbiology. 4th ed. Missouri Saunders Elsevier; 2011.
10. Ion TorrentTM Next-Generation Sequencing. Available from http://www.lifetechnologies.com/us/en/home/life-science/sequencing.html?s _kwcid=AL!3652!10!33369080 44!26566630335&ef_id=UysVxwAAAAep8zDk:20140320162252:s. Accessed 2014 March 20.
11. Illumina MiSeq Desktop Sequencer. Available from http://www.illumina.com/systems/miseq.ilmn. Accessed 2014 March 20
12. SMRT Technology. Available from http://www.pacificbiosciences.com/products/smrt-technology/. Accessed 2014 March 29.
13. Cholera pandemic's source discovered. Available from http://medicalxpress.com/news/2011-08-cholera-pandemic-source.html. Accessed 2014 March 20.
14. Rutvisuttinunt W, Chinnawirotpisan P, Simasathien S, Shrestha SK, Yoon IK, Klungthong C, et al. Simultaneous and complete genome sequencing of influenza A and B with high coverage by Illumina MiSeq Platform. J Virol Methods. 2013 Nov;193(2):394-404.
15. Vainio K, Steen TW, Arnesen TM, Rønning K, Ånestad G, Dudman S. Measles virus genotyping an important tool in measles outbreak investigation in Norway, 2011. Euro Surveill. 2012 Dec 13;17(50):pii=20340.