EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 12
161-163
Year: 2019

DNA SEQUENCING BY SANGER'S METHOD

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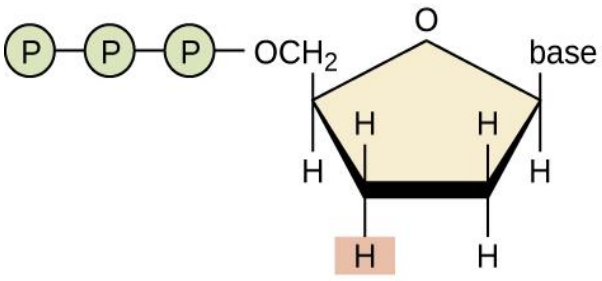
Article Received on 29/08/2019 **Article Revised on 19/09/2019** **Article Accepted on 09/10/2019**

ABSTRACT
The first developed method for DNA sequencing DNA were developed in middle 1970s by Frederick Sanger and Et al. It principally relied on termination of growing nucleotide chain (dNTPs) when a dideoxynucleotide triphosphate (ddNTPs) was inserted in it .Detection of labeled fragments was done by autoradiography on polyacrylamide gel electrophoresis (PAGE). Sanger sequencing given high quality sequences for relatively long stretches of DNA in many disease and genome project.

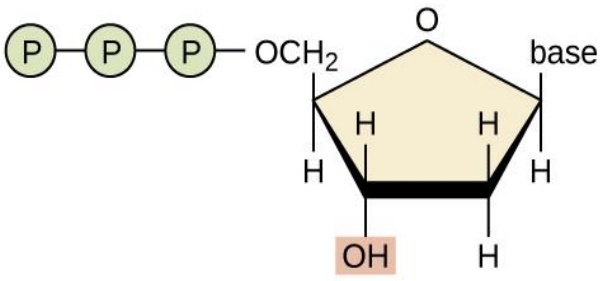
INTRODUCTION

As with recombinant DNA technologies and PCR, DNA sequencing exploit base pair complementarity together with an understanding of the basic biochemistry of DNA replication . Several techniques have been developed but one of them is by far the most used. It is called dideoxy sequencing or sometimes, sanger sequencing after its inventor. It invented by Frederick sanger and Et al in 1970-1977. So also called sanger sequencing after its inventor.

This technique base on enzymatic method because using of DNA polymerase. The term dideoxy comes from a special modified nucleotide, called a dideoxynucleotide triphosphate (generally, a ddNTP); this modified nucleotide is key to the sanger technique because of its ability to block continued DNA synthesis. A dideoxynuceotide lack the 3'- hydroxyl groups well as the 2'-hydroxyl group, which is also absent in deoxynucleotide (below given figure).



dideoxynucleotide (ddNTP)



deoxynucleotide (dNTP)

For DNA synthesis take place, the DNA polymerase must catalyze a condensation reaction between the 3'-hydroxyl group of the last nucleotide added to the growing chain and 5'-phosphate group of the next nucleotide to be added, releasing water and forming a phosphodiester linkage with the 3' carbon atom of the adjacent sugar. Because a dideoxynucleotide lacks the 3'- hydroxyl group, this reaction cannot take place and therefore DNA synthesis is blocked at the point of addition.

Procedure

We want to read the sequence of a cloned DNA segment. we use restriction enzyme for DNA segment and denature the DNA strands of this segment and use PCR.Copies of each template is divided in four batches and each batch is used for different replication reaction. Next we create a primer for DNA synthesis that will hybridize to exactly one location on the cloned DNA segment and then add a special "cocktail" of DNA polymerase. Normal deoxynucleotide triphosphates (dATP, dCTP, dGTP and dTTP) and a small amount of a special dideoxynucleotide triphosphates for one of the four bases (ddATP, ddTTP, ddCTP, ddGTP) in each batches. Four separate reactions are therefore performed in batches.

The polrmerase will begin to synthesize the complementary DNA strand, starting from the primer, but will stop at any point at which the dideoxynucleotide triphosphate is incorporated in the growing DNA chain in place of place of the normal deoxynucleotide triphosphate. Suppose the DNA sequence of the DNA segment of that we are trying to sequence is-

5'CTGACTTCGACAA3'

We would then start DNA synthesis from a complementary primer:-

5'CTGACTTCGACAA3'

3' TGTT5'

Direction of DNA synthesis

Using the special DNA sysnthesis cocktail "spiked" with ddATP, for example, we will create a nested set of DNA fragments that have the same starting point but different end points because the fragments stop at whatever point the insertion of ddATP instead of dATP halted DNA replication. The array of different ddATP arrested DNA chain looks like the list of sequences below (*A indicates the dideoxynucleotide.)

5'CTGACTTCGACAA3' Template DNA colne

TGTT5' Primer for synthesis

Direction of synthesis

*AGCTGTT5' Dideoxy fragment 1

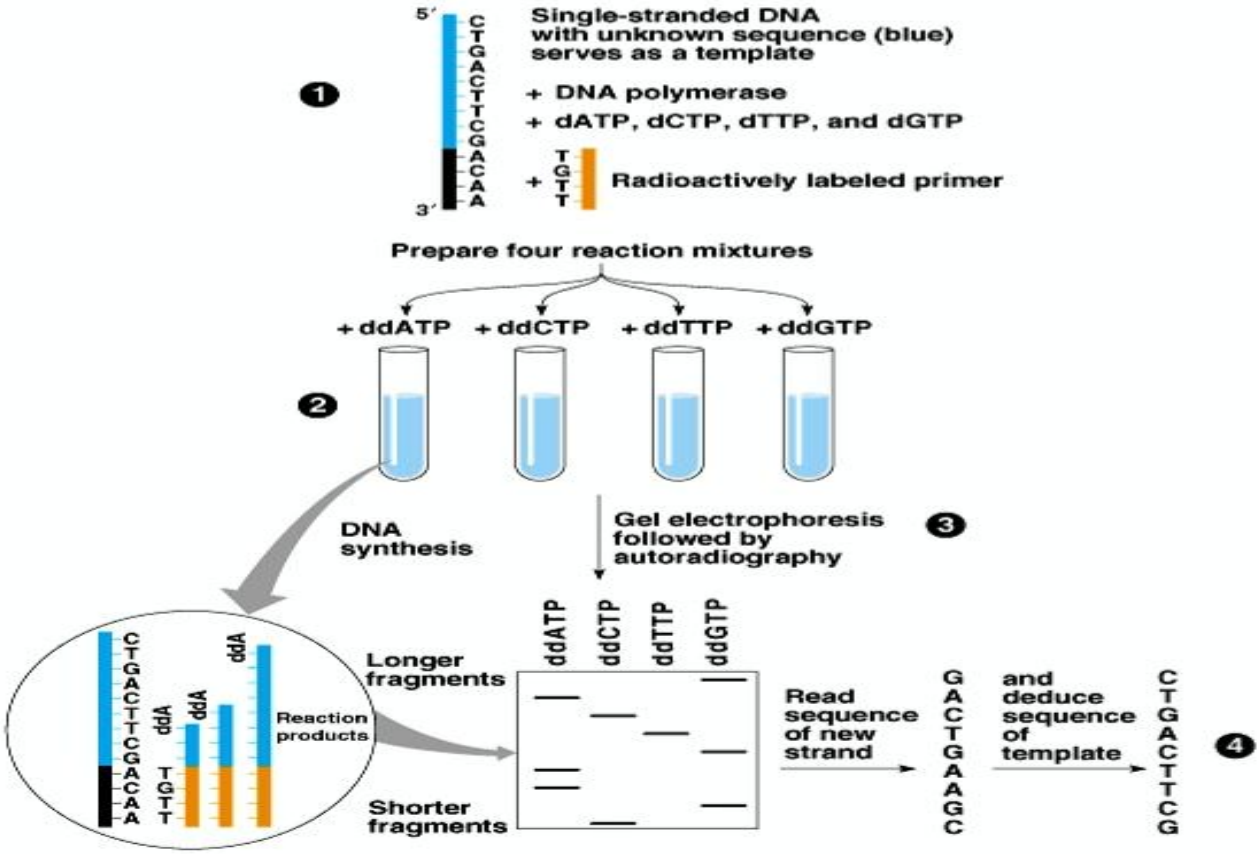
*AAGCTGTT5' Dideoxy fragment 2

*CTGAAGCTGTT5' Dideoxy fragment 3

We cangenerate an array of such fragment for each of the four possible ddNTPs in four separate cocktail (one spiked with ddATP, one with ddCTP, one with ddGTP, and one with ddTTP). Each will produce a different array of fragments with no two spiked cocktails producing fragments of the size.Further if we add up the results of all four cocktails, we will see that the fragments can be ordered in length, with the lengths increasing by one base at a time.

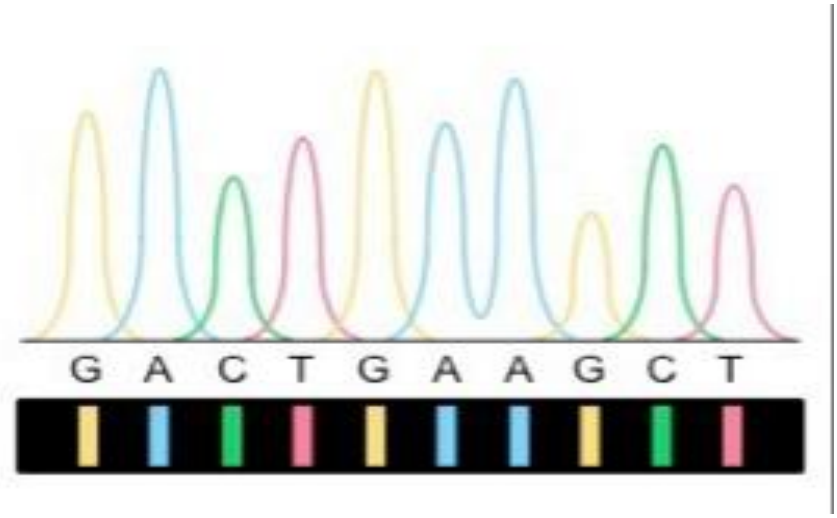
The final steps of the process are:-

- Display the fragments in order of size by using PAGE. They will appear in four separate columns labelled A, C, T and G.
- Labelled the newly synthesized strands.



So that they can be visualized after they have been separated according to size by PAGE. Do so by either radioactively or fluorescently laballing the primer (initiation labelling) or the individual dideoxynucleotide triphosphate (termination labelling).

Now we autoradio graphy of gel allows the sequence to be read directly. The order of bands on the autoradiograph is determined from the botton of the gel to the top. This correspondes to the sequence 5'-3' sequence of the strand synthesized in vitro and is therefore complementary to the template cloned insert.



Autoradiolabeled sequencer.

CONCLUSION

The data obtained will be use to enhance current knowledge and to further the development of potential treatments of disease. Sanger sequencing gives high quality sequences for relatively long stretches of DNA in many genome project. These include study of cancer of variants, detection of minimal residual disease, exone sequencing, detection of single nucleotide polymorphisms (SNPs) and for sequencing of microorganisms genome. This method is accurate for sequencing. however, Sanger sequencing is expensive and inefficient for larger-scale projects.

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