Genetic engineering
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Lab: 7

DNA Sequencing

Sequencing means finding the order of nucleotides on a piece of DNA. Nucleotide order determines amino acid order, and by extension, protein structure and function (proteomics). An alteration in a DNA sequence can lead to an altered or non-functional protein, and hence to a genetic disorder. DNA sequence is important to detect the type of mutations in genetic diseases and offer hope for the eventual development of treatment DNA.

Methods of sequencing

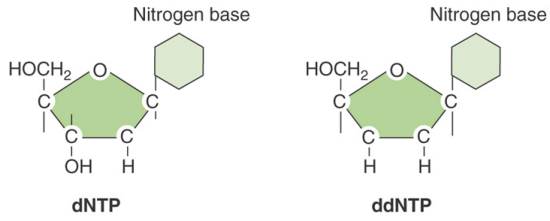
There are two main methods of DNA sequencing:

1-Sanger method (primer extension/chain-termination): most popular protocol for sequencing, very adaptable, scalable to large sequencing projects

2-Maxam-Gilbert chemical cleavage method: DNA is labeled and then chemically cleaved in a sequence- dependent manner.

The Sanger Technique Principle:

The Sanger Technique uses dideoxynucleotides ddNTP (ddATP,ddTTP,ddGTP,ddCTP). These are molecules that resemble normal nucleotides but lack the normal -OH group which allows nucleotides to join in a growing DNA strand then replication stops.



Requirements for Sanger Method

1- DNA to be sequenced must be in single strand form.
2- A primer complementary to the known region to start and direct chain synthesis. (15-30 nucleotides in length)
3- DNA polymerase.
4- 4 deoxynucleotide triphosphates (dNTPs).
5- 4 dideoxynucleotide triphosphates (ddNTPs) (small proportion**)**.

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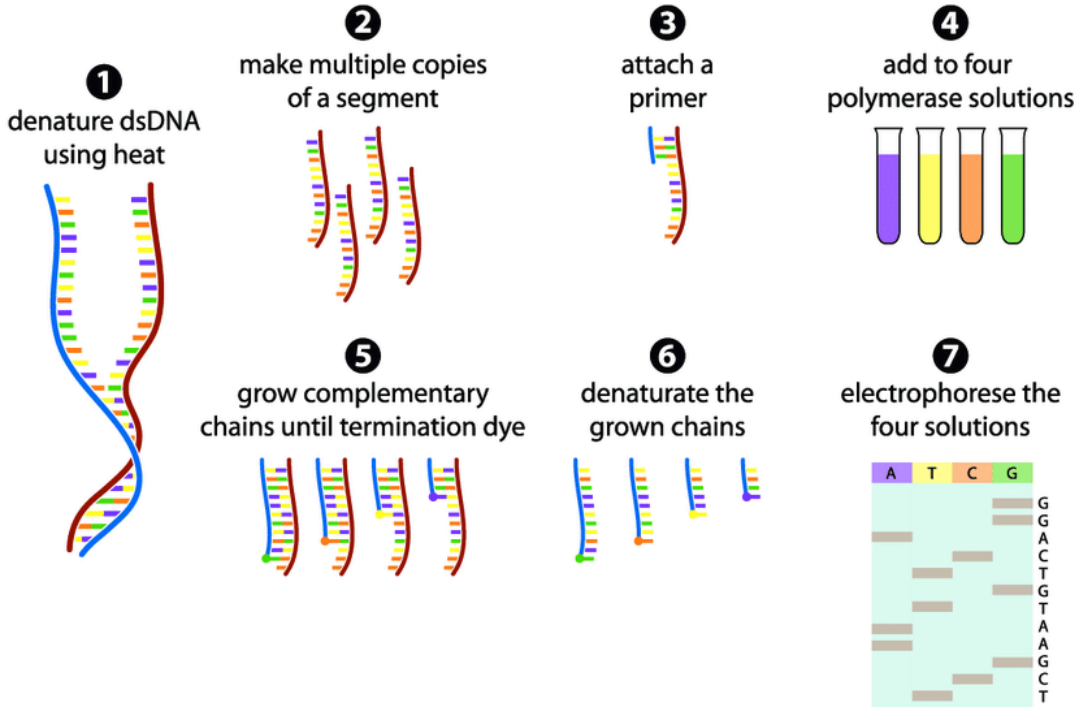
The template DNA pieces are replicated, incorporating normal nucleotides, but occasionally and at random dideoxy (DD) nucleotides are taken up and this stops replication on that piece of DNA. The result is a mix of DNA lengths, each ending with a particular labeled ddNTP. The different lengths 'travel' at different rates during electrophoresis, their order can be determined.

Visualization Methods

There are two forms of labeling:

1- Radioactive (Primer labeled (^{32}P or ^{33}P) or dNTP labeled (^{35}S or ^{32}P)
2- Nonradioactive (Fluorescence) (Primer labeled or ddNTP labeled)
Note: ddNTPs chemically synthesized to contain fluorescence. Each ddNTP fluoresces at a different wavelength allowing identification.



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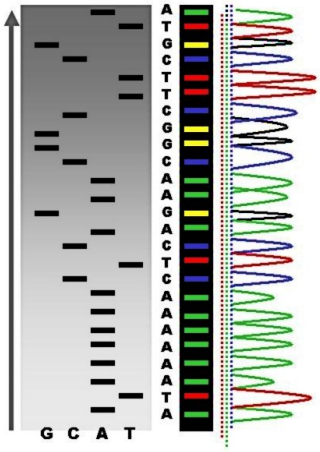
Example: What is the sequence of this DNA fragments using Sanger method?



Maxam-Gilbert method

The single stranded DNA fragment to be sequenced is labeled with radioactive phosphate at 5' end. The labeled DNA fragment is then divided into four aliquots, each of which is treated with a reagent, which modifies a specific base. This will results in strands of varying lengths. Strands separated out with electrophoresis. Gels read with radioautography.

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