DNA SEQUENCING

The DNA sequencing is a method by which the sequence of nucleotides at a particular strand of the DNA is determined. Fred Sanger and colleagues in mid-1970s devised a method for DNA sequencing called chain termination method.

Following are the methods for DNA sequencing:

1. Chain Termination Method: the singles strand of DNA that acts as a template is taken. The new strand synthesis is allowed with the help of enzyme polymerase that incorporates the deoxy-ribonucleotides (dATP, dCTP, dGTP and dTTP). The chain termination method involves the incorporation of some unusual nucleotides called di-deoxyribo-nucleotides (ddNTP) where the 3`OH is being replaced by H. When the polymerase enzyme adds the di-deoxy-ribonucleotides the chain, no further addition of ribonucleotides are possible causing termination of new strand synthesis at that position. In practice each ddNTPs are labelled with different fluorescent dyes that help in recognizing their position at the end of terminated chain. As a result different lengths of ssDNA are synthesized based on their point of its termination. The variable lengths DNA are separated by agarose gel electrophoresis.
2. Pyrosequencing: like chain termination method, the pyrosequencing also requires DNA is single stranded form. The single stranded DNA are obtained by alkali denaturation of DNA molecules or by cloning the DNA molecules into vectors such as M13 phage. The complementary strand is synthesized by enzyme DNA polymerase by adding deoxy nucleotides that is accompanied by release of a pyrophosphate that can be converted to a product that yields chemi-luminiscence by the enzyme sulfurylase. Each deoxynucleotides are sequentially added one after another in presence of an enzyme nucleotidase that degrades the nucleotide that is not incorporated into the growing chain. This procedure makes it possible to follow the order in which the nucleotides are added into the growing complementary strand.
3. Chain degradation method: in this method certain chemical compounds are used which degrades the strand at a particular nucleotide. So, four different enzymes are chosen that degrades upto the length of anyone of four nucleotides (A or T or G or C). This method is obsolete now.