

Notes(2) -BIOTECHNOLOGY : PRINCIPLES AND PROCESSES

Restriction Enzymes : the two enzymes responsible for restricting the growth of bacteriophage in Escherichia coli were isolated. One of these added methyl groups to DNA, while the other cut DNA. The later was called restriction endonuclease. The first restriction endonuclease—Hind II, whose functioning depended on a specific DNA nucleotide sequence was isolated and characterised five years later. It was found that Hind II always cut DNA molecules at a particular point by recognising a specific sequence of six base pairs. This specific base sequence is known as the recognition sequence for Hind II. Besides Hind II, today we know more than 900 restriction enzymes that have been isolated from over 230 strains of bacteria each of which recognise different recognition sequences.

Restriction enzymes belong to a larger class of enzymes called nucleases. These are of two kinds; exonucleases and endonucleases. Exonucleases remove nucleotides from the ends of the DNA whereas, endonucleases make cuts at specific positions within the DNA. Each restriction endonuclease functions by 'inspecting' the length of a DNA sequence. Once it finds its specific recognition sequence, it will bind to the DNA and cut each of the two strands of the double helix at specific points in their sugar -phosphate backbones . Each restriction endonuclease recognises a specific palindromic nucleotide sequences in the DNA.

the following sequences reads the same on the two strands in 5' à 3' direction. This is also true if read in the 3' à 5' direction.

5' — GAATTC — 3'

3' — CTTAAG — 5'

Restriction enzymes cut the strand of DNA a little away from the centre of the palindrome sites, but between the same two bases on the opposite strands. This leaves single stranded portions at the ends. There are overhanging stretches called sticky ends on each strand.