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Sub. Biology

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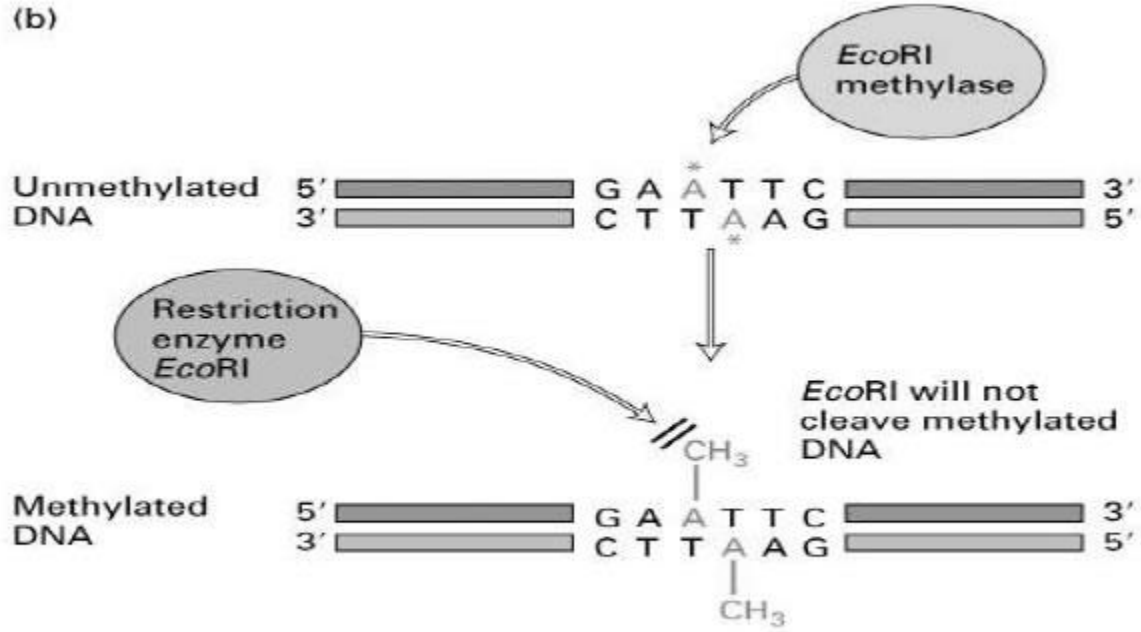
Chapter 11 Biotechnology Principles and Processes

• These enzymes **RESTRICT** the ability of foreign DNA (such as bacteriophage DNA) to infect/invade the host bacterial cell by cutting it up (degrading it)
• The host DNA is **MODIFIED** by **METHYLATION** of the sequences these enzymes recognise
o Methyl groups are added to C or A nucleotides in order to protect the bacterial host DNA from degradation by its own enzymes



Types of restriction enzymes

• Type I Recognise specific sequences but then track along DNA (~1000-5000 bases) before cutting one of the strands and releasing a number of nucleotides (~75) where the cut is made. A second molecule of the endonuclease is required to cut the 2nd strand of the DNA
o e.g. EcoK.

o Require Mg2+, ATP and SAM (S-adenosyl methionine) cofactors for function

Type II Recognise a specific target sequence in DNA, and then break the DNA (both strands), within or close to, the recognition site.Only they are used in rDNA technology as they recognize and cut DNA within a specific sequence typically consisting of 4-8 bp.

o e.g. EcoRI

o Usually require Mg2+

• Type III Intermediate properties between type I and type II. Break both DNA strands at a defined distance from a recognition site

o e.g. HgaI

o Require Mg2+ and ATP

Hundreds of restriction enzymes have been isolated and characterised

• Enables DNA to be cut into discrete, manageable fragments

• Type II enzymes are those used in the vast majority of molecular biology techniques

• Many are now commercially available

Many Type II restriction endonucleases recognise PALINDROMIC sequences (From Greek palindromos, running back again, recurring: palin, again)

A segment of double-stranded DNA in which the nucleotide sequence of one strand reads in reverse order to that of the complementary strand. (Always read from the same direction)

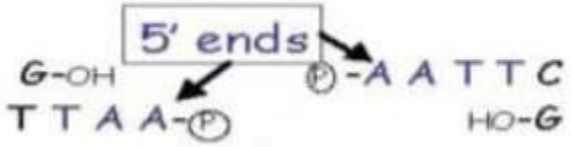
For example, EcoRI recognises the sequence

5’-G A A T T C-3’

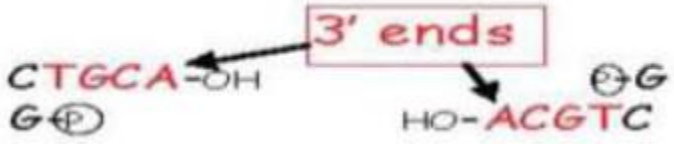
3’-C T T A A G-5’

Different enzymes cut at different positions and can create single stranded ends (’sticky ends’)

• Some generate 5’ overhangs - eg: EcoRI



• Some generate 3’ overhangs - eg: PstI



• Some generate 3’ overhangs - eg: PstI

Examples of restriction enzymes and the sequences they cleave

The 'sticky' overhangs are known as COHESIVE ENDS

• The single stranded termini (or ends) can base pair **(ANNEAL)** with any complementary single stranded termini

This is the basis for **RECOMBINANT DNA TECHNOLOGY**

• Inserting foreign DNA into a cloning vector

Restriction enzymes are a useful tool for analysing Recombinant DNA

After ligating a particular DNA sequence into a cloning vector, it is necessary to check that the correct fragment has been taken up. Sometimes it is also necessary to ensure that the foreign DNA sequence is in a certain orientation relative to sequences present in the cloning vector.