[26-11-20]

* Biotechnology *

Part III (H) & P.G. - Zoology

Dr. V. Kumar

* Biotechnology, we can get:

1. multiple copies of cloned DNA fragments
2. Large quantities of proteins produced by cloned genes.
3. Integration of gene in chromosome of host organism (gene transfer) so to repair the defective cistron or gene or develop new genotype.

* Recombinant DNA Technology:

- Recombinant DNA is the DNA formed by the union of foreign DNA (from eukaryotic cell) to vector DNA. It is called Heterologous DNA.

- Recombinant DNA technology involve because of following discoveries—

1. Denaturation and Renaturation of DNA
2. Artificial synthesis of genes in vitro
3. Discovery of enzyme restriction
4. Gene splicing and gene cloning technique.

1. Denaturation and Renaturation of DNA:

- The two strand of DNA easily separate on heating by breaking of H-bonds between their nitrogenous bases. This is called Denaturation.

- On cooling, the two complementary strands reunite to form double stranded DNA. (Renaturation).

- Because of this property single stranded DNA segments from different sources can joined, provided these have complementary base pairs. This is called Annealing.

2. Artificial synthesis of genes in vitro :

— Dr. Hargovind Khurana (1972) among with his colleagues first reported total synthesis of artificial genes Tyrosine-t-RNA. In vitro with a potential for functioning within a living cell.

3. Restriction endonuclease or Restriction enzymes :

- The restriction endonuclease are enzymes that recognised specific nucleotide sequence in DNA and cleave the DNA double helix at or near these restriction sites, called Target sites.
- These enzymes are apparently elaborated in bacteria as a protection against the entry of foreign or viral DNA. These enzymes are able to identified self and nonself DNA and cleave foreign DNA. These were first reported by W. Arber in 1962, who noticed that when DNA of a Bacteriophage entered a first Bacterium, it was cut into smaller pieces.
- However first restriction enzymes was isolated by Meselson and Yuan from E. Coli.
- Real break through came when restriction enzymes HINDII was isolated from bacterium Haemophilus Influenza Rd. by Kelly and Smith (1970) and Smith.

* Types :

1. Type I Restriction endonuclease :

- These interact with a modified recognition sequence in double stranded DNA. These cleave only one strand of DNA and at an apparantely random site. These enzymes create a gap of about 75 nucleotide in length by releasing acid soluble oligonucleotide. These enzyme requires mg^{++} ion, ATP and S-Adenosyl Methionine co-factor or Restriction.

[28-11-18]

2. Type II Restriction endonuclease :

- These enzymes recognise specific DNA nucleotide sequences.

- These cleave both Polynucleotide chains within or near to the Palindromic sequences.

- Palindromes are base pair sequences that read the same forward and backward in 5'-3' direction. in both strands of DNA.

eg. (a) 5'- N N G ↓ A A T T C N N - 3'
3'- N N C T T A A G N N - 5'

- These enzymes produces DNA fragments of definite length and sequence. These requires only Mg^{++} ions for restriction.
- Restriction enzyme Type II are used in gene manipulation.

3. Type III Restriction endonuclease:
- These cleave double stranded DNA at well defined sites and requires Mg^{++} and ATP, partially S-Adenosyl methionine. These enzymes are intermediate between Type I and Type II.

* Recognition sites or Target sites:
- Restriction endonuclease cleaves the DNA molecules only in the region where the nucleotides sequence ready the same on both the strands in 5'-3' direction.
- These regions in DNA are called Recognition site or Target site.

⊛ DNA cleavage Style :

- The restriction enzyme cuts DNA molecule by cleavage in any one of following two styles—

i) Sticky end style :

- In this style or cleavage the target sequence is asymmetrical.
- The staggered curves in two strands of DNA are same nucleotides apart producing complementary single stranded protruding ends.
- These unpaired protruding ends are called Sticky or cohesive end.

- Enzyme EcoR1, Hind II, BglII etc produce sticky ends.

eg: EcoR1

5'.... G A A T T C .... 3'

3'.... C T T A A G .... 5'

* cleavage products :

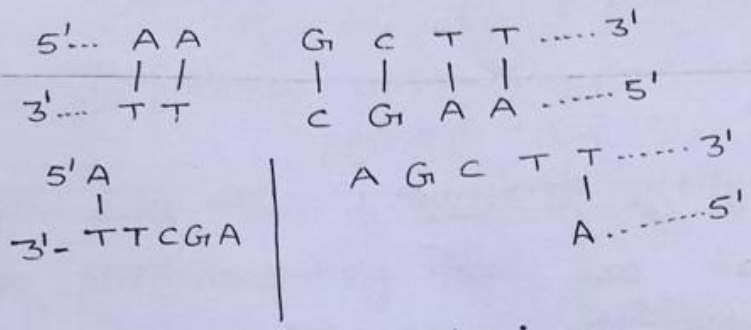
5'- G | AATTC ..... 3'

3'- CTTAA | G ..... 5'

Hind II

5'.... A A G C T T .... 3'

3'.... T T C G A A .... 5'



2. Blunt ends Style :

- Restriction enzymes like AluI, HaeIII, HindII, SmaI make cuts across both strands on DNA at the same position.

- The DNA fragments produced have blunt ends. This enable DNA of all kinds to be broken into a wide variety of fragment, depending on the restriction enzymes and also to join two defined fragments without introducing any additional material between them.