Revision Notes on Biotechnology

Recombinant DNA technology

Definition:

Genetic engineering, a kind of biotechnology, is the latest branch in applied genetics dealing the alteration of the genetic makeup of cells by deliberate and artificial means. Genetic engineering involves transfer or replacement of genes, so also known as recombination DNA technology or gene splicing.

Tools of genetic engineering:

(1) Two enzymes used in genetic engineering are restriction endonuclease and ligases.

(2) R.E. is used to cut the plasmid as well as the foreign DNA molecules of specific points while ligase is used to seal gaps or to join bits of DNA.

(3) The ability to clone and sequence essentially any gene or other DNA sequence of interest from any species depends on a special class of enzymes called restriction endonucleases.

(4) Restriction endonucleases are also called as molecular scissors or 'chemical scalpels'.

(5) Restriction endonucleases cleave DNA molecules only at specific nucleotide sequence called restriction sites.

(6) The first restriction enzyme identified from a bacterial strain is designated I, the second II and so on, thus, restriction endonuclease EcoRI is produced by Escherichia coli strain RY 13.

(7) Restriction enzyme called EcoRI recognizes the sequence

(8) It then cleaves the DNA between G and A on both strands. Restriction nucleases make staggered cuts; that is, they cleave the two strands of a double helix at different joints and blunt ended fragments; that is, they cut both strands at same place.

Steps of recombinant DNA technology

(1) Isolating a useful DNA segment from the donor organism.

(2) Splicing it into a suitable vector under conditions to ensure that each vector receives no more than one DNA fragment.

(3) Producing of multiple copies of his recombinant DNA.

(4) Inserting this altered DNA into a recipient organism.

(5) Screening of the transformed cells.

Vectors:

Vector in genetic engineering is usually a DNA segment used as a carrier for transferring selected DNA into living cells. These are as follows:

(1) **Plasmid:** Plasmid is extra chromosomal, closed circular double stranded molecules of DNA present in most eukaryotes. All plasmid carry replicons pieces of DNA that have the genetic information required to replicate. Plasmid pBR 322 was one of the first widely used cloning vectors, it contain both ampicillin and tetracycline resistance genes.

(2) **Phage:** It is constructed from the phage *l* chromosomes and acts as bacteriophage cloning vectors.

(3) **Cosmid:** The hybrids between plasmid and the phage *l* chromosome give rise to cosmid vectors.

(4) Beside all these there are artificial chromosomes like

(i) BACs (Bacterial Artificial chromosomes)

(ii) YACs (Yeast Artificial chromosomes)

(iii) MACs (Mammalian Artificial chromosomes) are very efficient vectors for eukaryotic gene transfers.

Application of recombinant DNA technology:

The technique of recombinant DNA can be employed in the following ways.

(1) It can be used to elucidate molecular events in the biological process such as cellular differentiation and ageing. The same can be used for making gene maps with precision.

(2) In biochemical and pharmaceutical industry, by engineering genes, useful chemical compounds can be produced cheaply and efficiently which is shown in table.

Applications of recombinant DNA products

Medically useful recombinant products	Applications
Human insulin	Treatment of insulin-dependent diabetes
Human growth hormone	Replacement of missing hormone in short stature people
Calcitonin	Treatment of rickets
Chronic gonadotropin	Treatment of infertility
Blood clotting factor VIII/IX	Replacement of clotting factor missing in patients with Haemophilia A/B
Tissue plasminogen activator	Dissolving blood clots after heart attacks and strokes
Erythropoitin	Stimulation of the formation of erythrocytes (RBCs) for patients suffering from anaemia during kidney dialysis or side effects of AIDS patients treated by drugs
Platelet derived growth factor	Stimulation of wound healing
Interferon	Treatment of pathogenic viral infections, cancer
Interleukins	Enhancement of action of immune system
Vaccines	Prevention of infectious diseases such as hepatitis B, herpes, influenza, pertussis, meningitis, etc.

Cloning:

Cloning is the process of producing many identical organisms or clones. In this process nucleus of ovum (n) is removed and replaced by nucleus of diploid cell of same organism. Now the egg with 2*n* nucleus is transferred to the uterus of mother to have normal pregnancy and delivers clone of itself.

Examples of organism cloning

(1) Cloning of sheep was done by **Dr. Ian Wilmut** (1995) of Roslin Institute, Edinberg U.K. and normal healthy lamb (DOLLY) was born in Feb, 1996. This lamb was exactly similar to her mother.

(2) The first cloned calves George and Charlie were born in January 1998.

(3) ANDI was the world's first genetically altered primate produced by inserting a jelly fish gene into the embryo of a rhesus

monkey.

(4) Scientist at Scotland cloned POLLY and MOLLY. Unlike Dolly, polly and molly were transgenic (they carried human protein gene) polly and molly were born in july 1997.

(5) **Brigitte Boissliar**, a 46-year old french chemist announced the creation of the world's first cloned human boby nicknamed "Eve" (December 2002).

Polymerase chain reaction (PCR):

(1) It was developed by **Kary Mullis** in 1983 and won Nobel Prize in 1993.

(2) PCR is a method for amplifying a specific piece of DNA molecule without the requirement for time-consuming cloning procedure.

(3) This process require Target DNA, a heat stable DNA polymerase, which work at optimum temperature of 70°C usually Taq DNA and four types of nucleotides with small single stranded strands of DNA of about 20 nucleotide called primers, produce multiple copy of desired DNA.