Recombinant DNA Technology

Defn

Genetic manipulation in the laboratory involving the isolation & Cloning of specific DNA are called Recombinant DNA Technology /Genetic Engineering

Gene of one species is transferred to another living organism by artificial means,is known as recombinant DNA Technology

Used in medicine& Agriculture

Applications

1)Large scale Quantitative preparation of biomolecules

Egs: Insulin,GH,FSH,LH
PTH,Erythropioetin,interferons,interleukins

FactorVIII,IX ,ADA For treating SCID

2)Prepn of Vaccines without contamination

3)Specific probes to detect

Bacterial& Viral infection in blood & tissues,
Oncogenes,mutations,DNA Fingerprinting,Gene mapping etc

4)Gene Therapy

5)In Agriculture for production of high yielding disease resistant crops

Recombinant DNA Technology

1) Isolation of Human desired gene & Selection of Vector

2)Preparation of Chimeric DNA (Cutting with RE, Incubate for Annealing, Add DNA ligase)—(Homopolymer tailing)

3)Cloning of Chimeric DNA

4)Transfection of Vector(Plasmid) into Host(E.Coli) (Incubation in Hypertonic medium containing Calcium for imbibing of plasmid into host cell)

5)Selection of Desired colony by culturing the tranfected host(E.coli) in medium containing Ampicillin &Tetracyclin, then transfer to a Replica plate (another agar plate) medium containing Chloramphenicol.(To select colonies killed by Chloramphenicol)

6)Gene amplification by adding sublethal dose of Protein synthesis inhibitor into medium & separate plasmid DNA from bacteria,& cut inserted foreign DNA from plasmid using same RE.

7)Vector Expression

To produce human protein bacterial promoter gene is added upsteam of Human gene.

Promoter gene added vector is called Expression Vector