Recombinant DNA Technology
(Genetic Engineering)

*Steps:-

→ prep^n of specific human gene
→ " " chimeric DNA
→ transfect^n / transformat^n of vector into the host.
→ select^n of colony containing the chimeric DNA.
→ expression of gene to produce the desired pdt.

i) prep^n of specific human gene:-

→ specific mRNA is first selected from the tissue.
→ from the specific mRNA, the cDNA (complementary DNA) is prepared using reverse transcriptase.

ii) prep^n of chimeric DNA:-

→ a vector carrying a foreign DNA is called chimeric DNA (hybrid DNA) (rDNA).
→ a vector DNA (eg:- plasmid) is cut backwards with a specific RE (restrict^n endo-nuclease).

sticky or blunt ends.
→ if EcoRI is used, sticky ends are produced with TTAA sequence on one strand & AATT sequence on the other strand.
→ human DNA is also cut with the same RE
→ same sequence are generated at the ends of the cut pieces.
→ now the sticky ends of both vector & human DNA have complementary sequences.
→ when vector DNA & human cut piece DNA are incubated together, annealing take place.
→ fragments are joined together by DNA ligase
→ a circular chimeric DNA is formed.

Restrict^n Endonuclease (RE) / Molecular Scissors

→ certain enzymes restrict the entry of phages into host bacteria, hence the name RE.
→ they prevent the replicat^n of phage by cleaving its DNA at specific points.
→ RE cut DNA at specific sites with palindrom (greek word means 'to run backwards') sequence - reads the same forward & backwards, eg:- malayalam, madam.
→ also called 'inverted repeat sequence'.
→ RE are named after the species & strain

of bacteria & the order of discovery. iv) select^{n} of colonies:-

eg:- EcoRI isolated from E.coli RY13

Eco - species of bacteria

R - strain

→ R - species

→ I - roman numeral one is the order of discovery.

→ RE cut DNA in 2 ways & produce either sticky or blunt ends. "expression vectors".

→ sticky end:- short overhanging ss sequence that are complementary in nature

→ blunt ends:- non-overhanging sequence. Applicat?

Expression Vectors

→ if a vector carrying foreign gene is translated to the protein, it is called

→ human proteins can be harvested from the bacterial culture.

→ human insulin, GH, hepatitis B vaccine, HPV vaccine etc can be produced by this technique.

→ human insulin is the first protein synthesized by genetic engineering.

→ monoclonal antibodies are used in diagnostic tests, to transport drugs, toxins & in cancer therapy.

→ recombinant HIV protein is used in HIV elisa testing.

→ gene therapy helps to treat genetic diseases by replacing a defective gene with normal one using cDNA technology.

iii) Transfect^{n}

→ process of introducing vector (plasmid) into a host.

→ the host E.coli & vectors are incubated in hypertonic medium with Ca for two min.

→ opens up Ca channels & vectors enters the cell.

* Cloning of chimeric DNA:-

→ bacteria are cultured

→ as the host cell multiplies, it forms a clone in which