Important terms

Genetic engineering- Introduction of manipulated genetic material into a cell where it replicates and passed on to progeny cells.

Recombinant DNA technology-Breakage of a DNA molecule at two desired places to isolate a specific segment and its insertion in another DNA molecule at appropriate place. The product is called as rDNA molecule. The procedure is called as recombinant DNA technology

Clone-Asexual progeny of a single cell or an individual.

History of Genetic engineering

1917	Karl Ereky coined the term 'Biotechnology'
1940	A Jost coins the term "Genetic Engineering"
1970	First restriction endonuclease isolated. The Nobel Prize in Medicine 1978 was jointly awarded to Werner Arber, Daniel Nathans and Hamilton O Smith for the discovery of restriction enzymes and their application to problems of molecular genetics in 1970
1973	Recombinant DNA technology was developed by Stanley N. Cohen and Herbert Boyer
1978	Genentech produced Human insulin in *E.coli*
1980	US Supreme court declared genetically modified micro-organisms can be patented (Anand Chakrabarty)
1982	First commercial automated DNA synthesized and sold (Kangas *et. al.*)
1988	Kary B Mullis publishes Amplification of DNA by Polymerase Chain Reaction (PCR)
1996	*S. cerevisiae* genome sequenced
2000	*Arabidopsis* genome sequenced
2001	Vitamin A rich golden rice developed
2001	Human genome sequenced

2002 Complete human gene microarrays commercially available

2006 Nobel Prize Medicine-The discoveries of Andrew Z. Fire and Craig C. Mello of RNA Interference (RNAi)

Steps of rDNA technology

I. Isolation of gene (vector and target gene)

II. Joining (Ligation) of target gene into vector DNA molecule

III Introduction into host cell (Transforming)

IV Cloning

i)Multiplication of rDNA molecule

ii)Division of host cell

iii) Numerous cell division resulting in a clone

V. Selecting (Screening of recombinants)

The basic procedure of rDNA technology

I Generating DNA Fragments

II Joining DNA into Vector (Ligation)

III Introduction of rDNA into Host (Transformation)

IV Screening of recombinants

I Generating DNA Fragments

1. Mechanical shearing- High speed mixing in blender, Sonication
2. Restriction digestion-Using restriction endonucleases

3. c-DNA synthesis- Alternative method (using reverse transcriptase)
4. Hybridization method- mRNA forms a complex with cDNA
5. Chemical Synthesis- Automated synthesizers- Kangas et al. 1982
6. PCR Amplification
7. RT-PCR

II Joining DNA into Vector (Ligation)

1. Blunt end ligation
2. Sticky end ligation
3. Linkers
4. Adaptors
5. Homopolymer tailing

III Introduction of rDNA into Host (Transformation)

Transformation of bacteria or Methods of gene transfer

I Biological methods

Target cells need to be infected by virus or bacteria

1. Transduction 2. Bactoinfection

II Non-biological methods

Vectors not required

1. Chemical transfection

a. Calcium chloride mediated DNA uptake

b. Lipofection

2. Physical transfection

a. Electroporation

b. Microinjection

c. Particle gun method or Biolistic gene transfer

d. Ultrasonication

e. Micro laser mediated gene transfer

IV Screening of recombinants

1. Genetic methods- Antibiotic resistance, Blue white screening
2. Immunochemical method
3. Hybridization method- Colony hybridization
4. Blotting techniques-Sourthern, Northern and Western