Biotechnology - Principles and Processes

Biotechnology is the field of molecular biology dealing with the techniques of using live microorganisms to produce products that are useful to humankind.

Genetic engineering

- It is a technique used by scientists for manipulating genetic material of living organisms.
- It involves artificial synthesis, isolation, modification and transfer of genetic material into a host organism to alter its phenotype.
- DNA cloning/Gene cloning: It is the method of making multiple identical copies of a single gene.
- Plasmid: It is a small, circular, extra-chromosomal genetic material that is capable of self replication.
- Characteristics of plasmid –
    - Has an origin of replication
    - Has a selectable marker
    - One or few cloning sites

Recombinant DNA technology

- It is a set of techniques for altering DNA. It includes the recognition and cloning of genes, the study of the expression of cloned genes and the production of a large number of gene products.

Construction of a Recombinant DNA

- Plasmid (autonomously replicating, circular, extra-chromosomal DNA) is isolated.
- Plasmid DNA is cut with a specific restriction enzyme at specific locations.
- The DNA of interest (to be inserted) is also cut with the same restriction enzyme.
- The DNA of interest is hybridised with the plasmid with the help of DNA ligase to form a **Recombinant DNA**.
- Recombinant DNA is then transferred into host for cloning.

i. **Tools of recombinant DNA technology –**

i. They are also known as molecular scissors.

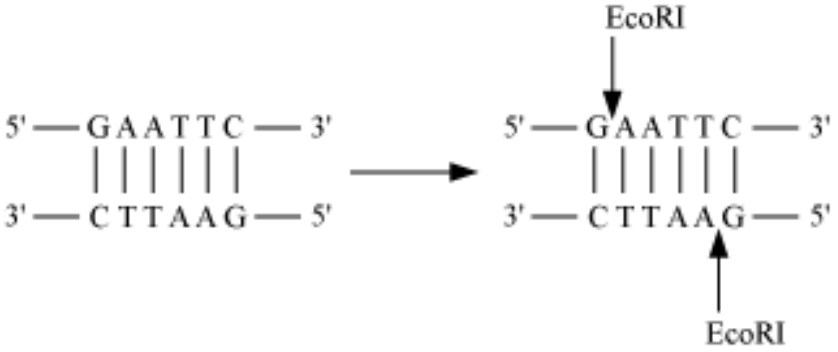
- There are two types of restriction enzymes –
  - **Endonuclease:** It removes the nucleotide from DNA fragments at specific positions within the DNA.
  - **Exonuclease:** It makes a cut at the 5' or the 3' end of DNA.
- **Naming of restriction endonuclease enzyme (for example, in EcoRI)**

- First letter represents the genus of bacteria
  - The next two letters represent the species from which the enzyme is isolated.
  - The second capital letter represents the name of the strain.
  - The roman number indicates the order in which the enzyme is isolated.

Restriction endonuclease recognises a particular sequence known as **palindromic sequence**.

- **Palindrome** is a sequence of base pairs that reads the same on the forward strand and the reverse strand.
- **Action of the restriction endonuclease enzyme**
  - The recognition sequence for the restriction enzyme EcoRI is

- EcoRI recognises this particular palindromic sequence and makes a cut between the adjacent guanine and adenine nucleotides, thereby producing overhanging, single-stranded pieces called sticky ends.



- The generated sticky ends can be joined by DNA ligase to form recombinant DNA molecule.
- **Gel electrophoresis:** It is a technique used for separating and isolating the DNA fragments generated by the action of the endonuclease enzyme.
- Electric field is applied to the electrophrosis matrix (commonly agarose gel) and negatively charged DNA moves towards anode.
- Fragments get separated according to their size.

Cloning vectors: Examples include plasmids and bacteriophage. Cloning vectors consist of origin of replication (ori), selectable marker and one or few cloning sites.

- **Insertional inactivation:** It is a technique to select recombinant DNA on the basis of their ability to produce colour in the presence of chromogenic substrate.
- *Agrobacterium tumifaciens* acts as a vector for cloning genes in plants.
- *Retroviruses* can be disarmed and used as cloning vectors in animals.

(iii) Transformation: It is the method of uptaking foreign DNA particle into a bacterial cell. The following methods are used for transformation.

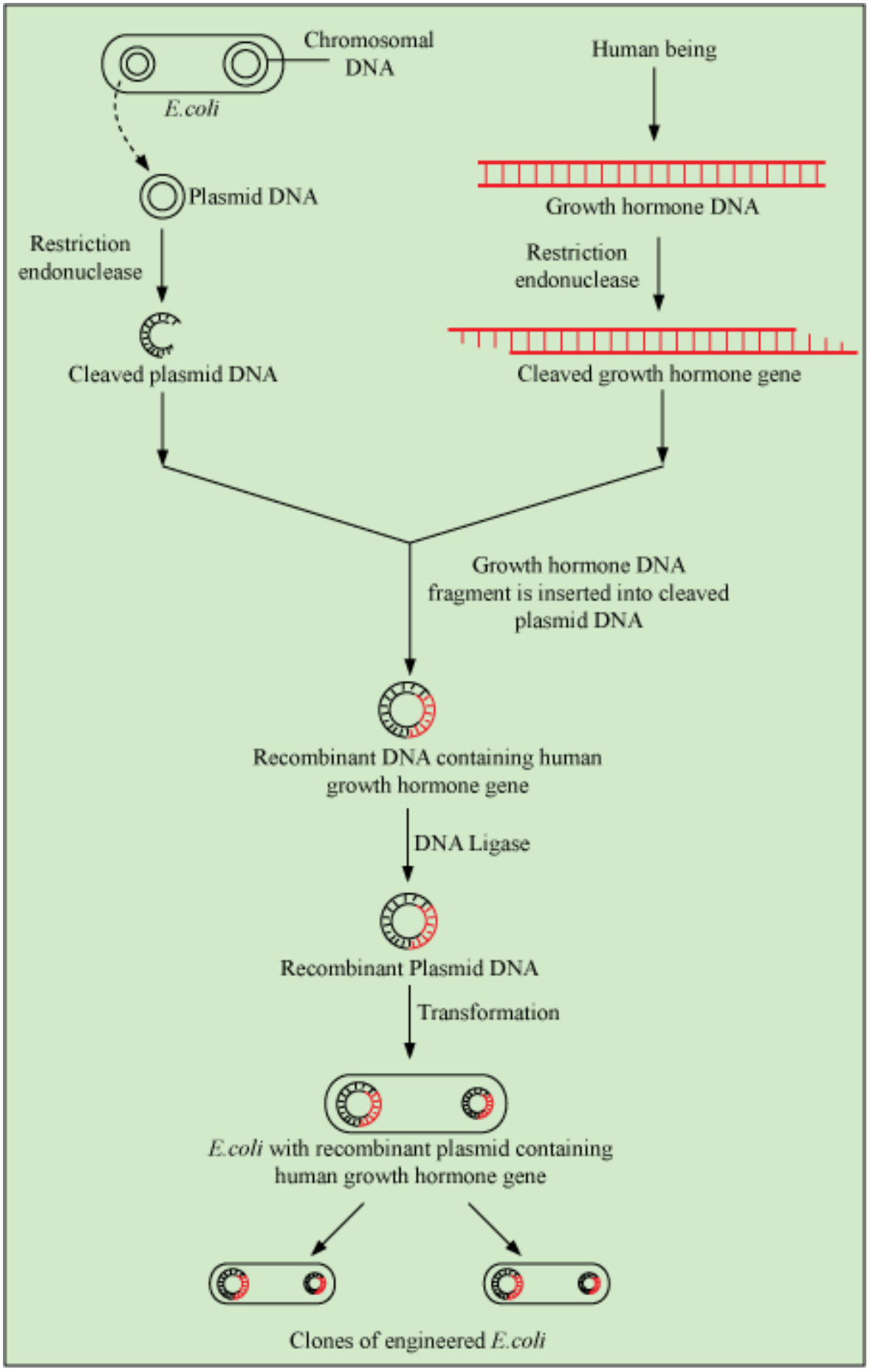
- **Microinjection:** It involves the injecting of recombinant DNA directly into the host cell using micropipettes.
- **Biolistics or gene gun:** It involves the bombardment of gold or tungsten particles coated with DNA into a plant cell.

Steps involved in recombinant DNA technology –

- **Isolation of genetic material:** This is the method of obtaining purified DNA. It is achieved by lysozyme in bacteria, by cellulase in plant cell and by chitinase in fungus.

- **Cutting of DNA at specific location:** It is performed by using restriction endonuclease and then separating the DNA fragments using agarose gel electrophoresis.

- **Amplification of gene:** The gene is amplified using polymerase chain reaction.
    - **Polymerase chain reaction** is a method of amplifying specific regions of DNA strand. It involves three steps – denaturation, annealing and extension.
    - This technique requires a set of primers and the thermostable DNA polymerase enzyme.
    - The thermostable DNA polymerase enzyme is isolated from the bacterium called *Thermus aquaticus.* The enzyme is resistant to denaturation by heat treatment.
- **Ligation of DNA fragments into the vector for cloning**
- **Insertion of recombinant DNA into recipient cells**
    - It is done by making the cell competent to take up DNA from its surroundings. This method is known as transformation.
- **Obtaining the gene product**
    - It involves culturing host cells in a medium and then extracting the desired product.
    - **Bioreactors**: Used for large scale production of desired protein; two types of bioreactors used –
        - Simple stirred-tank bioreactor
        - Sparged stirred-tank bioreactor
- **Downstream processing:** It is the process of separation and purification of recombinant protein so that product is ready for marketing.
- **Diagrammatic representation of recombinant DNA technology**


Chromosomal DNA
E.coli
Plasmid DNA
Restriction endonuclease
Cleaved plasmid DNA
Human being
Growth hormone DNA
Restriction endonuclease
Cleaved growth hormone gene
Growth hormone DNA fragment is inserted into cleaved plasmid DNA
Recombinant DNA containing human growth hormone gene
DNA Ligase
Recombinant Plasmid DNA
Transformation
E.coli with recombinant plasmid containing human growth hormone gene
Clones of engineered E.coli