

5. BIOTECHNOLOGY

PRINCIPLES, PROCESSES & APPLICATIONS

Single Page Simple Synopsis

Biotechnology utilises biomolecular processes and living organisms to develop products and technologies that improve our lives and the health of our planet.

It operates on two core principles 1) Genetic engineering (modifying DNA) and

2) Bioprocess engineering (large-scale sterile manufacturing).



FOUR CORE CONCEPTS:

1. **Genetic Engineering:** The manipulation of an organism's genetic makeup by introducing or altering DNA, changing its phenotype.

2. **Bioprocess Engineering:** The maintenance of sterile, controlled conditions to grow microbes or cells on an industrial scale for producing products like vaccines, antibiotics, and enzymes.

3. **Processes (Recombinant DNA Technology)**

The primary method of modern biotechnology involves creating Recombinant DNA (rDNA) through the following sequential steps:

i) **Isolation:** Extracting pure, high-quality genetic material (DNA) from the source organism.

ii) **Fragmentation:** Using *Restriction Endonucleases* (molecular scissors) to cut the DNA and isolate the specific gene of interest.

iii) **Amplification:** Using the *Polymerase Chain Reaction (PCR)* to make multiple copies of the desired DNA fragment.

iv) **Ligation:** Joining the target gene with a vector (like a bacterial plasmid) using the enzyme *DNA Ligase*.

v) **Transformation:** Introducing the rDNA into a suitable host organism **Ex:** *E. coli*.

vi) **Culturing:** Growing the transformed host cells on a large scale inside bioreactors to maximize yield.

vii) **Downstream Processing:** Extracting, purifying, and packaging the final biological product.

4. **Real-World Applications:**

i) **Agriculture:** Development of genetically modified (GM) crops such as *Bt Cotton* for natural pest resistance, and improved nutritional profiles like *Golden Rice*.

ii) **Medicine:** Large-scale production of life-saving therapeutics, including synthetic human insulin, gene therapy, and molecular diagnostics.

iii) **Environment:** Utilizing bioremediation techniques, where specialized microbes are used to clean up environmental pollutants, toxic waste, and oil spills.

ROOT POINTS

1. **Biotechnology** is a branch of modern biology that has brought qualitative improvements to human health and food production.
2. **Herbert Boyer** discovered in 1969 that certain restriction enzymes from *E. coli* could cut DNA strands in a specific fashion.
3. Techniques like **in vitro fertilisation** (“test-tube” babies), gene synthesis, DNA vaccines, and gene correction are all parts of biotechnology.
4. The **European Federation of Biotechnology (EFB)** defines it as the integration of natural science and organisms (or parts thereof) for products and services.
5. **Genetic engineering** involves techniques to alter genetic material (DNA/RNA) to change the phenotype of a host organism.
6. DNA replication is initiated at a specific sequence called the **origin of replication (ori)**.
7. **Cloning** is the process of making multiple identical copies of any template DNA.
8. **Restriction enzymes**, or “molecular scissors,” are used to cut DNA at specific locations.
9. **DNA ligase** is the enzyme used to join the cut DNA ends together.
10. **Plasmids** act as **vectors** to deliver alien DNA into a host organism, similar to how a mosquito transfers parasites.
11. **Transformation** is the procedure of introducing a piece of DNA into a host bacterium.
12. There are now over **900 restriction enzymes** isolated from more than 230 bacterial strains.
13. **Exonucleases** remove nucleotides from the ends of DNA, while **endonucleases** cut at specific internal positions.
14. A **DNA palindrome** reads the same on both strands when the orientation (e.g., 5' to 3') is kept the same.
15. **Gel electrophoresis** is used to separate DNA fragments, which are negatively charged and move toward the **anode**.
16. **Agarose**, a natural polymer from seaweeds, is the most common matrix for electrophoresis.
17. DNA is visualized by staining with **ethidium bromide** and exposure to **UV radiation**, appearing as bright orange bands.
18. **Elution** is the process of cutting out and extracting separated DNA bands from the agarose gel.
19. **Selectable markers** (like antibiotic resistance genes) help identify and eliminate non-transformants.
20. **Agrobacterium tumefaciens** uses a **Ti plasmid** to deliver “T-DNA” and transform plant cells into tumors.

21. **Micro-injection** is a method where DNA is directly injected into an animal cell's nucleus.
22. **Biolistics (gene gun)** uses high-velocity gold or tungsten micro-particles coated with DNA for plant cells.
23. **PCR (Polymerase Chain Reaction)** is used for in vitro **amplification** of a gene of interest.
24. **Downstream processing** refers to the separation and purification of a product before it is marketed.
25. **Tissue culture** allows whole plants to be regenerated from **explants** (any part of a plant) due to **totipotency**.
26. The first human hormone produced by recombinant DNA technology is **insulin**. [NEET-2014]
27. In Bt cotton, the Bt toxin present in plant tissue as protoxin is converted into active toxin due to **alkaline pH of the insect gut**. [NEET-2015]
28. The crops engineered for glyphosate are resistance/tolerant to **herbicides**. [NEET-2015]
29. Golden rice is a genetically modified crop plant where the incorporated gene is meant for biosynthesis of **vitamin A**. [NEET-2015]
30. The introduction of T-DNA into plants involves infection of the plant by **Agrobacterium tumefaciens**. [NEET-2015]
31. Activation of protoxin to active Bt toxin of *Bacillus thuringiensis* of boll worm [NEET-2019] is triggered by **Alkaline pH of gut**.
32. Bt cotton variety that was developed by the introduction of toxin gene of *Bacillus thuringiensis* (Bt) is resistant to **insect pests**. [NEET-2020]
33. DNA strands on gel stained with ethidium bromide when viewed under UV radiation appear as **Bright orange bands**. [NEET-2021]
34. A specific recognition sequence identified by endonucleases to make cuts at specific positions within the DNA is **palindromic nucleotide sequence**. [NEET-2021]
35. Separation of DNA fragments is done by a technique known as **Gel electrophoresis**. [NEET-2022]
36. Hind II always cuts DNA molecules at a particular point called recognition sequence and it consists of **6 base pairs (bp)**.
37. **Probe** is not a cloning vector. [NEET-2023]
38. In gene gun method used to introduce alien DNA into host cells, micro particles of **Tungsten or gold** metal as used. [NEET-2023]
39. Polymerase chain reaction (PCR) amplifies DNA following the equation **2^n** . [NEET-2025]