Vidya Bhawan Balika Vidyapeeth Lakhisarai

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Subject Biology

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d. **Vectors for cloning genes in plants and animals**– A*grobacterium tumefactions* (pathogen of dicot plant) is able to deliver a piece of DNA known as 'T-DNA" to transform normal plant cells into a tumor and direct these tumor cells to produce the chemicals required by the pathogen. Retroviruses in animals have the ability to transform normal cells into cancerous cells. The tumor inducing (Ti) plasmid of Agrobacterium tumefaciens has been modified into cloning vector having no more pathogenic to plant. Similarly retrovirus have been modified into cloning vector for animals.

Competent host (For Transformation with Recombinant DNA)

1) Simple chemical treatment with divalent calcium ions increases the efficiency of host cells (through cell wall pores) to take up the rDNA plasmids.
2) rDNA can also be transformed into host cell by incubating both on ice, followed by placing them briefly at 42oC (Heat Shock), and then putting them back on ice. This enables the bacteria to take up the recombinant DNA.
3) In **Microinjection** method, rDNA is directly injected into the nucleus of cells by using a glass micropipette.
4) **Biolistics / Gene gun method,** it has been developed to introduce rDNA into mainly plant cells by using a Gene / Particle gun. In this method, microscopic particles of gold / tungsten are coated with the DNA of interest and bombarded onto cells.
5) The last method uses *"Disarmed Pathogen"* Vectors (*Agrobacterium tumefaciens*), which when allowed to infect the cell, transfer the recombinant DNA into the host.

Processes of Recombinant DNA Technology

Recombinant DNA technology involves several steps in specific sequence-

a. Isolation of DNA

b. Fragmentation of DNA by restriction endonucleases

c. Isolation of a desired DNA fragment

d. Ligation of the DNA fragment into vector

e. Transforming the recombinant DNA into the host

f. Culturing the host cells in a medium at large scale

g. Extraction of the desired product.