

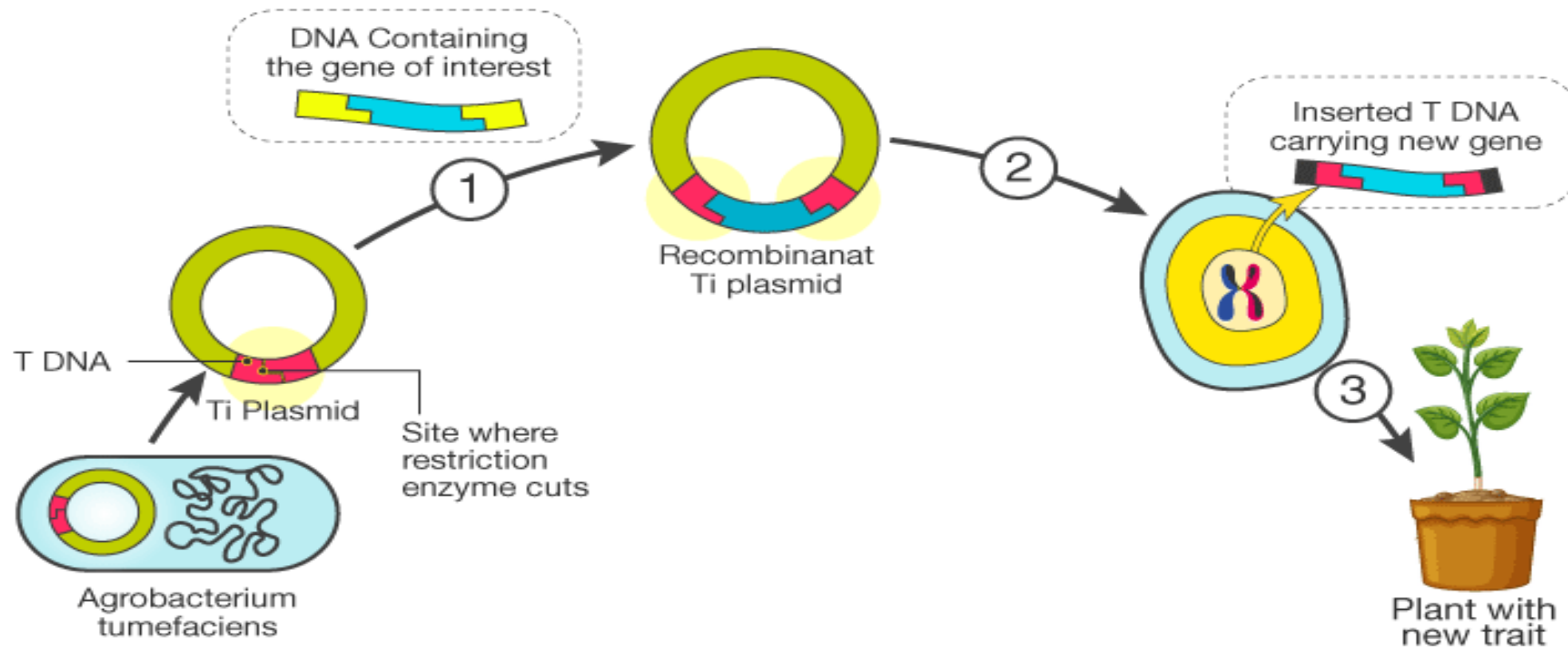
RECOMBINANT DNA TECHNOLOGY

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INTRODUCTION:

- Genetic engineering also known as recombinant DNA technology means altering the genes in a living organism to produce a genetically modified organism (GMO) with a new genotype.
- Various kinds of genetic modification are possible :
 1. Inserting a foreign gene from one species into another
 2. Forming a transgenic organism
 3. Altering an existing gene so that its product is changed.

RECOMBINANT DNA TECHNOLOGY



1 Treat foreign DNA and plasmid with restriction enzyme and DNA ligase.

2 Introduce the recombinant plasmid into cultured plant cells.

3 Regenerate new plant from cultured cells.

Tools Of Recombinant DNA Technology:

- The enzymes which include the restriction enzymes help to cut, the polymerases- help to synthesize and the ligases- help to bind.
- The restriction enzymes used in recombinant DNA technology play a major role in determining the location at which the desired gene is inserted into the vector genome.
- They are two types, namely Endonucleases and Exonucleases.

- The Endonucleases cut within the DNA strand whereas the Exonucleases remove the nucleotides from the ends of the strands.
- The restriction endonucleases are sequence-specific which are usually palindrome sequences and cut the DNA at specific points.
- They scrutinize the length of DNA and make the cut at the specific site called the restriction site. This gives rise to sticky ends in the sequence.
- The desired genes and the vectors are cut by the same restriction enzymes to obtain the complementary sticky notes, thus making the work of the ligases easy to bind the desired gene to the vector.

- The vectors – help in carrying and integrating the desired gene.
- These form a very important part of the tools of recombinant DNA technology as they are the ultimate vehicles that carry forward the desired gene into the host organism.
- Plasmids and bacteriophages are the most common vectors in recombinant DNA technology that are used as they have a very high copy number.
- The vectors are made up of an origin of replication- This is a sequence of nucleotide from where the replication starts, a selectable marker – constitute genes which show resistance to certain antibiotics like ampicillin; and cloning sites – the sites recognized by the restriction enzymes where desired DNAs are inserted.

- Host organism – into which the recombinant DNA is introduced. The host is the ultimate tool of recombinant DNA technology which takes in the vector engineered with the desired DNA with the help of the enzymes.
- There are a number of ways in which these recombinant DNAs are inserted into the host, namely – microinjection, biolistics or gene gun, alternate cooling and heating, use of calcium ions, etc.

Process of Recombinant DNA Technology:

- The complete process of recombinant DNA technology includes multiple steps, maintained in a specific sequence to generate the desired product.
- Step-1. Isolation of Genetic Material.
- The first and the initial step in Recombinant DNA technology is to isolate the desired DNA in its pure form i.e. free from other macromolecules.
- Step-2. Cutting the gene at the recognition sites.
- The restriction enzymes play a major role in determining the location at which the desired gene is inserted into the vector genome. These reactions are called 'restriction enzyme digestions'.

- Step-3. Amplifying the gene copies through Polymerase chain reaction (PCR).
- It is a process to amplify a single copy of DNA into thousands to millions of copies once the proper gene of interest has been cut using the restriction enzymes.
- Step-4. Ligation of DNA Molecules

- In this step of Ligation, joining of the two pieces – a cut fragment of DNA and the vector together with the help of the enzyme DNA ligase.
- Step-5. Insertion of Recombinant DNA Into Host.
- In this step, the recombinant DNA is introduced into a recipient host cell. This process is termed as Transformation. Once after the insertion of the recombinant DNA into the host cell, it gets

multiplied and is expressed in the form of the manufactured protein under optimal conditions.

- As mentioned in Tools of recombinant DNA technology, there are various ways in which this can be achieved. The effectively transformed cells/organisms carry forward the recombinant gene to the offspring.

- Application of Recombinant DNA Technology
- DNA technology is also used to detect the presence of HIV in a person.
- Gene Therapy – It is used as an attempt to correct the gene defects which give rise to heredity diseases.
- Clinical diagnosis – ELISA is an example where the application of recombinant

- Recombinant DNA technology is widely used in Agriculture to produce genetically-modified organisms such as Flavr Savr tomatoes, golden rice rich in proteins, Bt-cotton to protect the plant against boll worms and lot more.
- In the field of medicines, Recombinant DNA technology is used for the production of Insulin.