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For

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Unit 1

Principles of Gene Manipulation

- Recombinant DNA Technology
- Selection and identification of recombinant cells
- Restriction Enzymes, DNA modifying enzymes, Cloning Vectors, Ligation

Recombinant DNA Technology

Recombinant DNA technology involves using enzymes and various laboratory techniques to manipulate and isolate DNA segments of interest. This method can be used to combine DNA from different species or to create genes with new functions. The resulting copies are often referred to as recombinant DNA

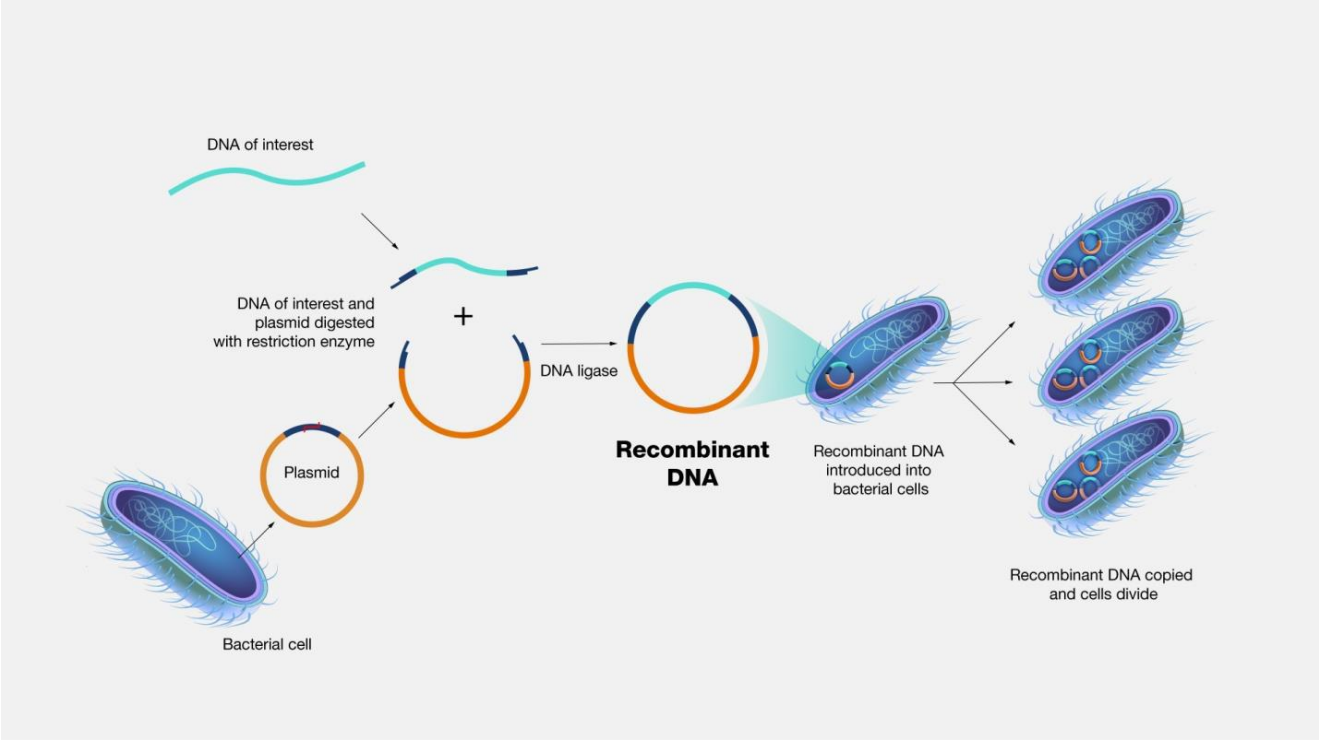

Fig.1 rDNA technology process

A technique mainly used to change the phenotype of an organism (host) when a genetically altered vector is introduced and integrated into the genome of the organism. So, basically, this process involves the introduction of a foreign piece of DNA structure into the genome which contains our gene of interest. This gene which is introduced is the recombinant gene and the technique is called the recombinant DNA technology.

The technology used for producing artificial DNA through the combination of different genetic materials (DNA) from different sources is referred to as Recombinant DNA Technology. Recombinant DNA technology is popularly known as genetic engineering. The recombinant DNA technology emerged with the discovery of **restriction enzymes** in the year 1968 by Swiss microbiologist Werner Arber, Inserting the desired gene into the genome of the host is not as easy as it sounds. It involves the selection of the desired gene for administration into the host followed by a selection of the perfect vector with which the gene has to be integrated and recombinant DNA formed. Thus the recombinant DNA has to be introduced into the host. And at last, it has to be maintained in the host and carried forward to the offspring.

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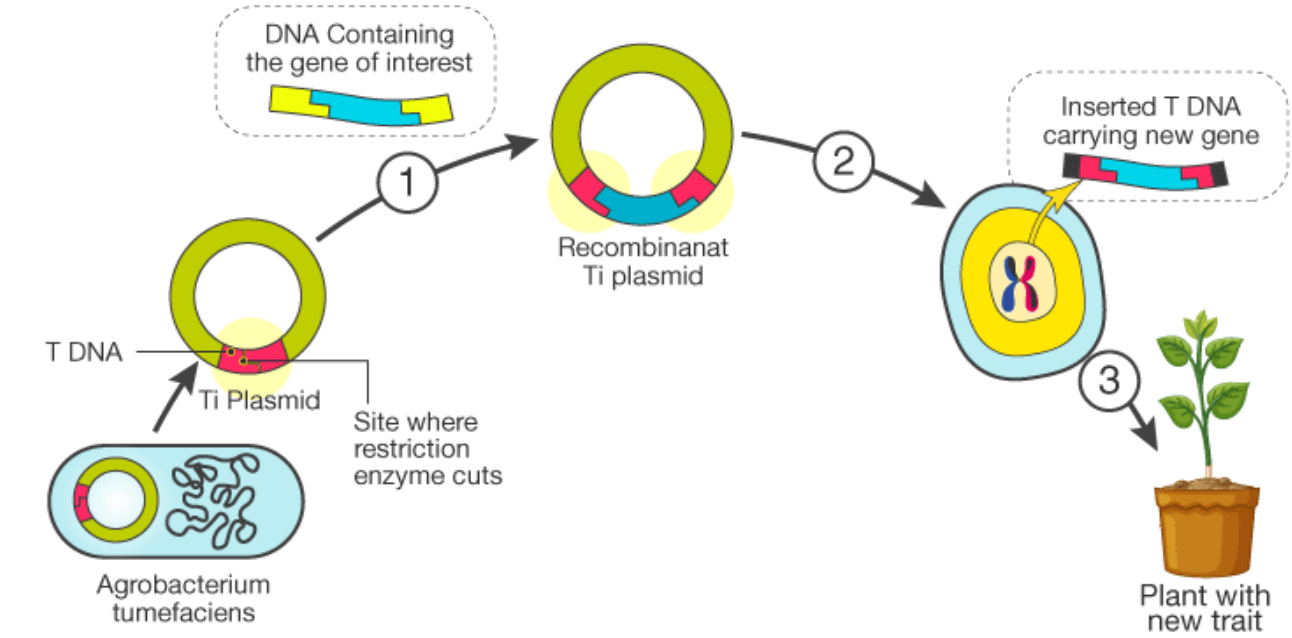

Fig.2 rDNA technology process in plant cell. 1. Foreign DNA and plasmid with restriction enzyme and DNA ligase. 2 introduce recombinant plasmid into cultured plant cells. 3. Regenerate new plant from cultured cells. .

Tools of Recombinant DNA Technology

The enzymes 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 nucleotides 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 restriction enzymes.

Step-4. Ligation of DNA Molecules.

In this step of Ligation, the 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 the recombinant DNA is inserted into the host cell, it gets multiplied and is expressed in the form of the manufactured protein under optimal conditions.

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, and Bt-cotton to protect the plant against ball worms and a lot more.

- In the field of medicines, Recombinant DNA technology is used for the production of Insulin.

DNA Cloning

A clone is a cluster of individual entities or cells that are descended from one progenitor. Clones are genetically identical as the cell simply replicates producing identical daughter cells every time. Scientists are able to generate multiple copies of a single fragment of DNA, a gene which can be used to create identical copies constituting a DNA clone. **DNA cloning** takes place through the insertion of DNA fragments into a tiny DNA molecule. This molecule is made to replicate within a living cell, for instance, a bacterium. The tiny replicating molecule is known as the carrier of the DNA vector.

Yeast cells, viruses, and Plasmids are the most commonly used vectors. Plasmids are circular DNA molecules that are introduced from bacteria. They are not part of the main cellular genome. It carries genes, which provide the host cell with beneficial properties such as mating ability, and drug resistance. They can be conveniently manipulated as they are small enough and they are capable of carrying extra DNA which is weaved into them.

Applications of Gene Cloning

Listed below are the applications of gene cloning:

- Gene Cloning plays an important role in the medicinal field. It is used in the production of hormones, vitamins and antibiotics.
- Gene cloning finds its applications in the agricultural field. Nitrogen fixation is carried out by cyanobacteria wherein desired genes can be used to enhance the

productivity of crops and improvement of health. This practice reduces the use of fertilizers hence chemical-free produce is generated

- It can be applied to the science of identifying and detecting a clone containing a particular gene which can be manipulated by growing in a controlled environment
- It is used in gene therapy where a faulty gene is replaced by the insertion of a healthy gene. Medical ailments such as leukaemia and sickle cell anaemia can be treated with this principle.

Unit 2

Applications of Genetic Engineering

- Single cell proteins
- Biosensors, Biochips
- Crop and live stock improvement, development of transgenics
- Development of DNA drugs and vaccines

Applications of Genetic Engineering

Medicine, research, industry and agriculture are a few sectors where genetic engineering applies. It can be used on various plants, animals and microorganisms. The first microorganism to be genetically modified is bacteria.

1. In Medicine: Genetic engineering can be applied to:

- Manufacturing of drugs
- Creation of model animals that mimic human conditions and,
- Gene therapy
- Human growth hormones
- Follicle-stimulating hormones
- Human albumin
- Monoclonal antibodies

- Antihemophilic factors
- Vaccines

2. In Research: Genes and other genetic information from a wide range of organisms can be inserted into bacteria for storage and modification, creating genetically modified bacteria in the process.

3. In Industry:

- Transformation of cells in organisms with a gene coding to get a useful protein.
- Medicines like insulin, human growth hormone, and vaccines, supplements such as tryptophan, aid in the production of food (chymosin in cheese making) and fuels are produced using such techniques.

4. In Agriculture:

- Genetically modified crops are produced using genetic engineering in agriculture.
- Such crops are produced that provide protection from insect pests.
- It is used or can be used in the creation of fungal and virus-resistant crops.

5. Genetic engineering can be applied to other areas:

- Conservation
- Natural area management
- Microbial art

Benefits of Genetic Engineering

1. The production of genetically modified crops is a boon to agriculture.
2. The crops that are drought-resistant, disease-resistant can be grown with it.
3. As described earlier, genetic disorders can be treated.
4. The diseases such as malaria, dengue can be eliminated by sterilising the mosquitoes using genetic engineering.
5. Therapeutic cloning

Single cell protein

Single cell protein (SCP) refers to dead and dry cells of microorganisms like yeast, bacteria, fungi, and algae. These SCPs serve as a food or feed supplement and can be an alternative to conventional protein sources. SCP includes a high content of protein with all essential amino acids

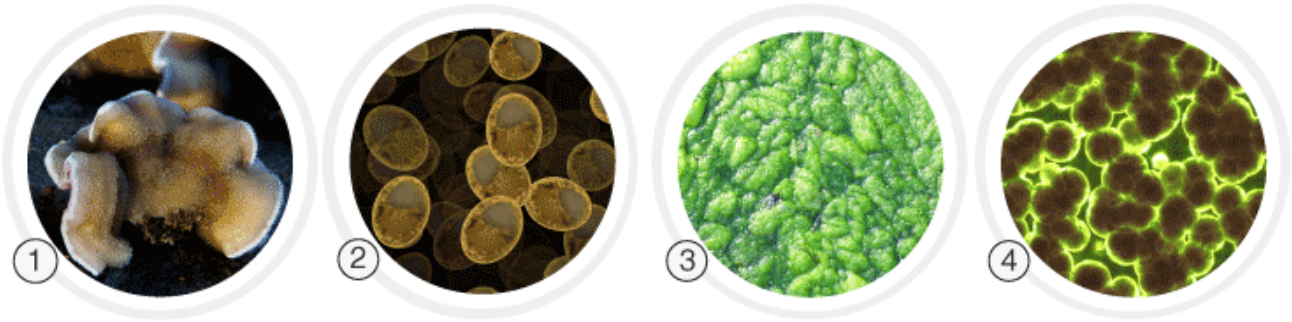

Fig.3 single cell proteins 1. Fungi, 2. Yeast, 3. Algae, 4. Bacteria.

With the continuous rise in the global population, the demand for food has also increased at an alarming rate. The conventional practices of agriculture and animal husbandry have been unable to fulfil the nutritional requirements of the present population. This has resulted in malnutrition owing to deficiency of protein in food.

Single-cell protein refers to the crude a refined or edible protein extracted from pure microbial cultures, dead, or dried cell biomass. They can be used as a protein supplement for both humans and animals. Microorganisms like algae, fungi, yeast, and bacteria have very high protein content in their biomass. These microbes can be grown using inexpensive substrates like agricultural waste viz. wood shavings, sawdust, corn cobs etc. and even human and animal waste.

The microorganisms utilize the carbon and nitrogen present in these materials and convert them into high-quality proteins which can be used as a supplement in both

human and animal feed. The single-cell proteins can be readily used as fodder for achieving fattening of calves, pigs, in breeding fish and even in **Animal Husbandry – Poultry** and Cattle Farming.

Single Cell Protein (SCP) offers an unconventional but plausible solution to this problem of protein deficiency being faced by the entire humanity.

Sources of Single Cell Protein

A list of the microorganisms used for the production of Single Cell Protein is as follows:

Yeast

- Saccharomyces cerevisiae
- Candida tropicalis
- Candida utilis

Algae

- Spirulina (spa)
- Chlorella pyrenoidosa
- Chondrus crispus

Bacteria

- Pseudomonas fluorescens
- Lactobacillus
- Bacillus megaterium

Fungi

- Aspergillus fumigatus
- Aspergillus niger
- Rhizopus cyclopean

Here are the average compositions of the different microorganisms present in the % dry weight of Single-cell protein.

Composition	**Fungi**	**Algae**	**Yeast**	**Bacteria**
Protein	30-45	40-60	45-55	50-65
Fat	2-8	7-20	2-6	1-3
Ash	9-14	8-10	5-10	3-7
Nucleic Acid	7-10	3-8	6-12	8-12

Production of Single-Cell Protein

The production is carried out in the following steps:

1. Selection of suitable strain.
2. Fermentation.
3. Harvesting.
4. Post-harvest treatment.
5. SCP processing for food.

Like any other microbial culture, production of pure microbial cultures for desired protein products requires a nitrogen source, sources of carbohydrates and other nutrients like phosphorus to support optimal growth of the culture. Contamination is prevented by maintaining strict sterile conditions throughout the process. The components of the culture media are either heat sterilized or filtered through microporous membranes. The selected microorganism is then inoculated in pure conditions. Most of the processes are highly aerobic, except algal fermentation; hence a good supply of oxygen is an indispensable requirement. After the multiplication of the biomass, it is recovered from the medium and purified further for enhanced usefulness and or storability.

Advantages of Single-Cell Protein

Large-scale Single-Cell Protein production has multiple advantages over conventional food production practices such as:

1. Microorganisms have a high rate of multiplication, which means a large quantity of biomass can be produced in a comparatively shorter duration.
2. The microbes can be easily genetically modified to vary the amino acid composition.
3. A broad variety of raw materials, including waste materials, can be used as a substrate. This also helps in decreasing the number of pollutants.
4. Production is independent of climatic conditions.

Applications of Single-Cell Protein

1. Provides instant energy.
2. It is extremely good for healthy eyes and skin.
3. Provides the best protein supplemented food for undernourished children.
4. Serves as a good source of vitamins, amino acids, minerals, crude fibres, etc.

Used in therapeutic and natural medicines for:

1. Controlling obesity.
2. Lowers blood sugar level in diabetic patients.
3. Reducing body weight, cholesterol and stress.
4. Prevents accumulation of cholesterol in the body.

Used in Cosmetics products for:

1. Maintaining healthy hair.

2. Production of different herbal beauty products, like- Biolipstics, herbal face cream, etc.

Used in Poultry:

1. As it serves as an excellent and convenient source of proteins and other nutrients, it is widely used for feeding cattle, birds, fishes etc.

Biosensor

A biosensor is a device that measures biological or chemical reactions by generating signals proportional to the concentration of an analyte in the reaction.

Biosensors are employed in applications such as disease monitoring, drug discovery, and detection of pollutants, disease-causing micro-organisms and markers that are indicators of a disease in bodily fluids (blood, urine, saliva, sweat). A typical biosensor is represented in Figure 1; it consists of the following components.

1. **Analyte**: A substance of interest that needs detection. For instance, glucose is an 'analyte' in a biosensor designed to detect glucose.

2. **Bioreceptor**: A molecule that specifically recognises the analyte is known as a bioreceptor. Enzymes, cells, aptamers, deoxyribonucleic acid (DNA) and antibodies are some examples of bioreceptors. The process of signal generation (in the form of light, heat, pH, charge or mass change, etc.) upon interaction of the bioreceptor with the analyte is termed bio-recognition.

3. **Transducer**: The transducer is an element that converts one form of energy into another. In a biosensor the role of the transducer is to convert the bio-recognition event into a measurable

signal. This process of energy conversion is known as signalisation. Most transducers produce either optical or electrical signals that are usually proportional to the amount of analyte–bioreceptor interactions.

4. **Electronics**: This is the part of a biosensor that processes the transduced signal and prepares it for display. It consists of complex electronic circuitry that performs signal conditioning such as amplification and conversion of signals from analogue into the

digital form. The processed signals are then quantified by the display unit of the biosensor.

5. Display: The display consists of a user interpretation system such as the liquid crystal display of a computer or a direct printer that generates numbers or curves understandable by the user. This part often consists of a combination of hardware and software that generates results of the biosensor in a user-friendly manner. The output signal on the display can be numeric, graphic, tabular or an image, depending on the requirements of the end user.

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