Chapter 20

BIOTECHNOLOGY

STUDENTS'LEARNING OUTCOMES

After studying this chapter, the students will be able to:

- Introduce genetic engineering.
- Outline the function of restriction enzymes.
- Describe plasmids as vectors and explain how recombinant plasmids can be formed.
- Explain polymerase chain reaction (PCR).
- Define genetically modified organism.
- Explain the formation of human insulin protein in bacteria.

The word "Biotechnology" means "technology based on biology". In scientific terms, it is defined as the use of organisms, biological processes, or systems to make products that improve human lives and environment. Its main focus is to solve complex challenges in medicine, agriculture, and industry. Biotechnology is a vast field but this chapter would deal with the most important component of biotechnology i.e., genetic engineering.

For information

Traditional biotechnology has been used for thousands of years in the form of fermentation to create food products like bread, cheese, and beer. Modern biotechnology emerged in the 20th century, after the discovery of the structure of DNA and advanced molecular techniques.

20.1- Genetic Engineering

Biotechnology is a broad "umbrella" term. However, the true "engine" of modern biotechnology is "Genetic Engineering". Genetic engineering is defined as the alteration of an organism's DNA to change its characters (traits) or capabilities. It involves inserting, deleting, or modifying genes, and sometimes transferring genes between different species. In genetic engineering, scientists rely on two basic technologies:

1. Recombinant DNA Technology: Physically cutting a piece of DNA from one organism and "pasting" it into the DNA of another.
2. Polymerase Chain Reaction (PCR): Rapidly amplifying a tiny segment of DNA into a large quantity.

20.2- Recombinant DNA Technology

Recombinant DNA technology means the cutting, splicing, and combining DNA molecules from different sources into a single hybrid molecule. It also involves the

www.ilmkidunya.com

introduction of recombinant DNA (rDNA) into a host (e.g., bacteria) to produce new genetic combinations or specific proteins.

20.2.1- Tools used in Recombinant DNA Technology

1- Gene of Interest

The gene of interest is the gene which is to be manipulated. The gene of interest can be obtained by one of the following three ways:

i. Direct **synthesis** of the gene of interest in the lab by using different enzymes (e.g., polymerases and ligases).
ii. Synthesis by doing **reverse transcription** of its mRNA by using reverse transcriptase enzymes.
iii. **Cleavage from chromosome** by using enzymes restriction endonucleases.

2- Restriction Endonucleases

These enzymes cut specific sequences of nucleotides in DNA. They are also called molecular scissors. There are many kinds of restriction endonuclease. Each kind recognizes a specific nucleotide sequence in a DNA. Such a sequence of nucleotides is called **restriction site** or recognition region. The enzyme binds to the DNA at that sequence, and cuts the DNA at a particular place within this site.

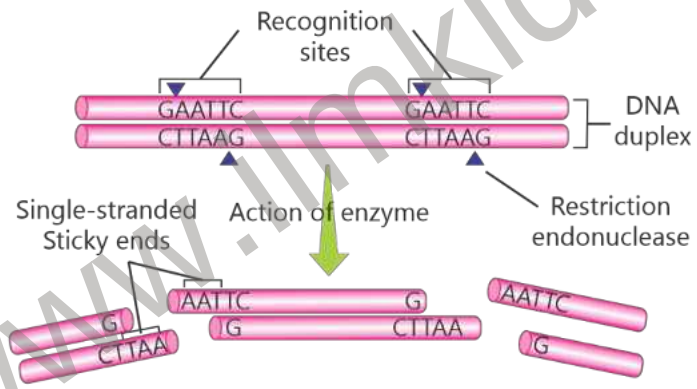


Figure 20.1: Action of restriction endonuclease

For information
Restriction endonucleases are valuable due to the fact that any two DNA fragments produced by the same restriction endonuclease can be joined together.

The restriction sites have **palindromic sequences** of four to eight nucleotides. This means the nucleotides at one end of the restriction site are complementary to those at the other end. So, the two strands of the DNA have the same nucleotide sequence running in opposite directions at the restriction site. Restriction endonuclease binds to and cleaves both strands. After cleavage, both DNA fragments have single-stranded ends (a few nucleotides long) that are sticky i.e., complementary to each other.

www.ilmkidunya.com

3- Vectors

Special molecules called vectors (or molecular carrier) are used for the introduction the of gene of interest into host cells. The gene of interest is attached to the vector and the vectors becomes a **recombinant DNA** (rDNA). Then, the rDNA is introduced into the host cells. The **plasmids** of bacteria are the most commonly used. Other vectors include;

i- Bacteriophages (e.g., phage lambda, bacteriophage P1)
ii- Cosmids (plasmids that contain a small region of bacteriophage DNA called the cos sequence)
iii- Bacterial artificial chromosomes – BACs
iv- Yeast artificial chromosomes – YACs.
v- P1 artificial chromosomes (have features of both P1 vectors and Bacterial Artificial Chromosomes)

Animal viruses (e.g., human cold virus) also serve as vectors to carry genes of interest into monkey and human cells.

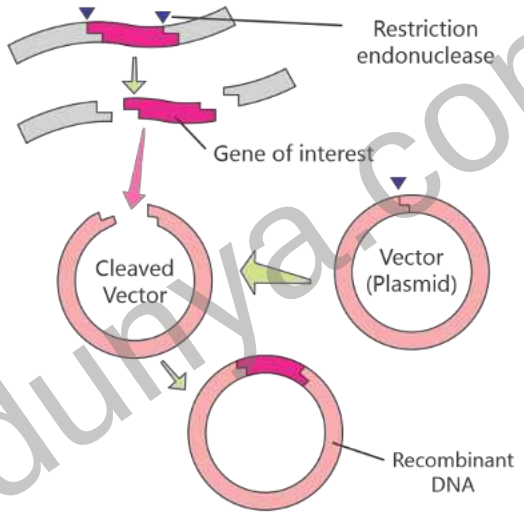


Figure 20.2: Use of Vector

4- DNA Ligase

This enzyme joins two DNA fragments. Therefore, it is called molecular glue. It forms the phosphodiester linkage between two adjacent nucleotides.

5- Expression System

Expression system means a suitable organism that can act as host for rDNA (gene of interest attached with vector) and can express the gene of interest. The selection of suitable expression system depends upon the type of vector used to make rDNA. Bacterial cells act as an ideal expression system because of their short generation time and simple genetic system.

20.2.2- Steps of Recombinant DNA Technology

1- Formation of Recombinant Vector (Plasmid)

i: DNA Cleavage: The source DNA of the organisms is **cleaved** by using a restriction endonuclease and the gene of interest is isolated. Similarly, the DNA of vector (e.g., plasmid or viral DNA) is also cleaved by using the same endonuclease. So, compatible sticky ends are produced at the gene of interest and the cleaved vector.

www.ilmkidunya.com

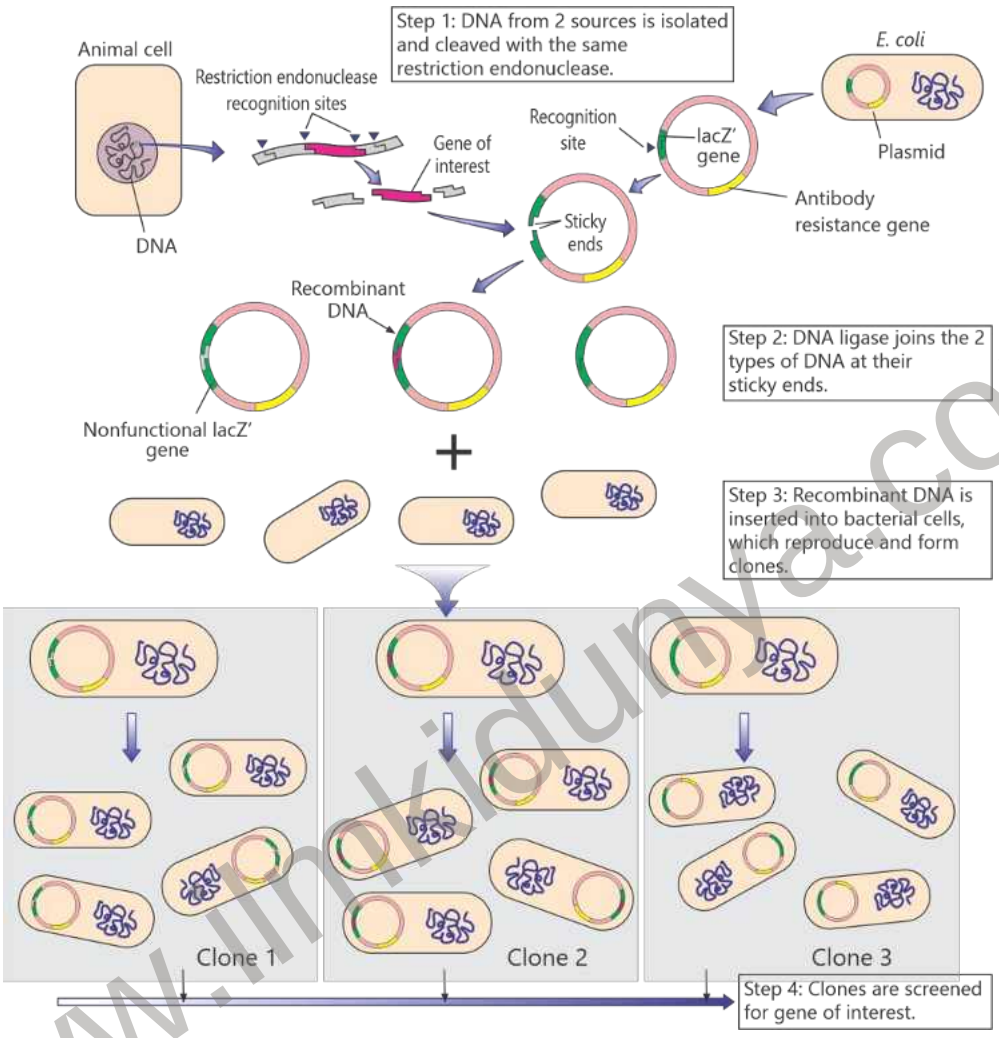


Figure 20.3: Steps of making Recombinant Vectors

ii: Production of Recombinant DNA (Vector): The gene of interest is inserted into the cleaved vector. For this purpose, the cleaved vector and the gene of interest are incubated together in the presence of DNA ligase. This enzyme connects them by forming phosphodiester linkages. So, the vector turns into recombinant vector (i.e., vector having the gene of interest).

2- Cloning of Recombinant Vector

The recombinant DNA (vector) and the expression system (usually bacteria) are incubated in the same medium. The bacterial cells take up recombinant DNA. All the incubated bacteria make bacterial clones which also contain the identical copies i.e., clones of the gene of interest.

www.ilmkidunya.com

3- Screening of Clone Library

Once the vectors have been inserted into host cells, scientists identify which bacteria actually took up the recombinant plasmid. This is done through:

i: Selecting for the Vector (Antibiotic Resistance): The plasmid contains an antibiotic resistance gene. The bacteria are grown on a medium containing that antibiotic (e.g., ampicillin). Only cells that took up a plasmid will survive and grow.

ii: Selecting for the Gene of Interest (Blue-White Screening): To distinguish between a "plain" plasmid and a "recombinant plasmid", the **lacZ' gene** of the plasmid is used. The lacZ' gene produces an enzyme which metabolizes a specific sugar and turns it blue

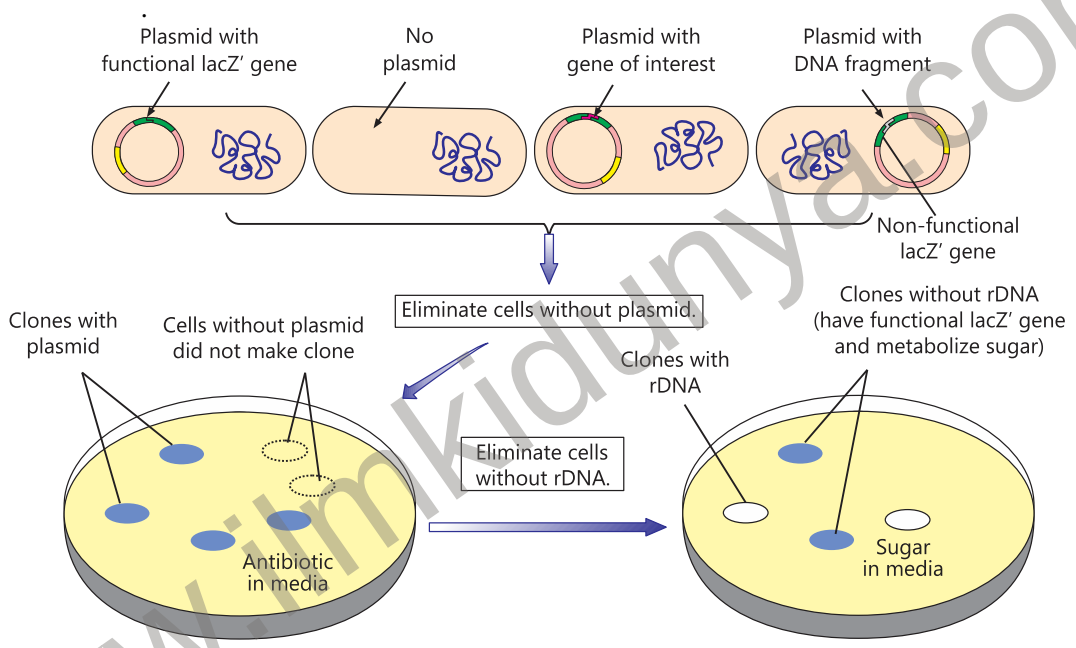


Figure 20.4: Screening of clones (Elimination of clones without vector (plasmid) and elimination of clones with vector (plasmid) but without recombinant DNA)

If the plasmid is not recombinant, the lacZ' gene remains functional and turns the bacterial colony blue. If gene of interest was successfully inserted, it "breaks" the lacZ' gene. Therefore, the desired recombinant clones will remain white.

iii: Identifying the Specific Gene (DNA Hybridization): To confirm the presence of gene of interest, a radioactive probe is used. It is a DNA strand complementary to gene of interest. The probe sticks (hybridizes) only to the colonies containing the gene of interest. Auto-radiography (photographic film) is used to locate these specific colonies on the master plate. The main steps of this technique are mentioned in the following diagram.

www.ilmkidunya.com

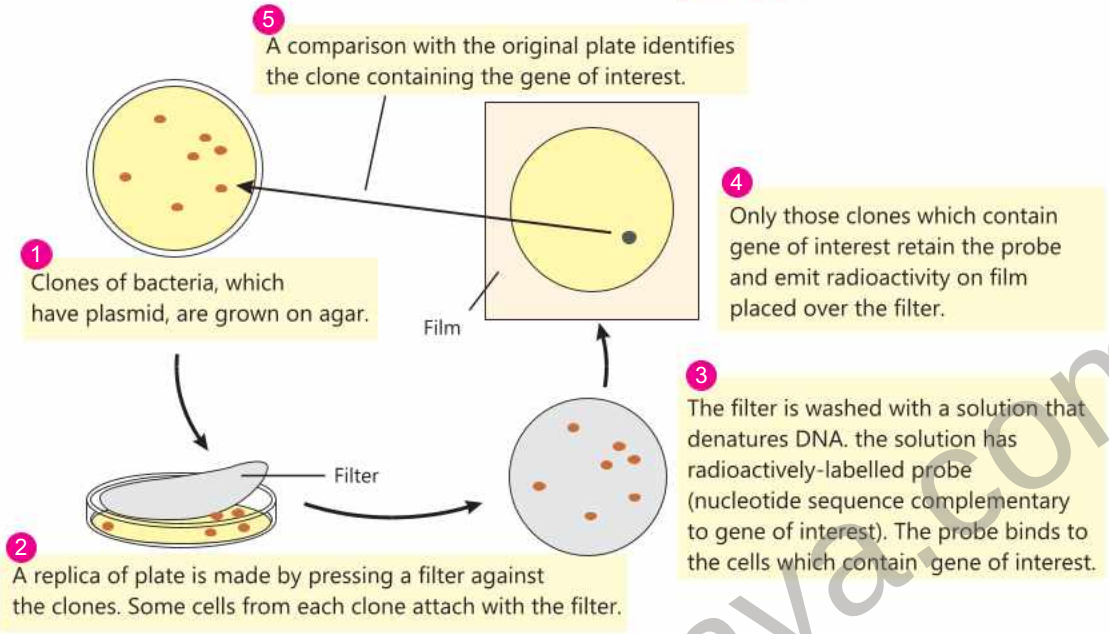


Figure 20.5: Identification of the clones that have gene of interest

20.3- Polymerase Chain Reaction (PCR)

In this technique, multiple identical copies of the gene of interest are made. The enzyme DNA polymerase is used to copy which polymerizes a given piece of DNA again and again, so that multiple copies are produced. That's why, the technique is known as polymerase chain reaction (PCR). Kary Mullis developed PCR in 1983. He won the Nobel Prize in Chemistry in 1993.

20.3.1- Steps of PCR

Step 1: Denaturation

First, an excessive amount of primer (a sequence of 20 to 30 nucleotides) is mixed with the DNA fragment (gene of interest). This mixture of primers and DNA is heated to about 98°C. At this temperature, the double-stranded DNA breaks into single strands. Each single stranded DNA can act as the template for the in vitro DNA synthesis.

Step 2: Annealing of Primer

The solution is allowed to cool to about 60°C. As it cools, the forward and the backward primers anneal or hybridize to the single-stranded DNA at its complementary regions. So, the rest of the fragment remains single-stranded.

Step 3: Extension of Primer

A very heat-stable DNA polymerase, called **Taq polymerase** is added.

www.ilmkidunya.com

All four types of nucleotides are also added. The Taq polymerase adds new nucleotides to the 3′ end of primer. In this way, it copies the rest of the fragment just like the replication of DNA. In this way, a complimentary copy of the entire single-stranded DNA has been added to the primer. Because both DNA strands are replicated, there are now two copies of the original DNA fragment.

Step 4: Repeating the Cycle

Steps 1 to 3 are repeated and the two copies become four. It is not necessary to add any more polymerase, as the heating does not harm this enzyme. Each cycle is completed in one or two minutes and it doubles the number of DNA molecules. After 20 cycles, a single fragment of DNA produces more than one million copies.

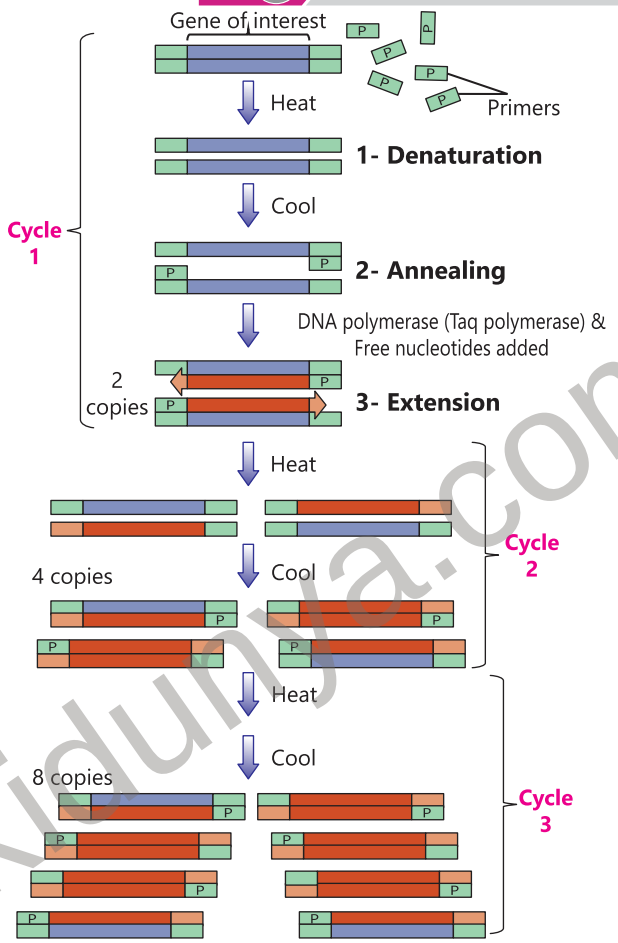


Figure 20.6: Steps of PCR

For information

PCR is also used to build genomic library. It is the collection of the total DNA from a single organism. Genomic libraries are commonly used for sequencing the DNA.

For information

The Taq polymerase is a temperature-tolerant enzyme isolated from *Thermus aquaticus*, a bacterium found in hot springs. This enzyme is stable and active at near-boiling temperatures.

For information

The optimum temperature for carrying out the extension of primer is 72°C. This step is completed in just one minute.

www.ilmkidunya.com

Applications of PCR

i. It is an efficient diagnostic technique used for detecting genotypes of infectious agents.
ii. It is used to detect genetic mutations responsible for certain genetic diseases and cancers.
iii. In criminal investigations, "DNA fingerprints" are prepared from the cells in a tiny sample of dried blood or at the base of a single human hair.
iv. Physicians can detect genetic defects in very early embryos by collecting a few sloughed-off cells and amplifying their DNA.
v. PCR is also used to examine the DNA of extinct species, as long as even a minuscule amount of their DNA remains intact.

20.4- Genetically Modified Organism (GMO)

A Genetically Modified Organism is a plant, animal or microbe in which the genome has been changed. Transgenic organisms are a specific subset of GMOs that have received DNA from a different, unrelated species. Approximately 90% of canola, cotton, corn, soybean, and sugar beets grown in the world are transgenic.

Methods of Gene Transfer

The gene (DNA) is transferred into the target cell through cell membrane or cell wall without destroying the cell. To produce a whole multicellular GMO, the gene is transferred into a gamete or gamete-producing tissue. Such gametes are then fused to produce GMOs. The following are common techniques used to transfer the gene of interest into a target cell.

(a) In **electroporation**, pores are created in the cell wall or cell membrane of target cell. The pores allow gene intake. Once the electrical current is discharged, the pores are closed.
(b) In **microinjection**, a fine needle, or injection, is used to directly inject the gene into the target cell nucleus.
(c) In **biolistic transformation**, the gene of interest coated with heavy metal ions is inserted into the target cell by mechanical force e.g., gene gun.
(d) In **protoplast mediated technique**, the cell wall and cell membrane are removed from the target cell. This protoplast is mixed with the medium containing the gene of interest.
(e) In **chemical methods**, the naked DNA is fused with a chemical agent. The chemical and DNA complex enters into the cell cytoplasm through diffusion or endocytosis.
(f) In **virus mediated gene transfer**, Adenovirus, Adeno-associated virus, HIV, lentivirus and other retroviruses are used for gene transfer.

www.ilmkidunya.com

20.4.1- GMO (Transgenic) Bacteria

Genes from a wide range of organisms can be added to a plasmid and inserted into bacteria. Transgenic bacteria can synthesize large amounts of the proteins encoded by the inserted foreign genes. Different forms of transgenic bacteria are being used to;

1. Produce pharmaceutical products e.g., insulin (to treat diabetes), clotting factors (to treat haemophilia), hepatitis B vaccine (against hepatitis B), Covid-19 vaccine (against Corona), human growth hormone (to treat dwarfism), interferon (to treat some cancers), erythropoietin (to treat anaemia), and tissue plasminogen activator (which dissolves blood clots).
2. Produce many food products e.g., alpha-amylase (converts starch to simple sugars), chymosin (clots milk protein for cheese making), and pectin-esterase (improves fruit juice clarity).
3. Produce enzymes which degrade toxins to convert pollutants into a less toxic form.
4. Leach copper from ore, clean up mercury pollution and detect arsenic in drinking water.

20.4.2- GMO (Transgenic) Animals

A transgenic animal carries a foreign gene to produce the desired protein. Transgenic animals serve various purposes e.g., producing drugs, enhancing yields, increasing resistance to disease etc. For example;

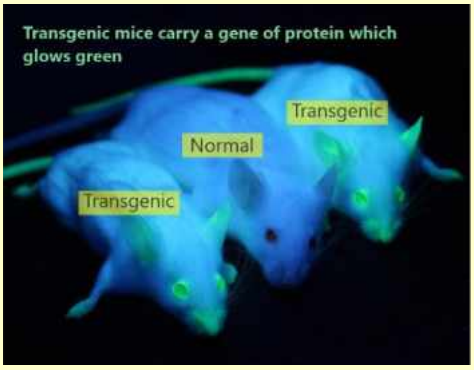
1. Transgenic dairy cows have genes from human beings to produce milk that is the same as human breast milk.

Tidbit

In theory, foreign genes may be inserted either in germline or in somatic cells of humans. If genes are inserted in germline cells, it results in inheritable changes. But, most research has focused on somatic cells because of risks and ethical issues associated with germline transformation.

Tidbit

Since the mid-1980s, transgenic mice are a key model for investigating diseases. Mice are the model for this purpose because there is complete analysis of its genome sequence and its genome is very much similar to humans.



2. Transgenic goats produce milk with strong spiderweb-like silk proteins.

www.ilmkidunya.com

3. Transgenic goats also produce human enzyme lysozyme in their milk to fight diarrhoea causing bacteria in humans.
4. Transgenic fish show better growth, reduced food intake, increased cold tolerance, and disease resistance.
5. In gene therapy, genetically modified viruses are used to deliver genes to humans to cure various inheritable diseases such as abnormal development of T and B cells and inherited blindness.
6. The first human biological drug produced from a transgenic goat was an anticoagulant to reduce the probability of blood clots during surgery or childbirth.
7. Malaria-resistant mosquitoes have been developed in the laboratory by inserting a gene that reduces the development of *Plasmodium* in them.

20.4.3- Transgenic Plants

Many plant cells are totipotent, meaning that a single cell can form a new plant. Biologists grow a new plant from a transgenic cell of plant. The main purposes of producing transgenic plants are;

1. Genes of *Bacillus thuringiensis* (a soil bacterium) have been inserted in many plants (e.g., cotton, rice, maize, potato, tomato, brinjal, cauliflower, cabbage etc.). These genes make proteins that kill insect pests but do not harm other useful insects.

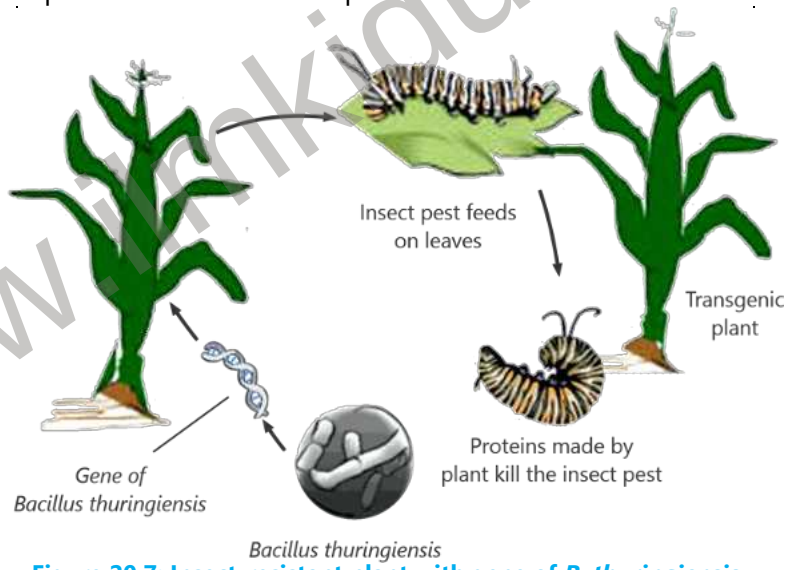


Figure 20.7: Insect-resistant plant with gene of *B. thuringiensis*

For Information
Each year, deficiency of iron affects 1.4 billion women and deficiency of vitamin A kills 670,000 children and causes 500,000 cases of irreversible childhood blindness.

2. Many crops have been modified to be resistant against glyphosate (an herbicide).

www.ilmkidunya.com

3. In developing countries, biologists have made GMO (transgenic) rice (golden rice) in which they have inserted genes to increase iron content. These genes were taken from beans, *Aspergillus fungus*, and wild rice. Similarly, the genes for making beta-carotene (a precursor of vitamin A) were taken from daffodil and inserted in rice. The transgenic rice is grown and consumed in areas with a shortage of dietary iron and vitamin A
4. GMO plants and plant cells are used for the production of biopharmaceuticals e.g., cytokines, hormones, antibodies, enzymes and vaccines etc.

20.5- Formation of Human Insulin in Bacteria

The production of human insulin was the first major triumph of genetic engineers. By using bacteria, scientists produce vast quantities of insulin that is identical to the insulin hormone naturally made in the pancreas. The process of creating synthetic human insulin involves producing the two polypeptide chains separately and then joining them chemically.

1. Preparation of the Two Genes: Since insulin consists of two distinct chains, scientists synthesise two different DNA sequences: one for Chain A and one for Chain B.

2. Construction of Recombinant Plasmids: Two sets of plasmids are obtained. The DNA for Chain A is inserted into one set of plasmids, and the DNA for Chain B is inserted into another. In both cases, the insulin gene is often attached next to a bacterial gene (like lacZ) to help the bacteria "recognize" and express the foreign DNA.

3. Transformation of Host Cells: The recombinant plasmids are introduced into two separate cultures of *Escherichia coli* bacteria. Culture 1 acts as a factory for Chain A. Culture 2 acts as a factory for Chain B.

4. Fermentation and Protein Expression: The bacteria are grown in large industrial bioreactors. As the bacteria multiply and perform their normal metabolic functions, they simultaneously "read" the human DNA and produce large quantities of the individual insulin chains.

5. Extraction and Chemical Joining: Once the chains are synthesized, the bacteria are harvested and the insulin chains are extracted and purified. In a laboratory setting, Chain A and Chain B are mixed together. They are linked by creating di-sulfide bonds (sulfur-to-sulfur bridges) between specific amino acids.

This final step creates the functional, three-dimensional human insulin molecule, identical to that produced by a healthy human pancreas.

www.ilmkidunya.com

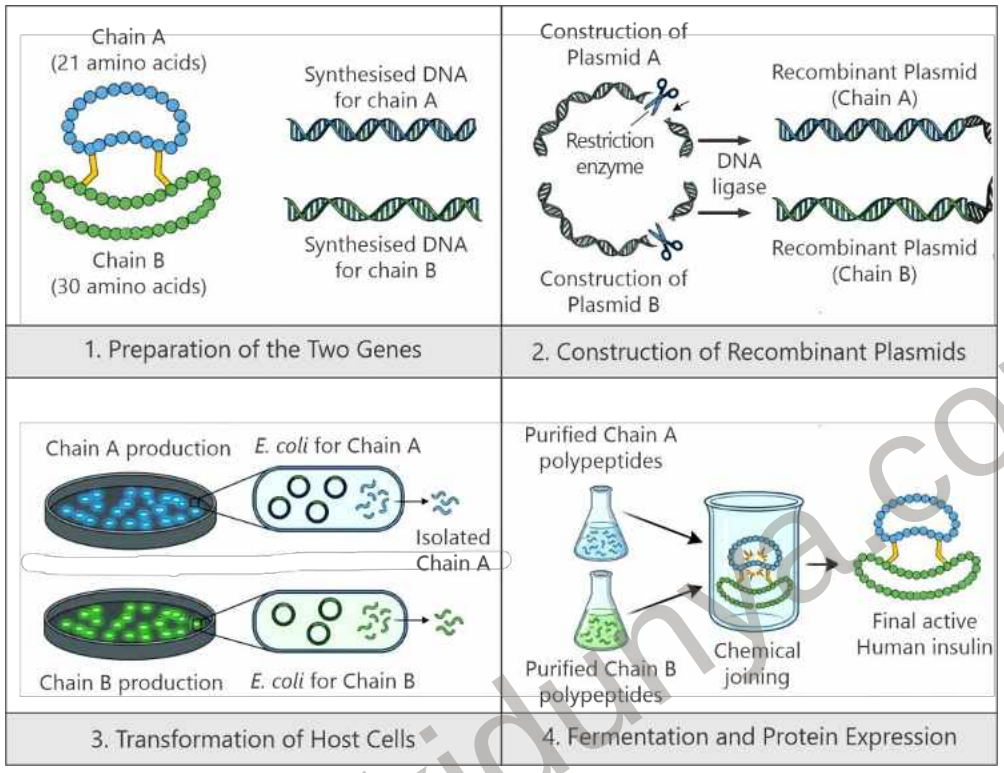


Figure 20.8: Formation of human insulin in bacteria

EXERCISE

SECTION 1: MULTIPLE CHOICE QUESTIONS

1. Identify the FALSE statement.
   (a) Restriction enzyme is used for the production of RFLPs.
   (b) DNA ligase enzyme is used to cut DNA, creating the sticky ends.
   (c) Reverse transcriptase enzyme is used for making cDNA from mRNA.
   (d) Electrophoresis means the separation of DNA fragments.
2. Which bacterium is used in the production of insulin by genetic engineering?
   (a) *Saccharomyces* b) *Rhizobium*
   (c) *Escherichia* (d) *Mycobacterium*
3. Which technique is used for cloning of gene/DNA?
   (a) RFLP analysis
   (b) Polymerase Chain Reaction (PCR)
   (c) Electroporation
   (d) Gel electrophoresis

www.ilmkidunya.com

4. Which method is used for obtaining the gene of interest?
   (a) Artificial gene synthesis (b) Reverse transcription
   (c) Cleavage from chromosome (d) All of these
5. When a gene of interest is attached to the vector, the new molecule is called;
   (a) Recombinant DNA
   (b) Restriction length fragment
   (c) Complimentary DNA
   (d) Genomic library
6. What is the use of plasmid in genetic engineering?
   (a) To carry DNA to a cell (b) To cut DNA
   (c) To join pieces of DNA (d) To replicate DNA
7. Which of these can serve as ideal expression system for gene cloning?
   (a) Bacteriophages (b) Bacterial cells
   (c) Plant cells (d) Animal cells
8. In PCR, optimum temperature for carrying out the extension of primer is;
   (a) 98° C. (b) 72° C. (c) 60° C (d) 37° C.
9. Which enzyme is used for the extension of primer in PCR?
   (a) DNA polymerase (b) Transcriptase
   (c) DNA ligase (d) Restriction endonuclease
10. What is true for a Genetically Modified Organism
   (a) Genes have been introduced (b) Genes have been enhanced
   (c) Genes have been deleted (d) All of these

SECTION 2: SHORT QUESTIONS

25. What is the role of restriction endonucleases and DNA ligases in gene cloning?
26. What is the role of vectors in recombinant DNA technology?
27. Describe how the gene of interest is selected and isolated during recombinant DNA technology.
28. State the steps for the integration of gene of interest into the vector.
29. State the role of GMO in making biotechnology products.
30. Differentiate between;
    - Endonuclease and ligase
    - PCR and Recombinant DNA Technology

SECTION 3: LONG QUESTIONS

32. Describe the components and steps of recombinant DNA technology.
33. Write a detailed note on restriction enzymes.
34. Write a note on polymerase chain reaction.
35. Describe the methods for the introduction of human DNA into bacteria.

www.ilmkidunya.com

INQUISITIVE QUESTIONS

13. Find and describe the accomplishments of the renowned genetic engineers working in private and public institutions in your province or city.
14. Analyse and interpret the DNA of a child by comparing it with that of two individuals in a case of disputed parenthood.
15. What may be the application of polymerase chain reaction?
16. The human genome project is regarded as the most ambitious project ever undertaken by man. Justify.
17. Investigate careers that require an understanding of biotechnology and genetic engineering.

www.ilmkidunya.com