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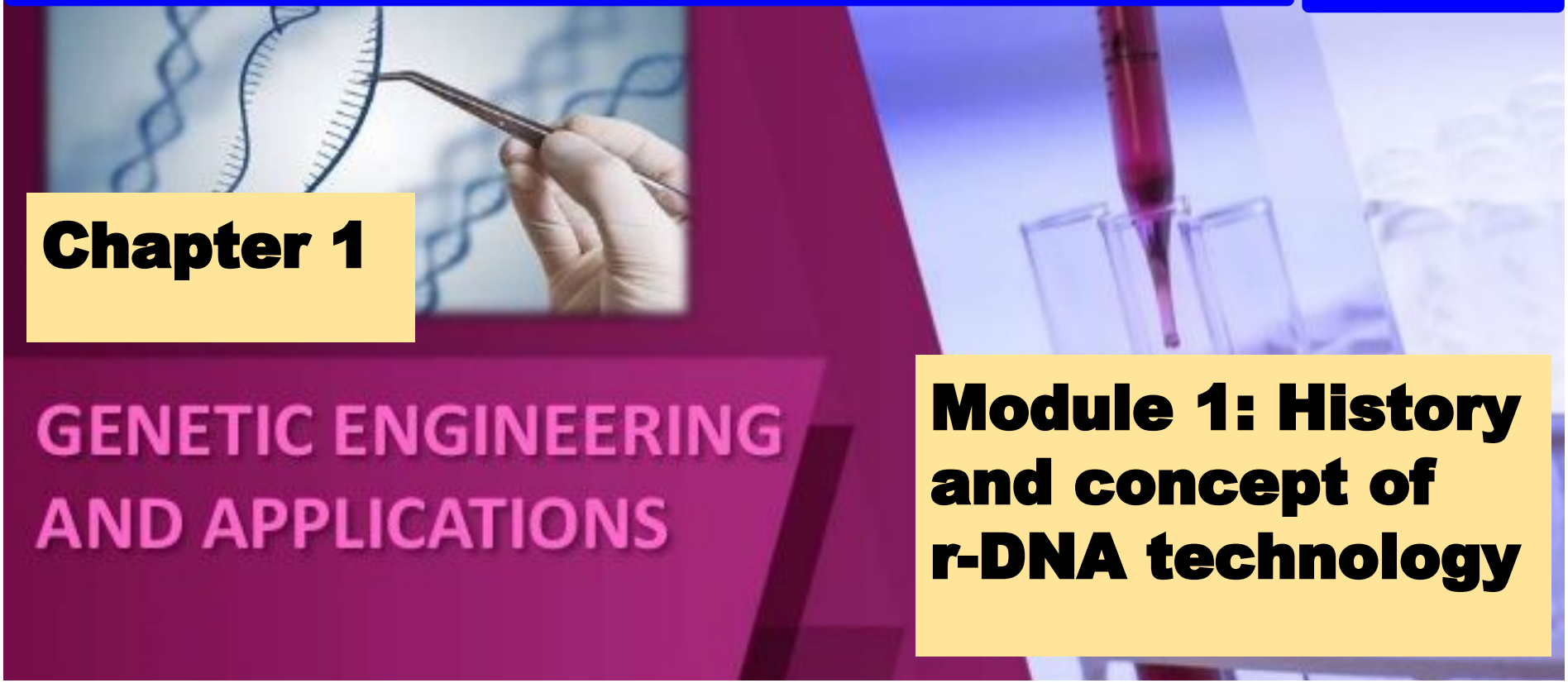

Chapter 1

GENETIC ENGINEERING AND APPLICATIONS

Module 1: History and concept of r-DNA technology

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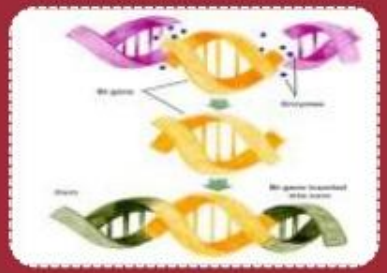
Assistant Prof. in Zoology

Genetic engineering

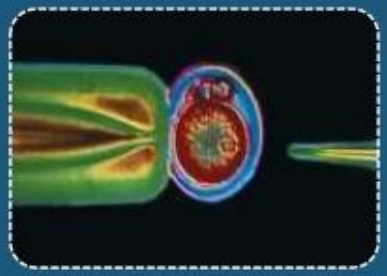


Definition -1

- A set of techniques capable to allow the identification, manipulation and multiplication of genes of living organisms.

Definition -2

- Changing of genes by using in vitro processes.



Other names

- Gene manipulation, gene cloning
- Recombinant DNA technology, genetic modification

INTRODUCTION

- Genetic engineering is a part of **biotechnology.**

- **Biotechnology** is the use of living systems and organisms to develop or make useful products, or "any technological application that uses biological systems, living organisms or derivatives thereof, to make or modify products or processes for specific use" (UN Convention on Biological Diversity, Art. 2).

INTRODUCTION continuation..

- ✓ Biotechnology is a huge topic.
- ✓ Its hard to define its exact boundaries.
- ✓ Some European scientists divide the field into :

1) **Red biotechnology**
2) **Green biotechnology**

- ✓ Some divides it into :

1) **White**
2) **Blue**

- ✓ Biotechnology falls under many umbrellas which is basically considered as life science.

Biotechnology

- Biology & Zoology
- Cell biology
- Microbiology
- Molecular Biology
- Physiology, Ecology, Embryology
- Genetics, Population genetics, Epigenetics
- Proteonomics & Bioinformatics

Book : Biotechnology & Genetic engineering

Objectives of Genetic Engineering

- Basic research on gene structure and function
- Production of useful products and services
- Generation of transgenic plants and animals
- Investigation of human genome for gene therapy

- The term "Genetic Engineering" was first coined by Jack Williamson in his science fiction novel.

- James Watson and Francis Crick showed that the DNA molecule has a double-helix structure.

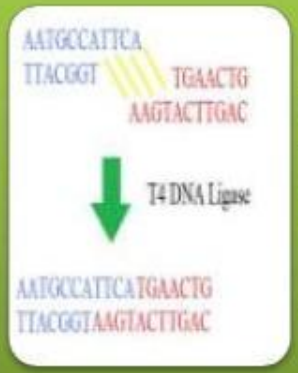
- In 1972, Paul berg created the first recombinant DNA molecules by combining DNA from the monkey virus SV40 with that of the lambda virus.

- In 1973 Herbert Boyer and Stanley Cohen created the first transgenic organism by inserting antibiotic resistance genes into the plasmid of an E.coli bacterium.

- The first trials of genetically engineered plants occurred in France and the USA in 1986, tobacco plants were engineered to be resistant to herbicides.

In 1970 **Hamilton Smiths** lab discovered restriction enzymes that allowed DNA to be cut at specific places and separated out on an electrophoresis gel.

- This enabled scientists to isolate genes from an organism's genome.



DNA ligases, that join broken DNA together, had been discovered earlier in 1967 and by combining the two enzymes it was possible to "cut and paste" DNA sequences to create recombinant DNA.

Plasmids, discovered in 1952, became important tools for transferring information between cells and replicating DNA sequences.

Frederick Sanger developed a method for sequencing DNA in 1977, greatly increasing the genetic information available to researchers

Polymerase chain reaction (PCR), developed by **Kary Mullis** in 1983, allowed small sections of DNA to be amplified and aided identification and isolation of genetic material

Artificial competence was induced in *Escherichia coli* in 1970 when **Morton Mandel and Akiko Higa** showed that it could take up bacteriophage λ after treatment with calcium chloride solution ($CaCl_2$).

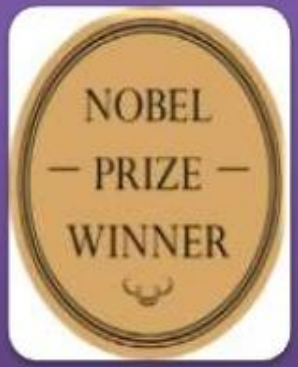
Two years later, **Stanley Cohen** showed that $CaCl_2$ treatment was also effective for uptake of plasmid DNA.

Transformation using **electroporation** was developed in the late 1980s, increasing the efficiency and bacterial range

In 1972 **Paul Berg** utilised restriction enzymes and DNA ligases to create the first recombinant DNA molecules.

- **Herbert Boyer** and **Stanley N. Cohen** took Bergs work a step further and introduced recombinant DNA into an bacterial cell.

In 1981 the laboratories of **Frank Ruddle, Frank Constantini and Elizabeth Lacy** injected purified DNA into a single-cell mouse embryo and showed transmission of the genetic material to subsequent generations.

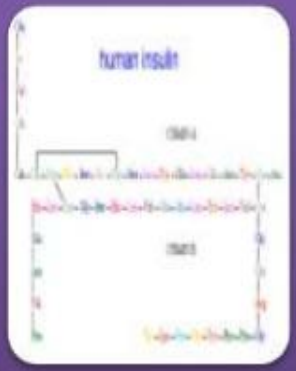


On June 19, 2013 the leaders of three research teams who originated the technology, Robert T. Fraley of Monsanto; Marc VanMontagu of Ghent University in Belgium and founder of Plant Genetic Systems and CropDesign ; and Mary-Dell Chilton of Washington University in St. Louis and Syngenta were awarded with the World Food Prize

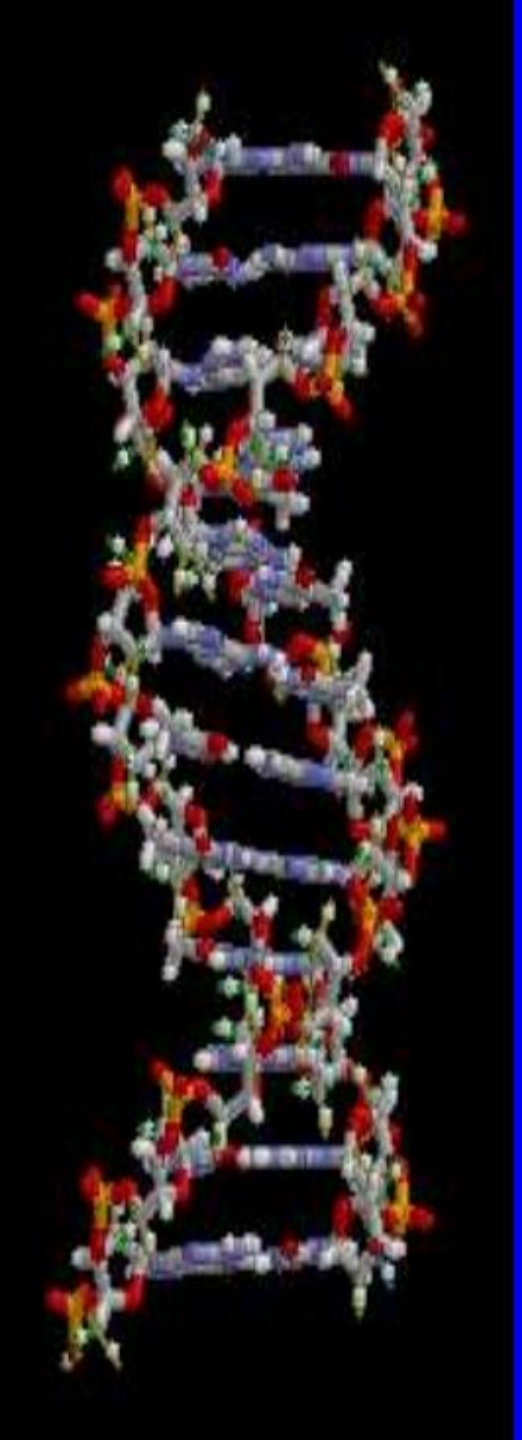
The first recorded knockout mouse was created by **Mario R. Capecchi, Martin Evans and Oliver Smithies** in 1989. They are used to study gene function and make useful models of human diseases.

In 1992 onco-mice with tumor suppressor genes knocked out were generated.

Creating Knockout rats are much harder and has only been possible since 2003



Bacteria synthesising **human insulin** were developed in 1979, being used as a treatment for the first time in 1982



"The Father of Cloning"

Hans Spermann

1. What is genetic engineering?

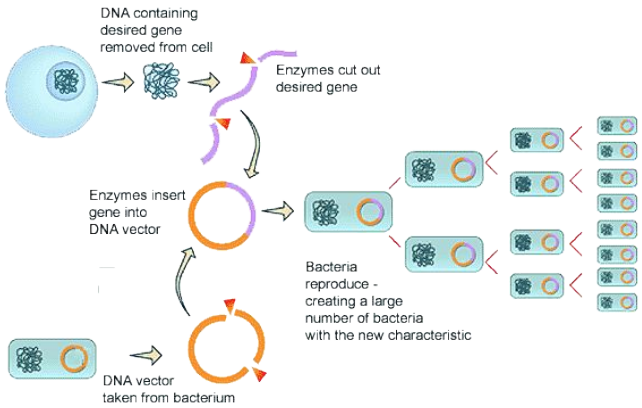
Genetic engineering is a technique that makes it possible to transfer DNA sequences from one organism to another

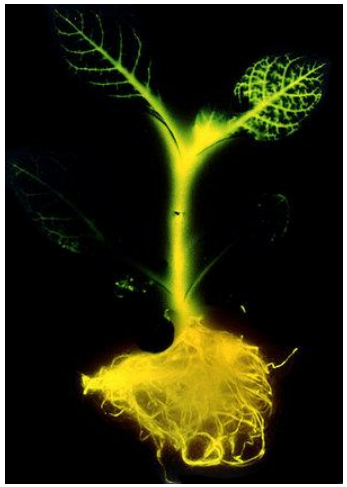
2. What are transgenic organisms?

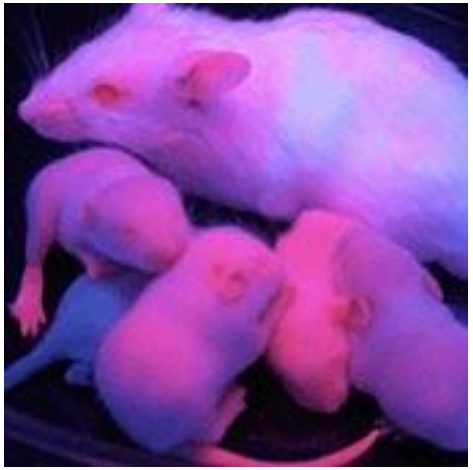
Organisms that contain genes from other species

Examples of Transgenic organisms

Transgenic microorganisms	Transgenic plants	Transgenic animals

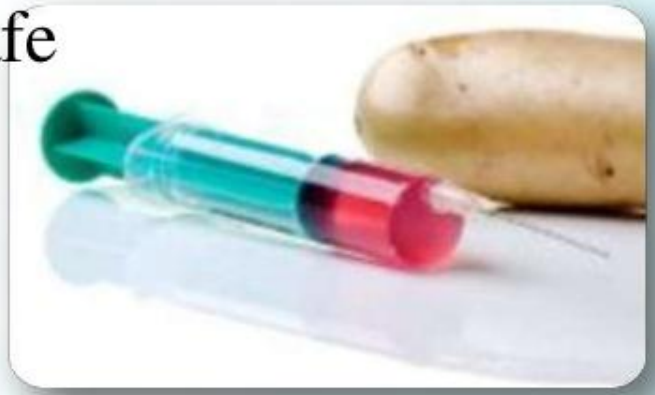






- The Flavr Savr tomato was a tomato engineered to have a longer shelf life.

- In 1995, Bt Potato was approved safe by the Environmental Protection Agency.



- Bt-Cotton is a genetically modified cotton which is resistant to pests.

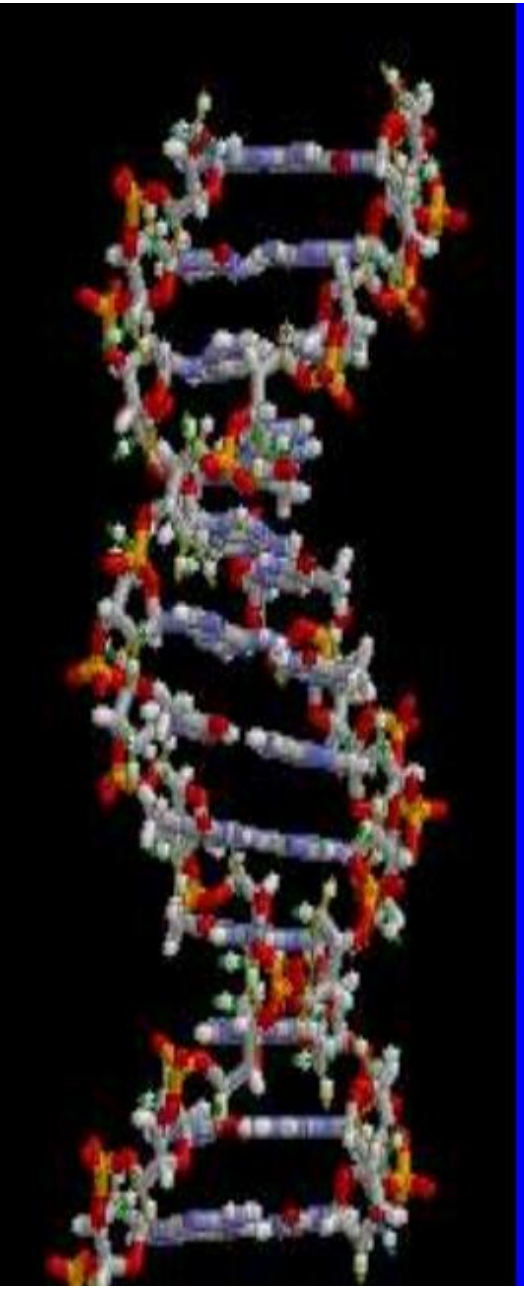
- **Golden Rice genetically modified to contain beta-carotene (a source of Vitamin A).**

The GloFish was the first genetically modified animal to become available as a pet. It is a natural Zebrafish which has genetic information from bioluminescent jellyfish added to its DNA.

Using Modern DNA and cross fertilization techniques; the Dolion i a cross between a lion and a dog.

The Zorse is a cross between a zebra and a domestic horse.

The crosses were originally done in England and Africa to try to produce a domestic horse like animal that was resistant to diseases spread by a fly in Africa.



BASICS OF GENETIC ENGINEERING

- Different terms used for genetic engineering :

1) Gene manipulation
2) Gene cloning
3) Recombinant DNA technology
4) Genetic modification
5) New genetics

BASICS OF GENETIC ENGINEERING CONTINUATION

- Direct manipulation of an organism's genome using biotechnology .

First isolating and copying the genetic material of interest using molecular cloning methods

Generate a DNA sequence

New DNA inserted in the host genome

An Introduction to Genetic Engineering (Desmond S. T. Nicholl) Edi :3rd 2008 Chapter 2.

Objectives of r DNA Technology

- Artificially synthesize new genes.
- Altering the genome of an organism.
- Bring about new gene combinations not found in nature.
- Understanding the hereditary diseases and their cure.
- Improving human genome.

Chemical knives in molecular carpentry

Enzymes are chemical knives in r DNA technology

- **DNA or RNA polymerase-** replicating or annealing a DNA chain.
- **Reverse transcriptase** – synthesize c DNA from RNA template.
- **DNA ligase** – joining DNA strands together.
- **Nuclease**-breaks phospho-diester bonds within free ends (exonucleases) or in an interior position(Endonucleases).
- **Restriction endonuclease** – recognize a specific base sequence and cuts the DNA.

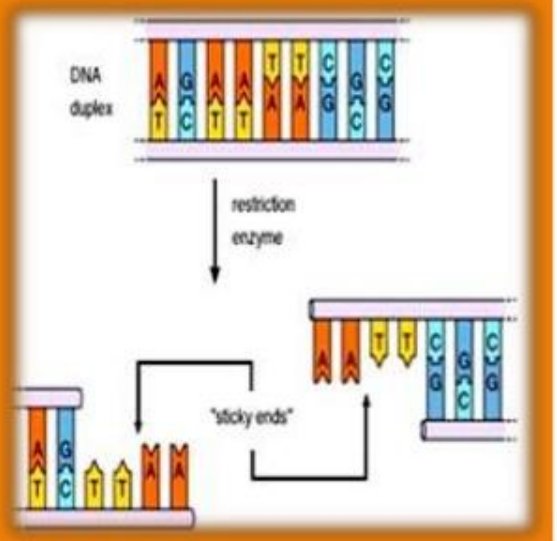
Restriction endonucleases (RE ases)

- A special class of sequence – specific enzymes
- Found in bacteria which protect its genetic material from the invasive attacks of viruses.
- Site-specific - cleave DNA molecules only at specific nucleotide sequences.
- REases recognize DNA base sequences that are palindromes.
- REases make two single stranded breaks, one in each strand.
- RE ases make staggered cuts with complementary base sequences for easy circularization.

Restriction Enzymes- Types of cuts

- Recognition sequences of RE ases are *palindromes*
  - A *palindrome* is a word, phrase, number or other sequence of units that can be read the same way in either direction
- **Cohesive (sticky) ends** – overhanging single-stranded ends
- **Blunt ends** – double-stranded, non-overhanging ends

Salient features of Restriction endonucleases (RE ases)



- Endonucleases
- Bacterial enzymes
- Different REases Isolated from different bacteria
- Names of REases are derived from source bacteria.
- Cut DNA into defined and reproducible fragments
- Basic tool of Genetic Engineer

Restriction endonucleases

Over 3000 restriction enzymes have been studied in detail, and more than 600 of these are available commercially.

Restriction endonuclease	source	Type ends formed
EcoRI	Escherichia coli	Forms sticky ends
BamHI	Bacillus amyloliquifaciens	Forms sticky ends
HaeIII	Hemophyllus aegypticus	Forms blunt ends
HindIII	Hemophyllus influenza	Forms sticky ends
NotI	Nocardia otitidis	Forms sticky ends

NOMENCLATURE FOR RESTRICTION ENDONUCLEASES

- EcoRI
  - Escherichia (E) (genus)
  - coli (co) (specific epithet)
  - strain Ry13 (R) (strain)
  - first endonuclease (1) (order of identification)

- HindIII
  - Haemophilus (H) (genus)
  - influenzae (in) (specific epithet)
  - strain Rd (d) (strain)
  - third endonucleases (III)(order of identification)

Restriction mapping

- A map showing the unique sites of cutting of the DNA of a particular organism by a single REase enzyme.
- A particular REase generates a unique set of DNA fragments with specific base sequence.
- Another enzyme will generate a different set of DNA fragments from the same DNA molecule.
- The family of DNA fragments generated by a single enzyme can be detected easily gel electrophoresis.

Applications of Restriction endonucleases (RE ases)

RE ases catalyze sequence – dependent double-stranded breaks in DNA yielding a homogeneous population of DNA fragments.

- **Preparing a restriction map of DNA**
- **Fragmenting genomic DNA prior to Southern Blotting.**
- **Generating DNA fragments that can be sub-cloned in appropriate vectors.**
- **Generating DNA fragments for labeled probes**

DNA ligases-DNA joining enzymes

- The cut DNA fragments are covalently joined together by DNA ligase.
- These enzymes were originally isolated from viruses, E.coli and eukaryotic cells.
- DNA ligases actively participate in cellular DNA repair process.
- DNA Ligase joins (seals) the DNA fragments by forming a phosphodiester bond between the phosphate group of 5'-carbon of one deoxyribose with the hydroxyl group of 3'-carbon of another deoxyribose.

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THE END

THANKS FOR LISTENING

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