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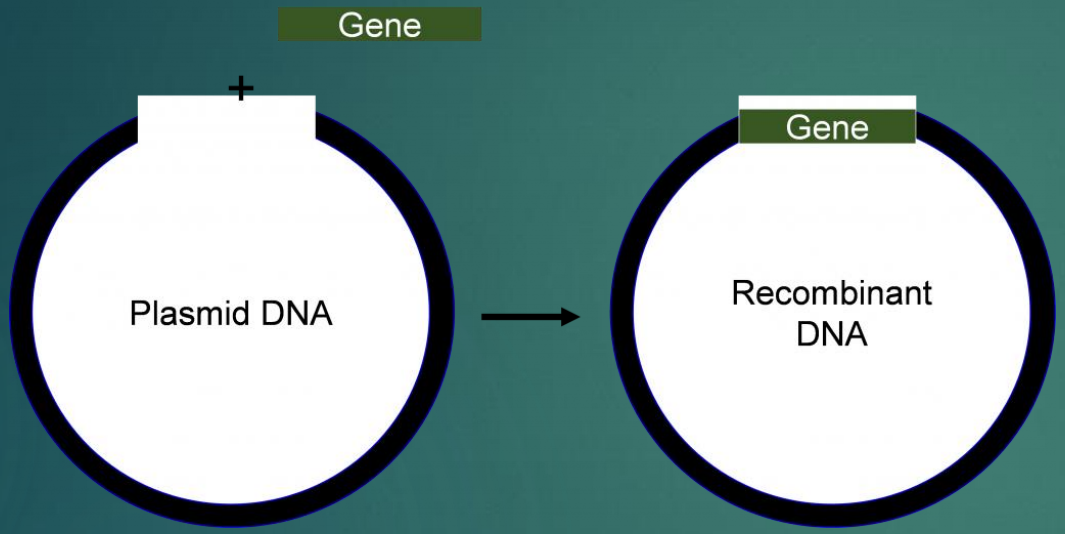
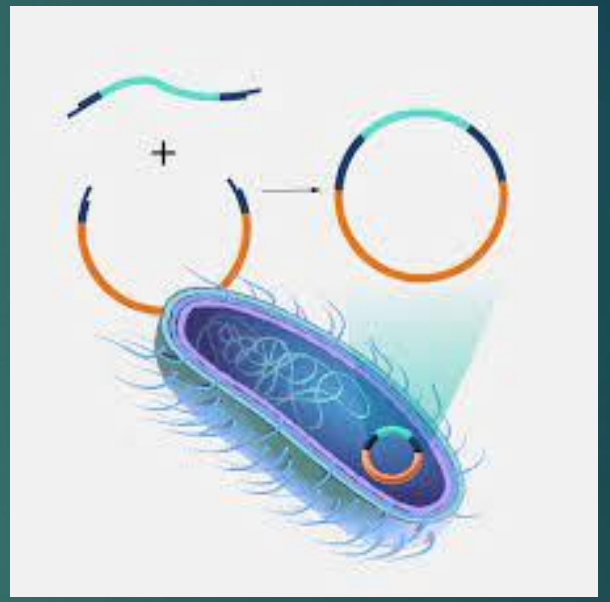
Basic Principles of Recombinant DNA Technology:

- INTRODUCTION
- HISTORY
- PRINCIPLES
- APPLICATIONS

Introduction:

- Recombinant DNA technology, also known as genetic engineering, is a field of biotechnology that involves the manipulation of DNA molecules to create new combinations of genetic material. This technology allows scientists to introduce foreign DNA into an organism's genome, resulting in the expression of new traits or the modification of existing ones. Recombinant DNA technology involves several techniques, including DNA cloning, polymerase chain reaction (PCR), and gene editing. DNA cloning involves the insertion of a DNA fragment into a bacterial or yeast cell, which can then replicate the DNA and produce large quantities of the desired product. PCR is a technique that amplifies a specific segment of DNA, making it possible to study and manipulate small quantities of DNA. Gene editing techniques, such as CRISPR/Cas9, allow scientists to modify specific genes within an organism's genome. Recombinant DNA technology has many applications in medicine, agriculture, and industry. For example, it is used to produce human


Gene
+
Plasmid DNA
Gene
Recombinant
DNA

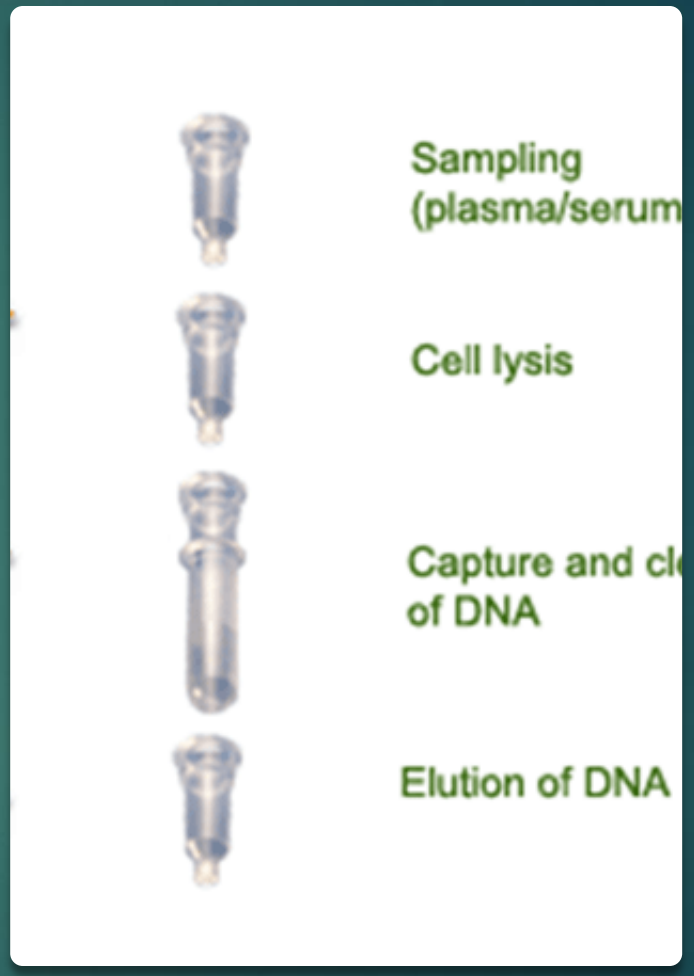


HISTORY:

The first recombinant DNA (rDNA) molecules were generated in 1973 by Paul Berg, Herbert Boyer, Annie Chang, and Stanley Cohen of Stanford University and University of California San Francisco. In 1975, during "The Asilomar Conference" regulation and safe use of rDNA technology was discussed.Paul Berg, a biochemist at Stanford who was among the first to produce a recombinant DNA molecule in 1972, wrote a letter shortly afterwards, along with ten other researchers, to the journal Science

Principles:
Isolation of DNA:

The first step is to extract the DNA containing the gene of interest from the organism of interest. This can be achieved by various methods, such as cell lysis and purification techniques.



- **DNA Isolation Steps:**
- DNA can be isolated from various sources, such as blood, tissue, cells, saliva, and hair follicles, using different methods depending on the sample source and the purpose of the isolation. However, the general steps involved in DNA isolation are as follows:
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- **Sample collection:** The first step is to collect the sample containing DNA. The sample can be collected using various methods, such as blood collection using a venipuncture needle, swabbing the inside of the mouth to collect saliva, or collecting tissue samples using a biopsy.

- **DNA purification:** After cell lysis, the DNA is purified from other cellular components, such as proteins, lipids, and RNA. This can be achieved by adding a series of alcohol solutions, such as ethanol or isopropanol, to the sample to precipitate the DNA. The DNA can then be washed with a solution to remove any remaining impurities.
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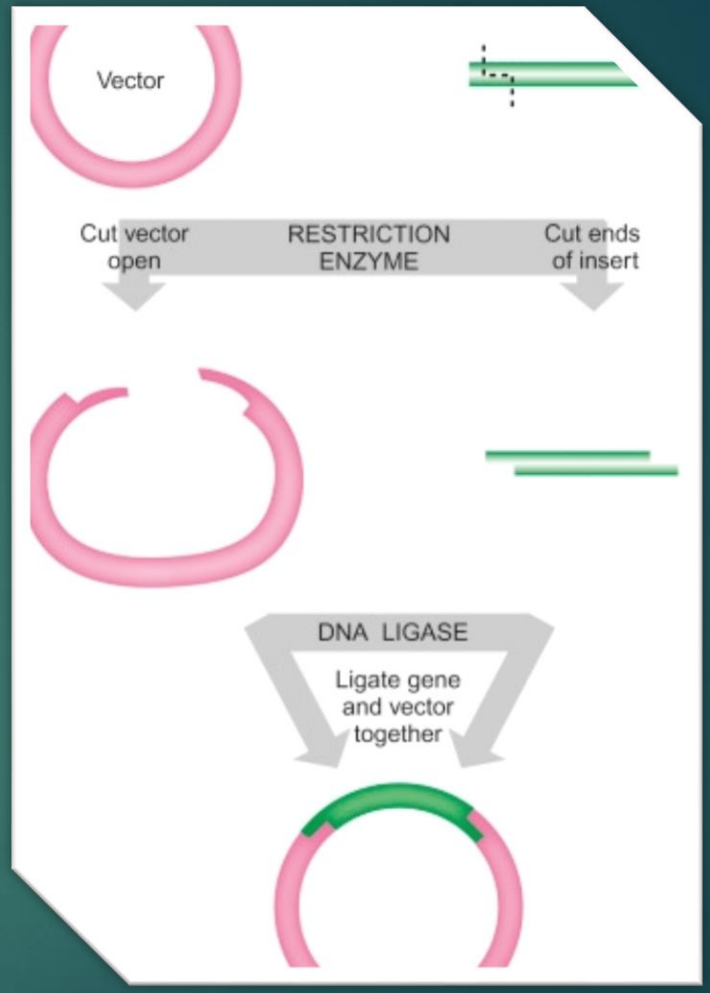
- **DNA quantification:** The final step is to quantify the amount of DNA isolated. This can be done using a spectrophotometer, which measures the absorbance of the DNA sample at a specific wavelength. Alternatively, a fluorometer can be used to measure

Cutting DNA:

The isolated DNA is then treated with restriction enzymes, which are proteins that recognize specific DNA sequences and cut the DNA at those sites. These enzymes produce DNA fragments with sticky ends or cohesive ends, which have unpaired nucleotides that can later be joined with other DNA fragments.



- Restriction Digestion: Restriction enzymes are used to cut DNA into smaller fragments at specific sites. This technique is often employed in molecular cloning, DNA sequencing, and other DNA manipulation procedures.
- Polymerase Chain Reaction (PCR): PCR is a widely used method to amplify specific DNA sequences. To initiate the PCR process, the DNA template is often first cut into smaller fragments using restriction enzymes. This step helps in the efficient amplification of the target DNA region.

- **Genetic Engineering:** In genetic engineering, DNA can be cut and recombined to introduce new genes or modify existing ones. Restriction enzymes are used to cut the DNA at desired sites, and the resulting fragments can be joined with other DNA molecules to create recombinant DNA.
- **DNA Fragment Analysis:** Cutting DNA with restriction enzymes can be used for DNA fragment analysis. By cutting DNA from different individuals or samples with the same enzyme, the resulting fragments can be compared and analyzed to detect variations in DNA sequences.
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Gene of interest and vector preparation:

- The gene of interest, which may come from the same organism or a different organism, is obtained by cutting its DNA using the same restriction enzymes.
- A vector, typically a plasmid or a viral DNA, is also cut with the same restriction enzymes.
- The vector acts as a carrier for the gene of interest.

Joining DNA fragments:

- Joining DNA fragments is the process of connecting two or more separate pieces of DNA together to form a longer DNA molecule. This is commonly done in molecular biology research to create recombinant DNA molecules.
- There are several methods for joining DNA fragments,

DNA LIGATION

.The most common method of joining DNA fragments involves the use of enzymes called DNA ligases. DNA ligases catalyze the formation of phosphodiester bonds between the 3' end of one DNA fragment and the 5' end of another DNA fragment, resulting in a continuous DNA molecule.

Polymerase chain reaction

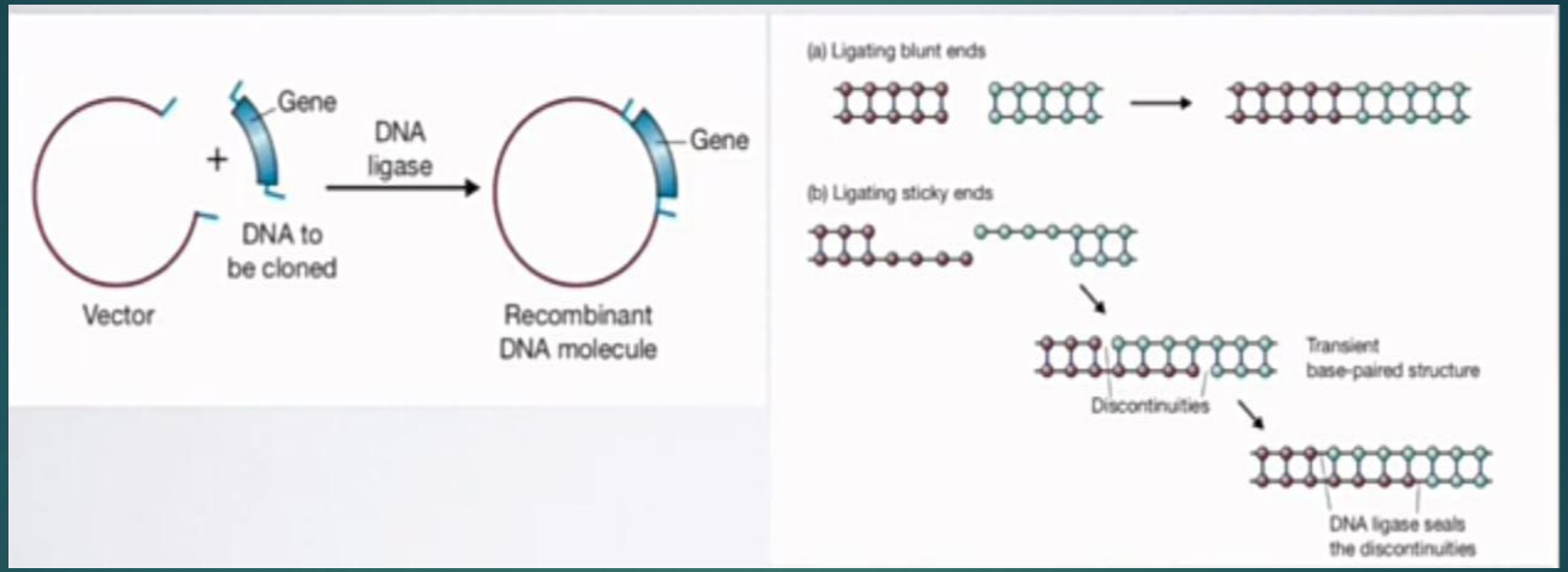
PCR is a technique used to amplify specific DNA sequences. It can also be employed to join DNA fragments.

Restriction Enzyme

Restriction enzymes are enzymes that can recognize specific DNA sequences and cut the DNA at those sites.

Homologous Recombination:

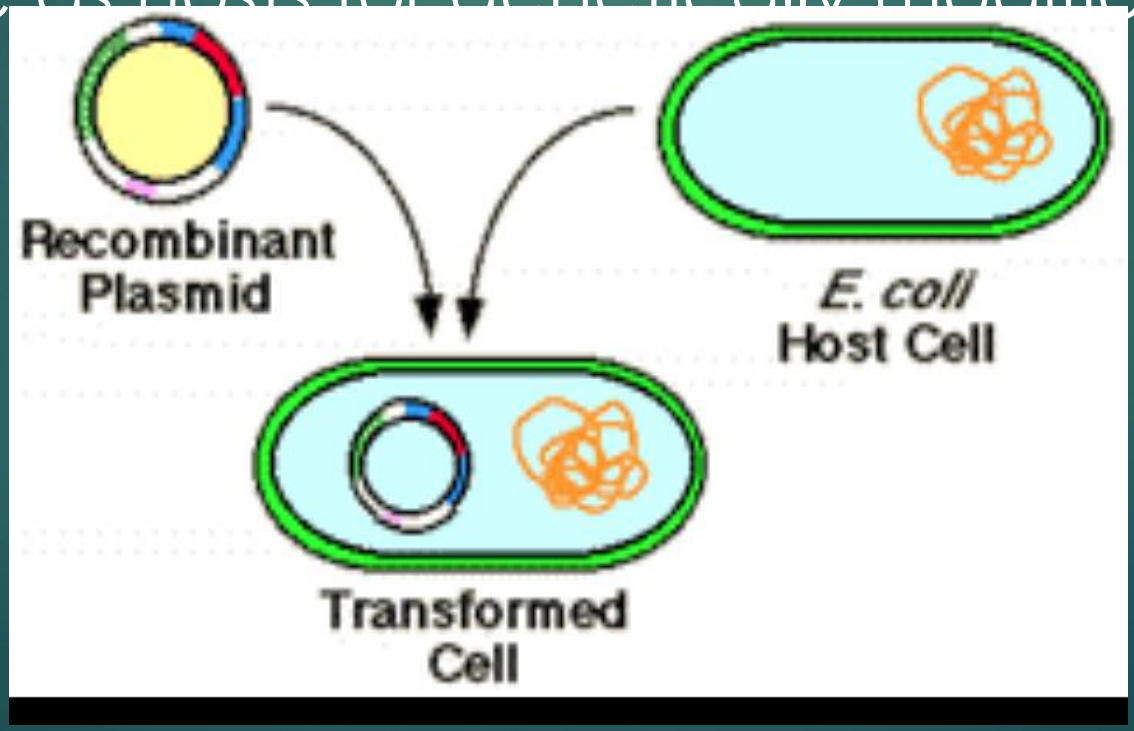
Homologous recombination is a natural DNA repair mechanism that can also be harnessed to join DNA fragments. In this method, the ends of the DNA fragments to be joined are designed in homologous sequence


Gene
DNA
ligase
Gene
+
DNA to
be cloned
Vector
Recombinant
DNA molecule
(a) Ligating blunt ends
(b) Ligating sticky ends
Transient
base-paired structure
Discontinuities
DNA ligase seals
the discontinuities

INTRODUCTION into HOST ORGANISMS:

- The introduction of recombinant DNA into host organisms is a key step in genetic engineering. The DNA fragment of interest is cloned into a vector, which is then introduced into the host cell by transformation, transfection, or infection. Once inside the host cell, the recombinant DNA can be expressed, allowing for the synthesis of proteins or other molecules with desired properties.

- Host organisms are also essential in genetic engineering and biotechnology. Scientists often use host organisms to produce desired substances or manipulate their genetic material. For example, bacteria like Escherichia coli (E. coli) are commonly used hosts for the production of proteins, while plants may serve as hosts for genetically modified crops.

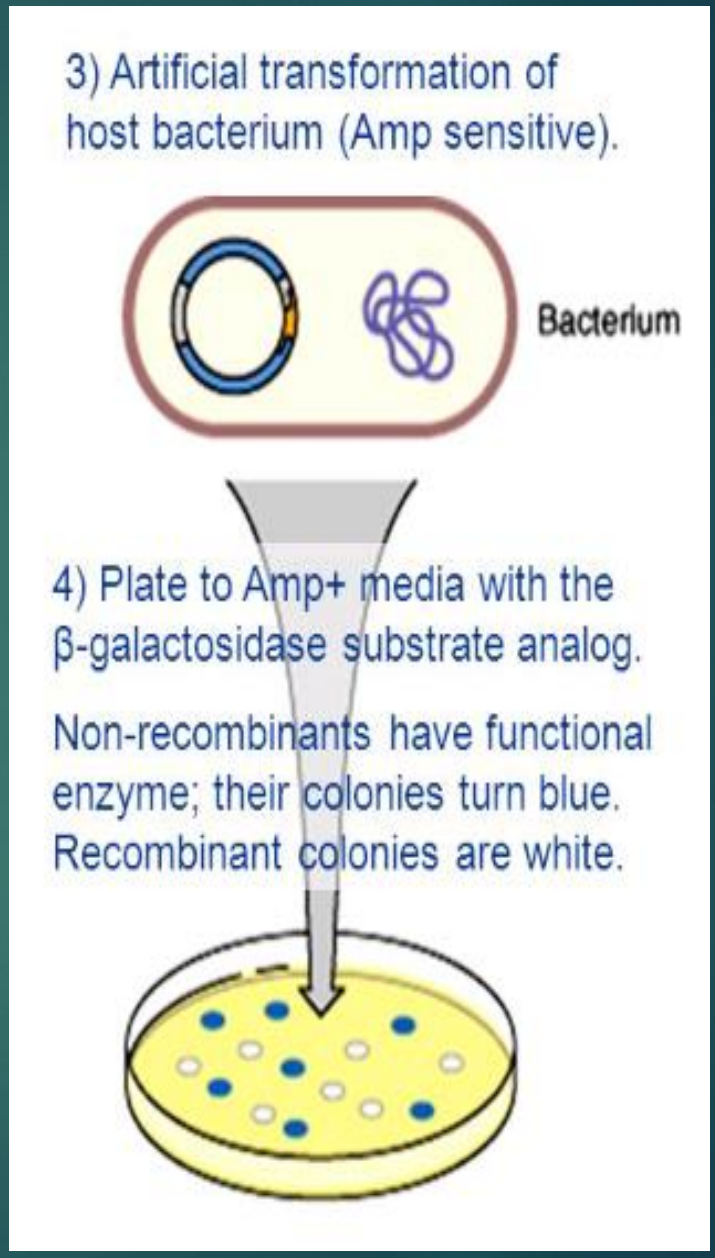


SELECTION:

- It is where some sort of pressure the presence of an anti biotic is applied during the growth of HOST cell, containing the recombinant DNA . The cells with the desired characteristics are therefore selected by their ability to survive. This approach ranges in sufestication from simple selection for the presence of a vector up to direct selection of cloned genes by complementation of defined mutatioins.

SCREENING:

- A genetic screen or muta genesis screen is an experimental technique use to identify and select for individuals who posses a phenotype of edinterest in mutagenized population.



PHARMACEUTICAL Applications:

PHARMACEUTICAL APPLICATIONS REFER TO THE VARIOUS USES OF DRUGS AND MEDICATIONS IN THE TREATMENT AND PREVENTION OF DISEASES. HERE ARE SOME APPLICATIONS OF RECOMBINANT DNA AS FOLLOWS:

1. Gene therapy:

- It is an important applications of RD-TECHNOLOGY.
- Normal gene could be introduced into patient so that genetic disease can be cured.

2. Vaccines:

- VACCINE WHICH MAKE USE OF RECOMBINANT DNA TECHNOLOGY CALLED RECOMBINANT VACCINES.
- GENE ENCODING FOR IMMUNOGENIC PROTEIN IS ISOLATED AND USE TO PRODUCE RECOMBINANT DNA WHICH ACT AS VACCINE. E.G. VACCINA VIRUS
- RECOMBINANT PROTEIN VACCINE: THE SPECIFIC PROTEIN USED IN PREPARE PROTEIN VACCINE

3. Treatment of diseases:

- Recombinant DNA technology had made it possible to treat different diseases by inserting new genes in human body.
- It is widely used in biotechnology, medicine
- and research.Today recombinant protein and other products that result from the use of rDNA technology

4. Recombinant Human growth hormone:

Produced by pituitary gland (HGH) essential for proper growth in children.Some children, however have disorders that cause reduce level of human growth hormone (HGH).

- In 1979 Somatostatin was first introduced.

5. Recombinant monoclonal Antibody:

- Synthetic antibodies called called recombinant antibodies(rAbs)created using antibody genes from human cells.
- It is used to treat cancer, various auto immuno disorders and other disease.
- The Food and Drug Administration approved 22 monoclonal antibodies.

6. Recombinant Blood clotting factor:

- Coagulation f8 is very essential and important factor.
- Less amount of f8 present in human body called haemophilia.
- rDNA technology also made possible production of human blood clotting factor.

THANK YOU SO MUCH…

Any question??