BASIC PRINCIPLES OF GENETIC ENGINEERING

PRESENTED BY:-

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Introduction

- Gregor Mendel is known as “the Father of Genetics”
- Erwin Chargaff noticed that there is a pattern in the 4 bases: Adenine, Guanine, Cytosine and Thymine. (Adenine paired with Thymine and Guanine paired with Cytosine)
- Genetic engineering is the artificial manipulation or alteration of genes

OR

It is the process by which pieces of DNA are transferred from one organism to another (i.e. from Human to Bacterium or from Bacterium to Plant or from Human to Sheep)

- Genetic engineering involves these steps:-

-removing a gene (target gene) from one organism

-inserting target gene into the DNA of another organism

-”Cut and paste process”

- Genetic engineering allows the DNA from different species to be joined together.
- Process:-

1. DNA carrying a gene of interest is taken from a (e.g., a human) cell
2. The gene is inserted into the DNA of another (host) cell
3. The host cell now contains recombinant DNA
4. The host cell multiplies

- This often results in combinations of DNA that would never be possible in nature!!! For this reason genetic engineering is not a natural process.
- If DNA is transferred from one species to another the organism that receives the DNA is said to be transgenic (Genetically Modified organism).
- Examples of cross-species transfer of genes:
  - a human gene inserted into a bacterium
  - a human gene inserted into another animal
  - a bacterial gene placed in a plant

Important Terms

- **Recombinant DNA-** altered DNA is called recombinant DNA (recombines after small section of DNA is inserted into it)

  OR

  DNA made from 2 different organisms e.g. human gene and bacterial plasmid

- **Genetically Modified Organism (GMO)-** Organism with the altered DNA

Tools Used in Genetic Engineering

- **Restriction Enzymes**:

  - are special enzymes used to cut the DNA at specific places.

  - different enzymes cut DNA at specific base sequences known as a recognition site. For example

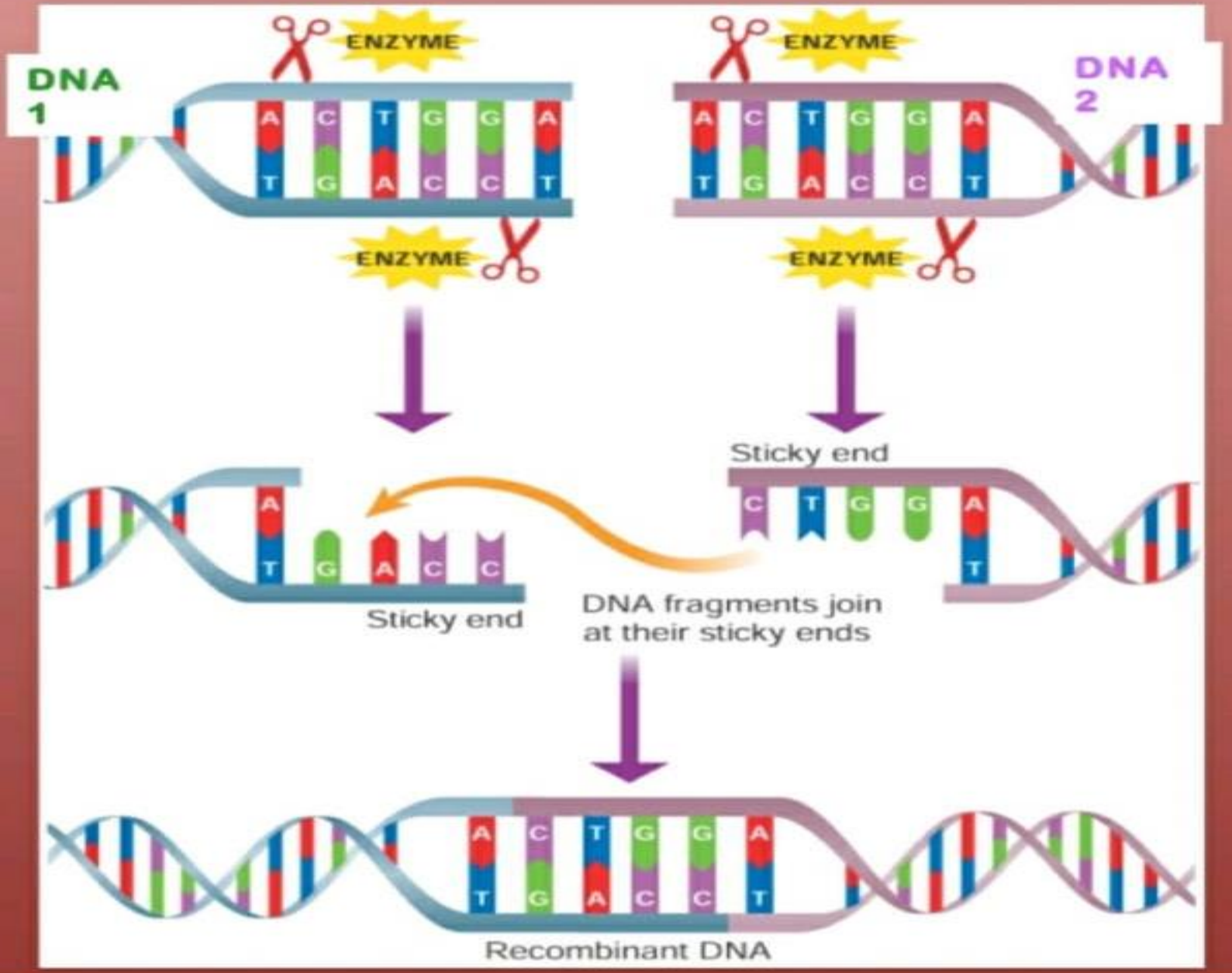
    i) One restriction enzyme will always cut DNA at the base sequence: GAATTC.

    ii) Another restriction enzyme only cuts at the sequence: GATC.

  - If DNA from two different organisms is cut with the same restriction enzyme the cut ends from both sources will be complementary and can easily stick together.

- Restriction endonucleases can cut the DNA crosswise at selected positions
- Has the capability to recognize and cut at the palindromes
- Can make a blunt cut or a “sticky end”
- The pieces of DNA produced are called as restriction fragments.
- Differences in the cutting pattern of specific restriction endonucleases give rise to restriction fragments of differing lengths- restriction fragment length polymorphism.

Restriction enzymes



- **DNA Ligase**:

  -enzyme which acts like a glue sticking foreign DNA to DNA of the cloning vector.

  -enzyme necessary to seal the sticky ends together.

  -will only work if DNA from the two DNA sources has been cut with the same restriction enzyme i.e. sticky ends of cut DNA will be complementary to each other.

- **Reverse Transcriptase**:

  -enzyme that is required when RNA has to be converted into DNA

- **VECTOR** - plasmids (A plasmid is a circular piece of DNA in a bacterium) or viruses are vectors

  -A vector is needed to transfer a gene (piece of donor DNA) into a host cell

  -readily accepted by cloning host

  -vector such as plasmid or bacteriophage should have an origin of replication (ORI) which is a sequence somewhere on the vector from where replication starts and any piece of DNA when linked to this sequence can be made to replicate within the host cell

  -must accept DNA of desired size

  -contain a gene that confers drug resistance to their cloning host

Process of Genetic Engineering

- Five steps involved in this process:
  1. Isolation
  2. Cutting
  3. Insertion (Ligation)
  4. Transformation
  5. Expression

Applications of Genetic Engineering

- You must know three applications: one involving a plant, one animal and one for a micro-organism.
- **PLANTS**: "Weed killer-resistant crops"

  -many types of crop plants have bacterial genes added to them.

  -these genes make the plants resistant to certain weed killers (herbicides).

  -this means that the weed killers kill the weeds but do not affect the transgenic plants.

- **ANIMALS**: There is a growing trend to experiment with inserting human genes into the DNA of other mammals. The transgenic animals formed in this way will then produce a human protein and secrete it into their milk or even into their eggs.
- E.g.:- “Sheep produce human clotting factor”

  - A human gene has been inserted into the DNA of sheep.

  - This allows the adult sheep to produce a clotting chemical needed by haemophiliacs to clot their blood – produced in the milk of the sheep.

  - **Pharming**: is the production of pharmaceuticals by genetically modified animals i.e. sheep, cows, goats etc.

- **MICRO-ORGANISMS**: "Bacteria make insulin"

- before genetic engineering, insulin came from pigs and cows

-Insulin is produced by genetically modified bacteria. The human gene able to make insulin is inserted into bacteria.

- The human insulin gene has been inserted into a bacterium (E-coli).

-This allows the bacterium to produce insulin for use by diabetics.

PRODUCTION OF INSULIN USING RDNA TECHNOLOGY

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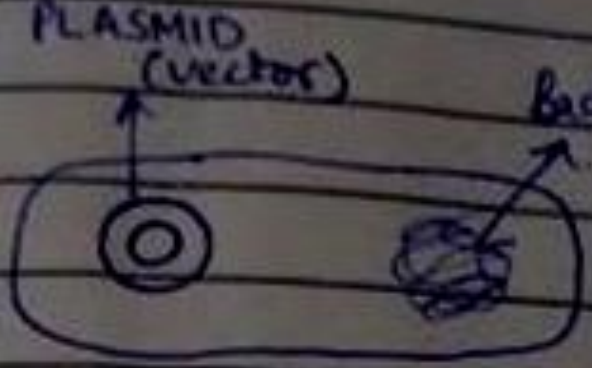


E. coli

PLASMID

RESTRICTION ENDONUCLEASE

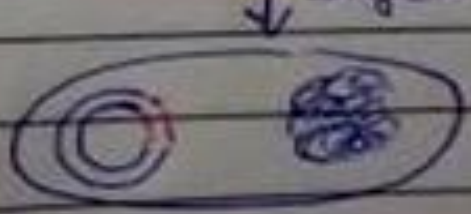
Human cell

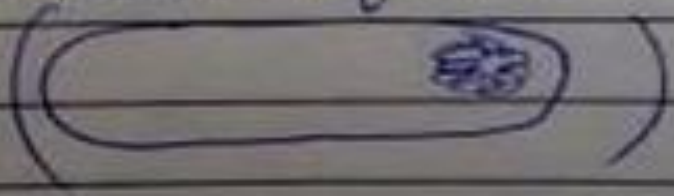
INSULIN GENE

RESTRICTION ENDONUCLEASE

RECOMBINANT DNA

↓ Inject into plasmid free E. coli





RECOMBINANT DNA

E. coli CELL

↓

Culturing of this bacteria and extractio
of insulin

↓

- Insulin injected into patients

OTHER APPLICATIONS OF GENETIC ENGINEERING

- To make growth hormone to treat dwarfs
- To prepare vaccines
- To make plants resistant to disease Tomato mosaic virus causes great damage
- To make pigs, cows or fish grow faster
- Higher production of milk by cows
- To make pigs with less fat - leaner meat.

- **Gene therapy**-when a gene is inserted into a patient to treat a genetic disease.

- The new DNA usually contains a functioning gene to correct the effects of a disease-causing mutation.

- It repairs or reconstructs the defective genetic material. In future, this technique may allow doctors to treat a disorder by inserting a gene into patient's cell instead of using drugs or surgery.

- A gene that is directly inserted into the cell usually doesn't function. So a vector acting as a carrier is genetically engineered to deliver the gene.

-Certain viruses are used as vectors because they can deliver the new gene by infecting the cell. The viruses are modified so they can't cause disease when used in people.

-The vector may be directly injected or given intravenously into a specific tissue in the body, where it is taken up by the individual cells. Alternatively, a sample of patient's cells can be removed and exposed to the vector in lab setting. The cells are then returned to the patient.

- Golden Rice: is genetically modified (rich in vitamin A)
- Drought-Resistant Seeds
- Pest-Resistant Cabbage- Gene that programs poison in scorpion tails was combined with cabbage. These genetically modified cabbages produce scorpion poison that kills caterpillars when they bite leaves — but the toxin is modified so it isn’t harmful to humans.
- GM Trees Grow faster, yield better wood. Australian eucalyptus trees have been altered to withstand freezing temperatures. Loblolly pines have been created with less lignin – better to make paper.

POSSIBLE HAZARDS OF GENETIC ENGINEERING

- Risks for human health, e.g. higher chances for cancer
- Risks for animal health, e.g. higher chances of infection
- Risk of creating new microbes that are difficult to kill by antibiotics

THANKS