

GENETIC ENGINEERING

Definition:- The branch of Biological Science which deals with the addition, subtraction or Replacement of genes from an organism to bring permanent heritable change for Mankind - is called G.E. ✓

OR

Isolation of gene from one organism and its transfer to another genome (organism) to produce recombinant DNA of desirable combination is possible by Modern enzymatic technique. That is why G.E. is popularly called Recombinant DNA Technology.

APRIL
THURSDAY

28

To know the Process of Genetic Engineering following terms must be explained

① R.E. (Restriction Endonuclease):- It is specific enzyme which cuts DNA at a specific site - called PALINDROMIC SITE.

In genetic engineering such group of enzymes are called SCISSORS or cut enzymes. It was discovered by SMITH &

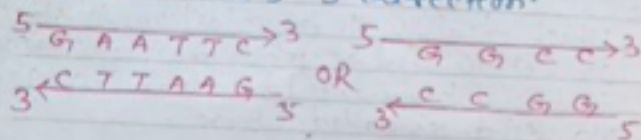
WILCOX (1970) from Haemophilus influenzae Bacteria. Through R.E. isolation of DNA segment is possible. It is also known as

HIN MALE STIMULANT

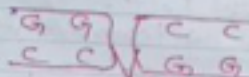
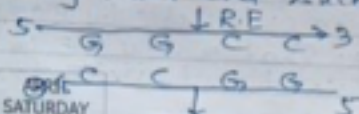


4	11	18	25
5	12	19	26
6	13	20	27
7	14	21	28
8	15	22	29
9	16	23	30

(2) PALINDROMIC SITE! - is that in which sequence of 4 to 6 nucleotides reading are same in 5-3 direction.

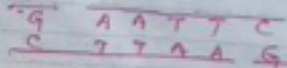
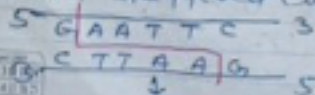


(3) BLUNT END:- Some R.E. cuts the two strands of DNA at the same place, generating blunt end such as:-

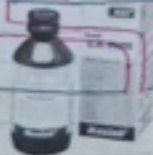


Blunt end.

(4) STICKY END:- Some R.E. cuts the two strands of DNA at different places generating sticky end. Two cuts ends are separated by few nucleotides called staggered cut.



Staggered Cut



A NERVE TONIC WITH VITAMIN B

(5) VECTOR + PLASMIDS + TRANSFORMATION:-

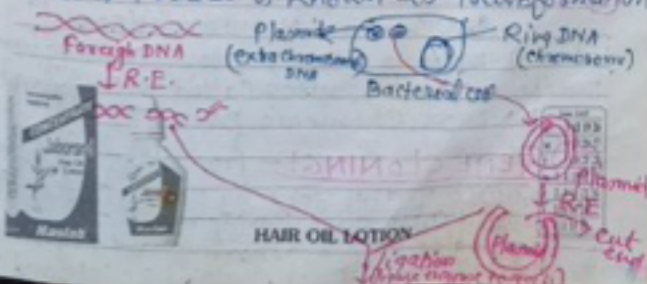
Plasmids are extra chromosomal DNA found in bacterial cell. These DNA can self replicate. They arise from chromosomal DNA.

It is possible to isolate these plasmids in large quantity.

Vectors are vehicles through which foreign gene is carried into desired organism. Plasmids and viruses are used as a carrier of foreign DNA. So, plasmids (foreign gene) and viruses (vehicle) are commonly known as vectors.

Transformation:- Thus by interchanging plasmid DNA (foreign bacterial DNA) and viral DNA fragments several new vectors have been synthesized.

When such new fragments (DNA) are introduced into appropriate host cells, then such cells are called transformed and process is known as transformation.



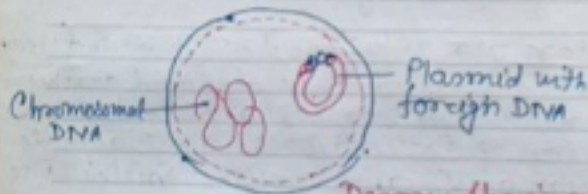
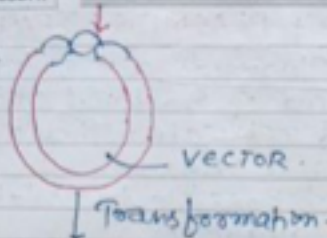


Diagram Showing

Vectors & Transformation

Thus, Plasmids are defined as self replicating extra chromosomal DNA present in one or more many copies and vectors are small segment of DNA which are carry any gene to 'recipient Bacterium'

6 RECOMBINANT DNA (CHIMERIC DNA):-

Association of two different types of DNA as shown above coming from Bacteria and viruses called recombinant DNA or Chimeric DNA.

7 GENE CLONING!:- Production of many copies of any gene by using plasmid DNA and Bacteria. Cloning of Entire genome of an organism in form of small DNA Placed in suitable recipient cells.



Such as Bacteria is called host Gun Experiment
8 GENE LIBRARY:- The entire genome of an organism can be fragmented and each fragment can separately be cloned to produce many copies of each fragment. This is referred to Gene library.

TECHNIQUES AND ACHIEVEMENT OF GENETIC ENGINEERING

Following Techniques are application in Genetic Engineering

- (1) Transformation
- (ii) Parasexual Hybridization or Protoplast Fusion
- (iii) Transduction
- (iv) Recombinant DNA Technology.

1 TRANSFORMATION:- The process by which direct introduction of DNA into any cell. Such as:-

(a) In Bacteria:- In prokaryotic cell like Bacteria desired gene and Bacteria are placed in incubator. Then there is uptake of DNA. Such produced Bacteria is called transformed Bacteria.



Diagram illustrating the process of transformation in bacteria. A 'Desired Gene' is added to 'Bacteria Placed in Incubator'. The process results in 'Transformation' and 'Uptake of DNA'. The final product is 'Bacteria Transformed'.

6) In Plant: - To introduce the desired gene in plant cell action of enzyme Pectinase and cellulase are must to dissolve the cell wall and cell membrane.

Desired gene + Plant cell $\xrightarrow[\text{Incubator}]{\text{Pectinase, Cellulase}}$ Placed in

Transformed Plant cell $\xleftarrow[\text{Uptake of DNA}]{\text{Removal of cell wall and membrane}}$

Such plant cell (transformed) can multiply in suitable medium called Tissue culture. Such plant produced by tissue culture is called Transgenic plant. These plant will appear with new characters.

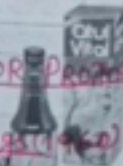
7) Animal cell: - Because cell wall is absent, so only treatment of cellulase is required to remove cell membrane.

Desired gene + Animal cell $\xrightarrow[\text{In Incubator}]{\text{Cellulase}}$ Uptake of DNA

Transgenic Animal (mouse) $\xleftarrow[\text{Transformed Animal cell}]{} \leftarrow$

8) PARASEXUAL HYBRIDIZATION OR PROPLAST FUSION

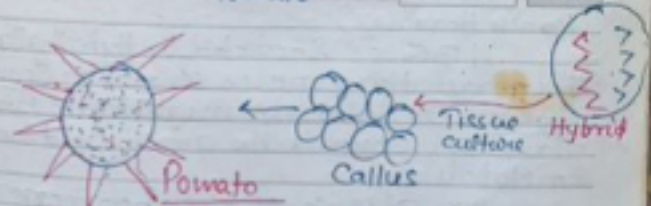
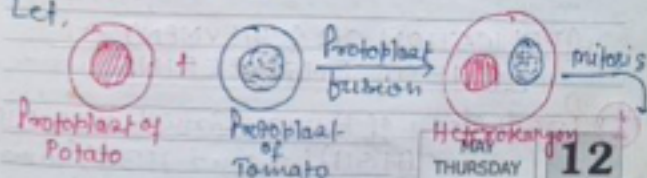
Recorded by Edward Cocking (1960) FOR VIGOR & VITALITY NON-SUGAR SYRUP



Plant cell after treatment with Pectinase and cellulase their protoplasts are separated. Then Protoplast of two different species are ~~not~~ placed together called Protoplast fusion forming Heterokaryon.

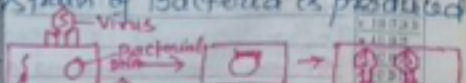
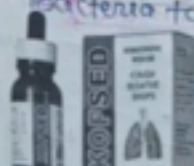
Potato is an example of Protoplast fusion produced by Potato and Tomato. By such Techniques Genetic Barrier can be crossed.

Let,



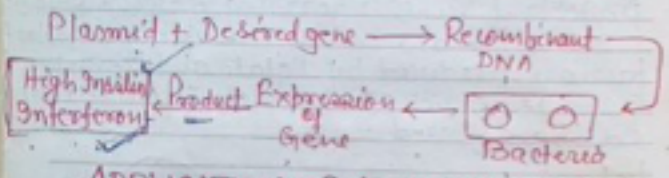
8) TRANSDUCTION: -

Transfer of Genetic material from one bacteria to another is called Transduction. Thus two types of different strain of Bacteria is produced.



Thus two types of different strain of Bacteria's produced.

④ RECOMBINANT DNA TECHNOLOGY:-



APPLICATION OR ACHIEVMENT OF GENETIC ENGINEERING.

① Production of Human Growth Hormone

14 MAY SATURDAY

(HGH):- and HBH (Human Brain Hormone) -

HGH is secreted by Hypothalamus. This brain hormone is known as Somatostatin. It later on migrates to Pituitary gland. It controls the growth and sugar metabolism.

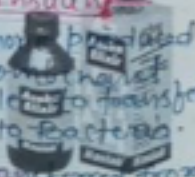
In absence of this hormone man suffers with a disease called Pituitary Dwarfism.

② Synthesis (Production) of Insulin

15 SUNDAY

Insulin is a Hormone produced by cattle. By DNA recombinant technology, it is possible to transfer genes from mammals to Bacteria.

Such Insulin gene is transferred from cattle to Bacteria to produce INSULIN.



③ Production of Interferon (Animal Protein + Anticancerous):-

Interferons are "viral induced" protein produced by cells infected by Virus. Charles Weismann isolated the gene for Interferon from Human Lymphocytes and cloned it in E. coli and yeast.

It is potential antiviral drug used for the treatment of certain types of Cancer - specially Breast cancer. It is also used as Natural defence against viral disease like Influenza and Hepatitis.

④ Production of Urokinase

MAY TUESDAY 17

It is an enzyme responsible for dissolution of Blood Clots. Urokinase is Genetically synthesized using microorganism.

⑤ Production of Hybridomas = (Monoclonal Antibody)

Hybridomas are Genetically engineered long lived Hybrid cells, produced by fusion of B-cell Single lymphocyte of mouse and Single Bone marrow tumor cells.



The technique involved is called Hybridoma Technique and as Monoclonal Antibodies.

This Hybridoma is used for production of Antibodies which can be used against treatment of several disease and also for diagnosis.

⑥ Production of Suberbug (*Pseudomonas putida*)

It is genetically altered Bacterium used in control of environmental pollution to consume Hydrocarbon.

This new Bacteria combine the Genetic elements of four (4) different Bacteria and has the ability of breaking up the oil spills - commonly called oil eating.

⑦ NIF Gene: - (Nitrogen-Fixing Gene) -

A set of altogether 20 genes called Nitrogen fixing genes have been recognised in Bacteria - *Klebsiella pneumoniae*.

They are nitrogen fixing genes. These genes are transferred to the crop plants with a view to overcome the use of costly fertilizers.

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MAY
THURSDAY

⑧ DNA Finger Printing (Southern Blotting): -

Southern blotting is a technique evolved by E.M. Southern for isolation of Eukaryotic genes.

DNA fragments produced by restriction enzymes are obtained as bands on an Agarose gel by gel electrophoresis.

These fragments are then transferred to Nitrocellulose filter and identified by using DNA or RNA probes.

Probes: - Small segments of DNA or mRNA - used to detect the presence of

a gene containing particular sequence of nucleotides are called probes.

DNA finger printing is the technique used in Forensic Science to identify criminals or any kind of suspect by so-called blotting. DNA can be isolated from blood stains, hairs or semen stains.

⑨ **Northern Blotting:** - Isolation and identification of RNA molecule by above technique is called Northern blotting.

⑩ **Gene Therapy:** - It is possible to cure genetic diseases (defects) in man by altering the genetic materials of the female. It is possible to cure several chronic disease through such therapy.

⑪ Production of Factor VIII-C for Hemophilia:

The cloning of DNA for Human blood clotting called Factor VIII-C has been successfully achieved. This DNA expressed in mammalian cell and produced the protein VIII-C, which is responsible for clotting of human blood.

Since this protein is absent in Hemophiliacs, so, it is used in treatment of Hemophiliacs.

⑫ Genetic engineering is also used for transfer of disease resistance and insecticide genes into the crop plant.