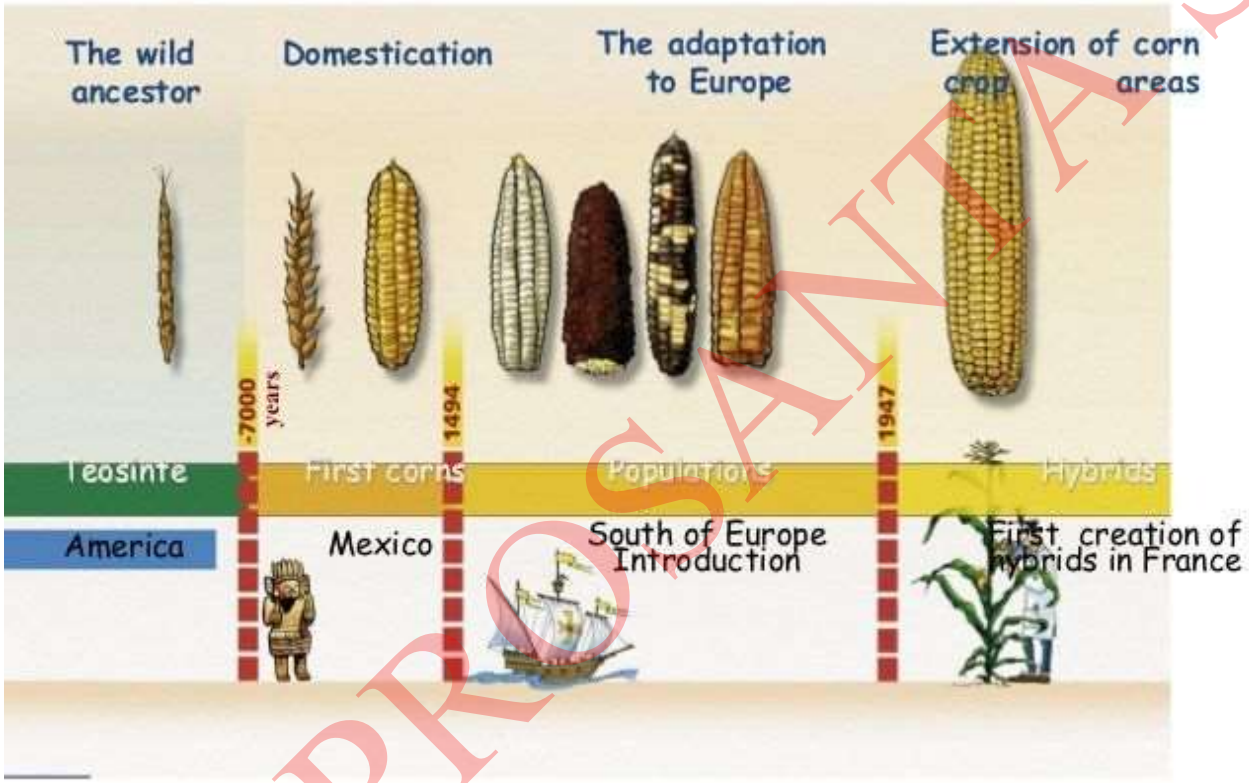
Plant Biotechnology

-Overview

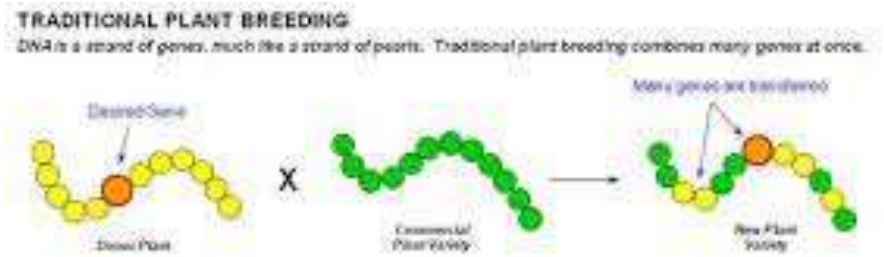
Prosanta Saha

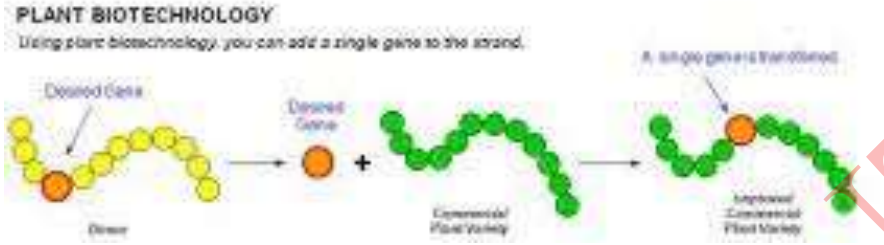
Domestication for crop improvement

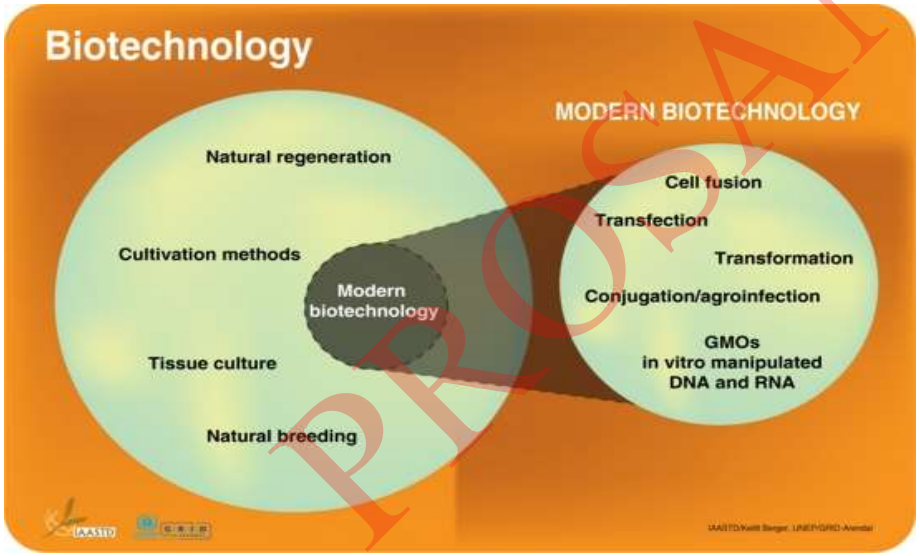
- A process over thousands of years... Since human civilization began
- Mainly done through conventional breeding approaches



plant breeding and biotechnology contd...







Comparing Genetic Modification Techniques

	Conventional Breeding	Mutation breeding	Plant Biotechnology
Level	Whole organism	Molecule	Molecule
Precision	Thousands of genes	Unknown	Single gene
Certainty	Genetic change poorly characterized	Genetic change poorly characterized	Gene function well understood
Limits	Between species and genera	Not applicable	No limitations

Genetic Engineering

The process of manipulating and transferring instructions carried by genes from one cell to another

Why do scientists want to change gene instructions?

- **to produce needed chemicals**
- **to carry out useful processes**
- **to give an organism desired characteristics**

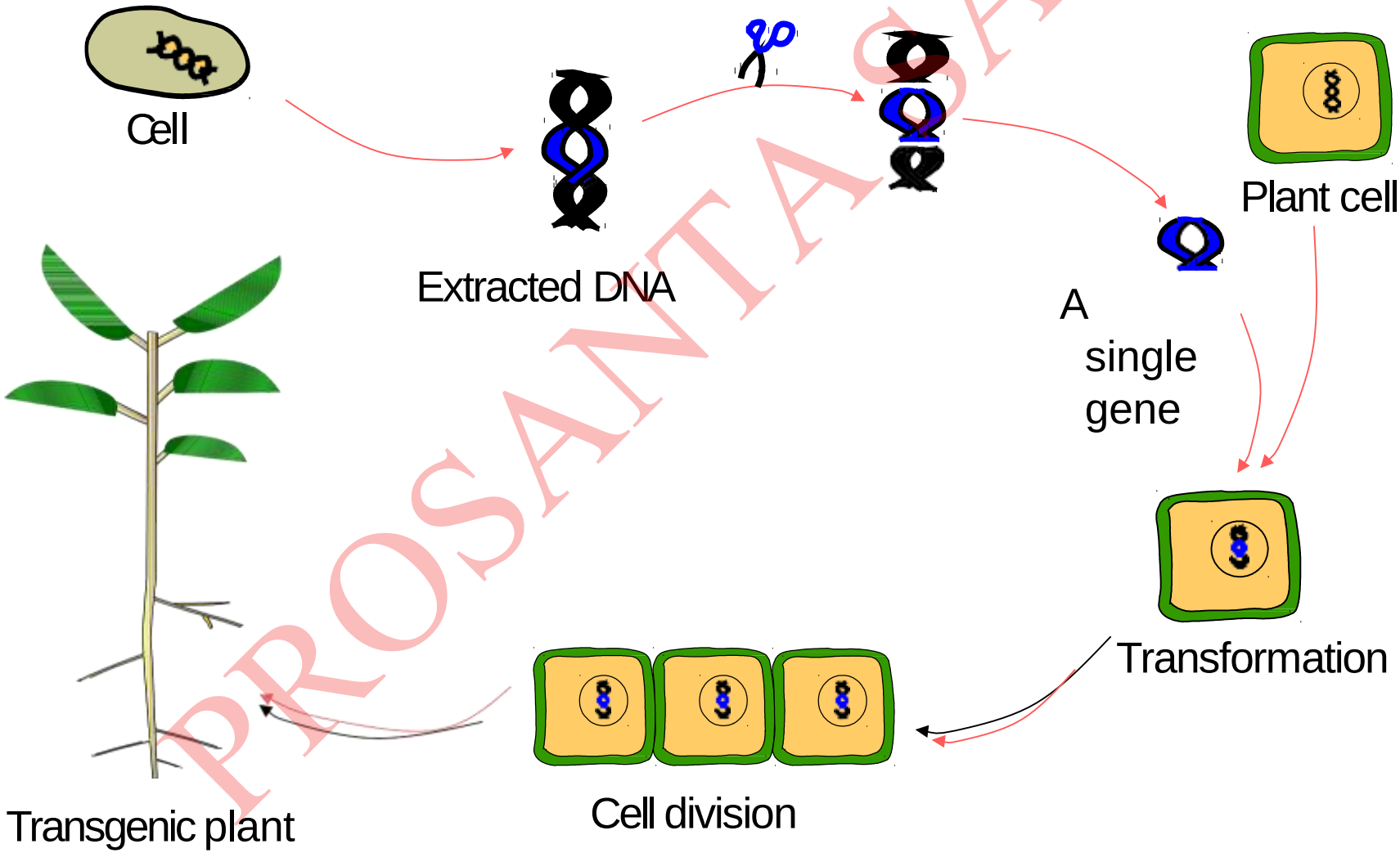
What About the Term Genetic Engineering?

Genetic engineering is the basic tool set of biotechnology

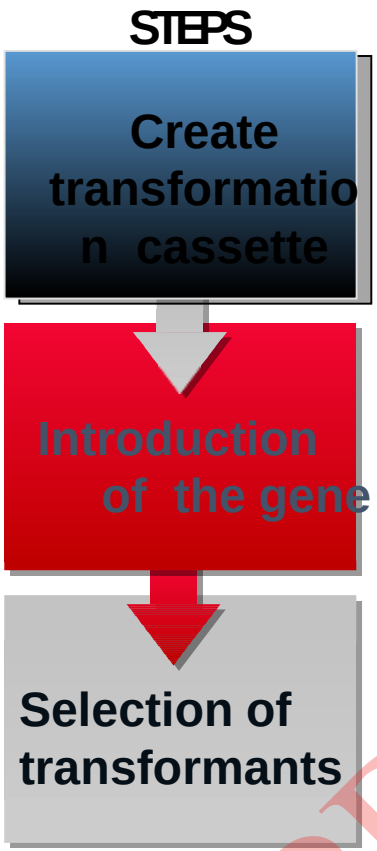
Genetic engineering involves:

- Isolating genes
- Modifying genes so they function better
- Preparing genes to be inserted into a new species
- Developing transgenics

Plant Genetic Engineering Process



Introducing the Gene - Requirements



- **Prepare tissue for transformation**
  - Tissue must be capable of developing into normal plants
  - Leaf, germinating seed, immature embryos
- **Introduce DNA**
  - *Agrobacterium* or gene gun
- **Culture plant tissue**
  - Develop shoots & Roots
- **Screening of putative transformants**
  - Field test the plants

- **DNA delivery systems must be**
  - *Simple*
  - *Efficient and preferably inexpensive*
  - *The method must be available for use either because it is in the public domain or because it can be licensed*
- **System of choice depends on**
  - *the target plant*
  - *its regeneration system*

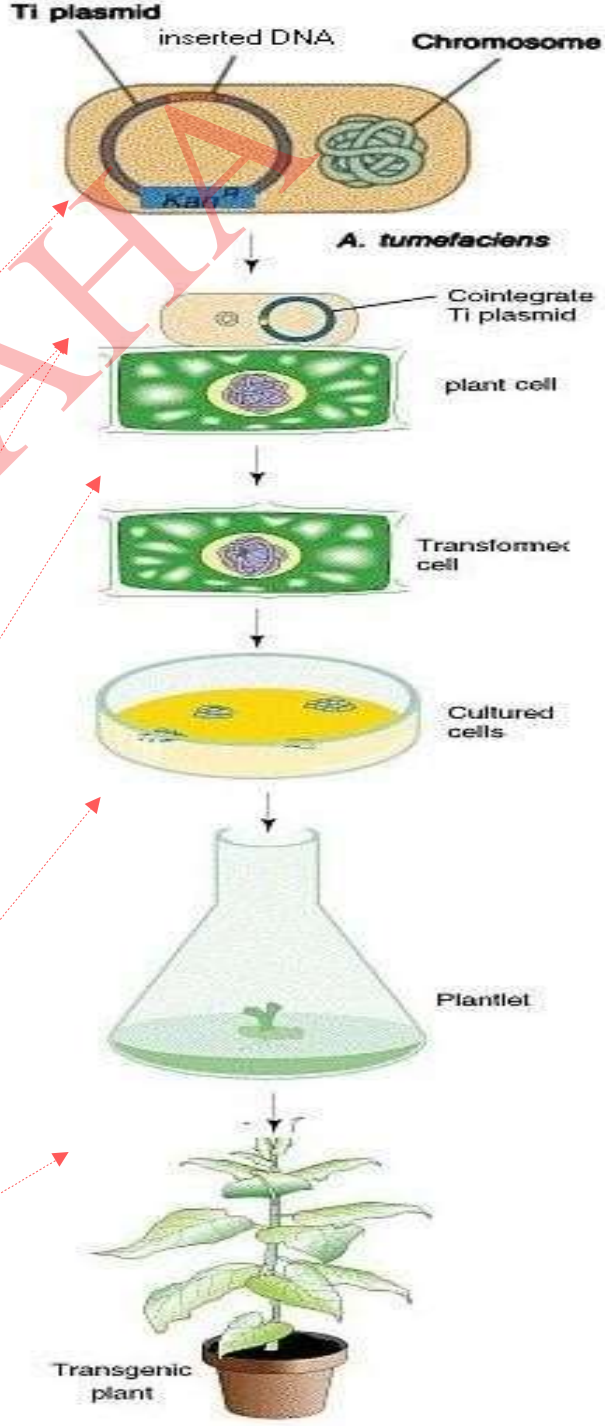
Isolate and clone gene of interest

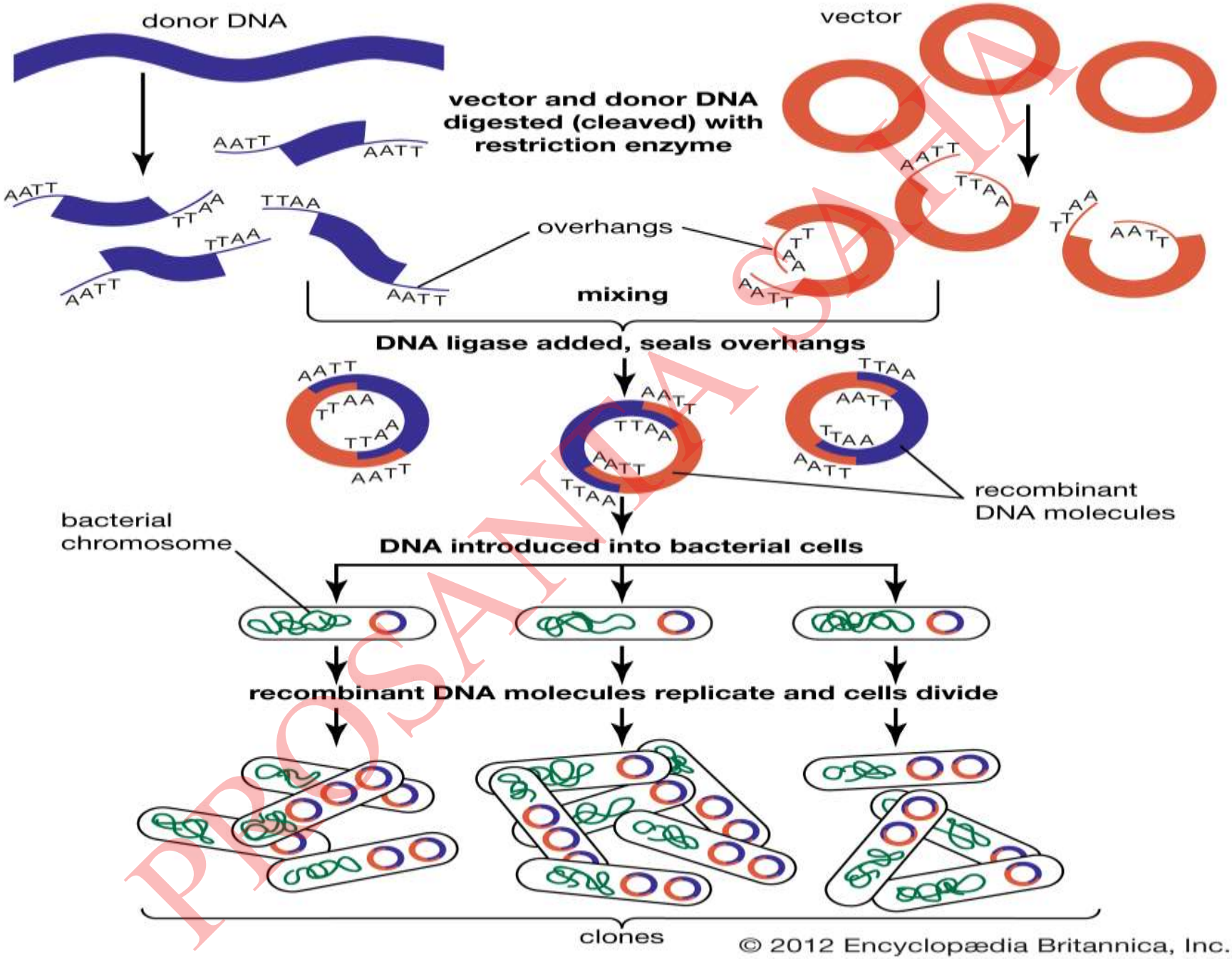
Add DNA segments to initiate or enhance gene expression

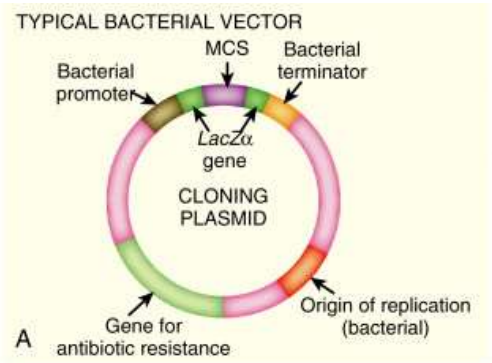
Add selectable markers Introduce gene construct into plant cells (transformation)

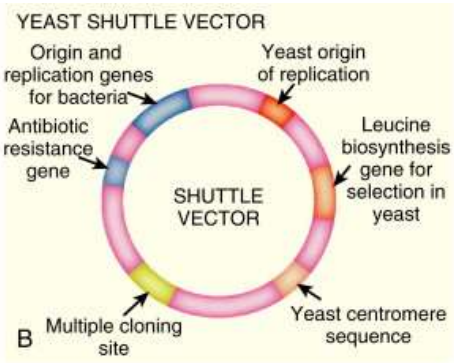
Select transformed cells or tissues

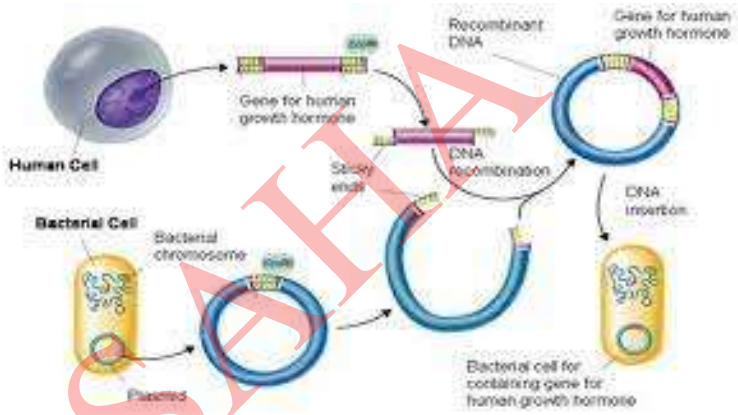
Regenerate whole plants

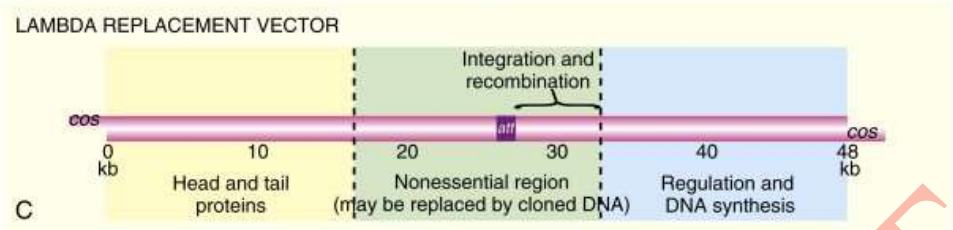


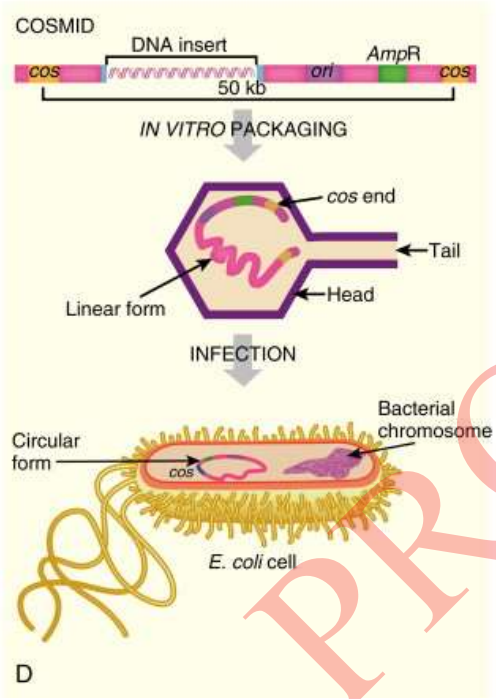


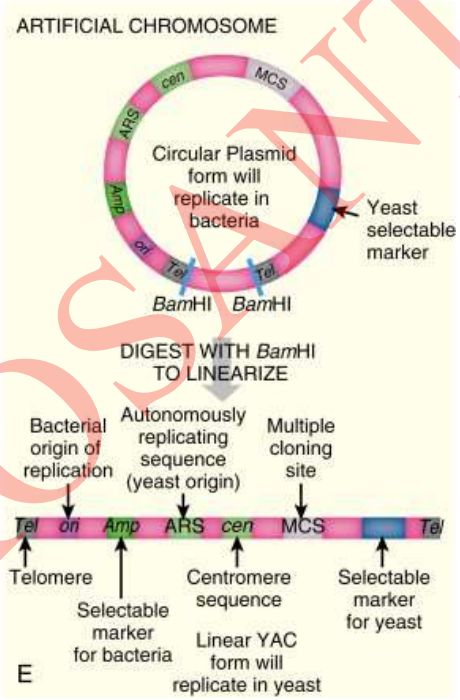


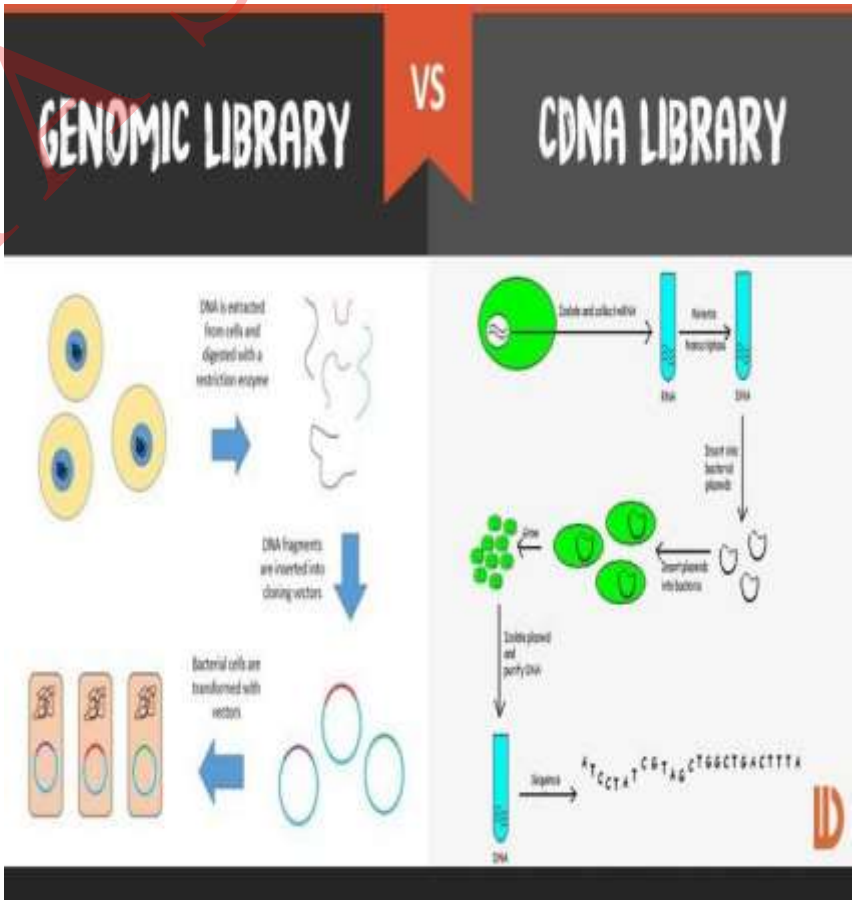




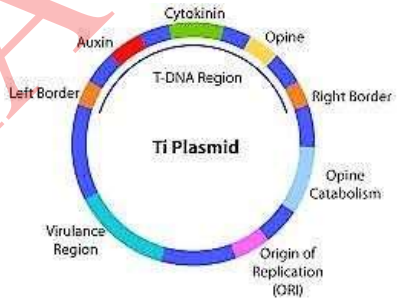








Commonly used promoters



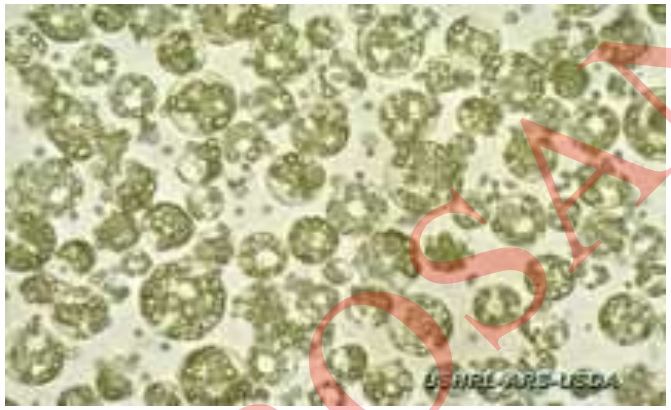
- **Constitutive promoter**
  - **CaMV 35S** : suitable for expression of foreign genes in dicots:
  - The maize **ubiquitin** promoter, also a constitutive promoter which
  - drives strong expression of transgenes in monocots.
- **Organ/ tissue specific promoters**
  - **Vicilin** and **phytohemaglutinin**, **glutenin promoters** seed specific expression
  - a-**amylase** promoter for expression in the aleurone of cereal grains;
  - **Patatin** promoter for tuber specific expression in potatoes and the **RuBisCo** promoter for green tissue specificity

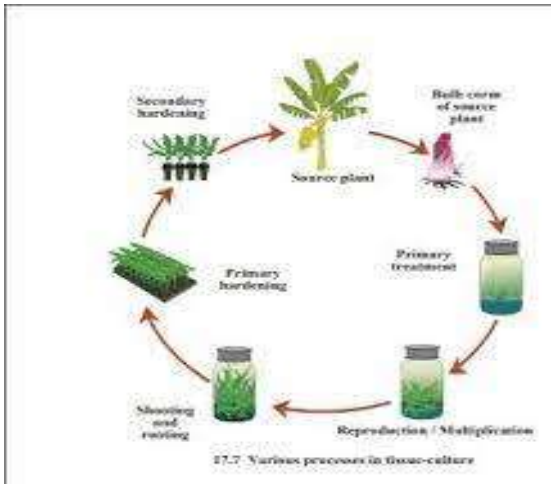
Regeneration

We use tissue culture techniques to regenerate whole plants from single cells

getting a plant back from a single cell is important so that every cell has the new DNA

Plant tissue culture uses growth regulators and nutrients to regenerate plants *in vitro*





17.7 Various processes in tissue culture

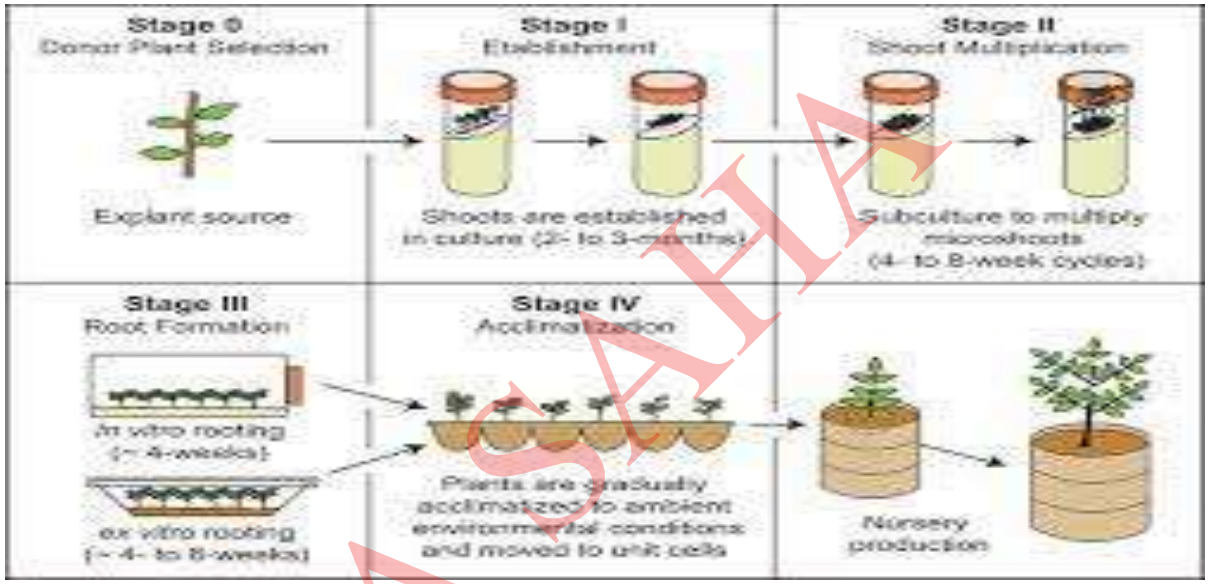


Figure 5.8: Protoplast Culture

Transformation methods

Indirect

Agrobacterium tumefaciens mediated, Virus mediated

Direct

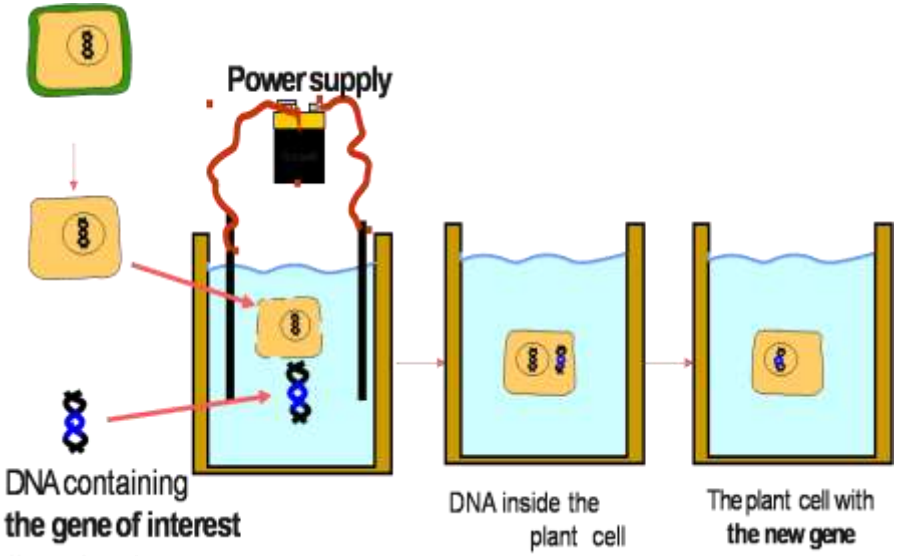
Particle bombardment

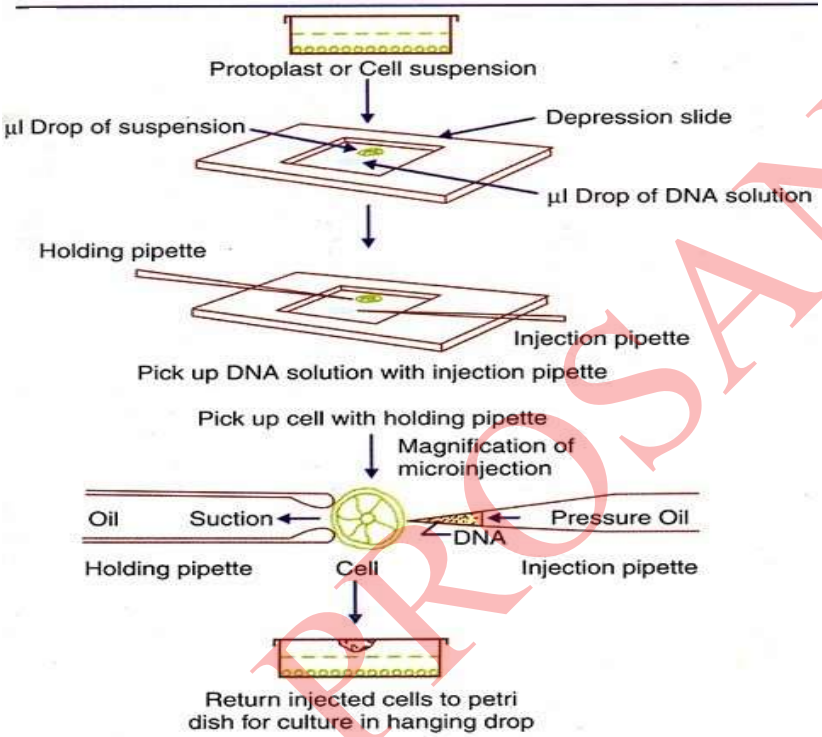
Polyethylene glycol (PEG)-mediated protoplast transformation.

Electroporation Microinjection

Silicon Carbide Whiskers (SCW)

Method depends on plant type, cost, application





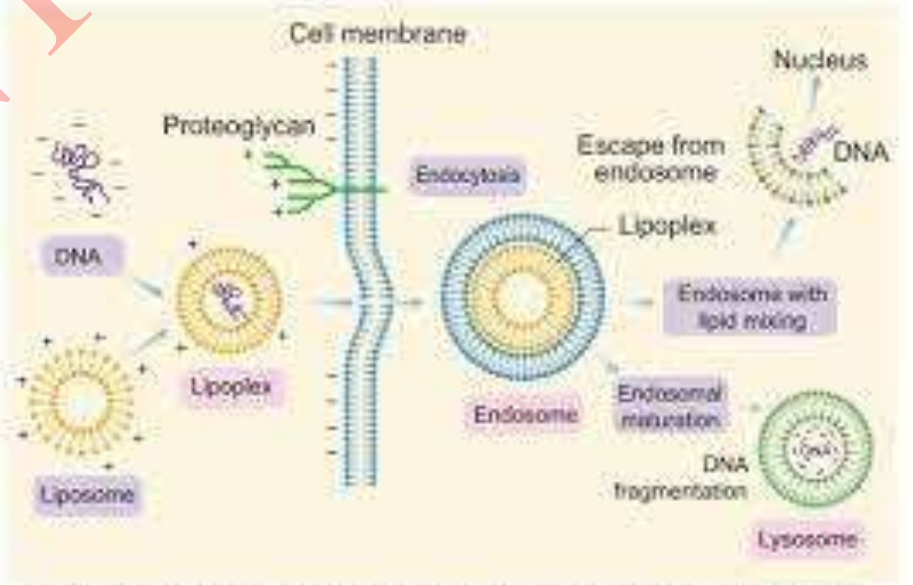
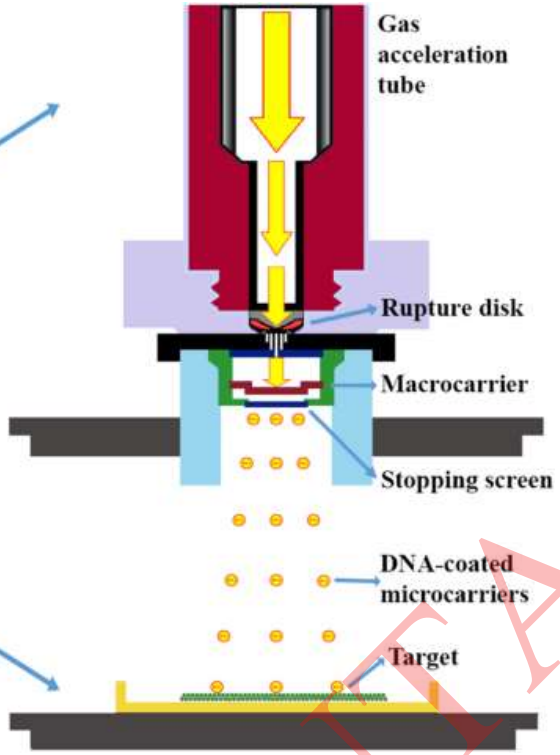
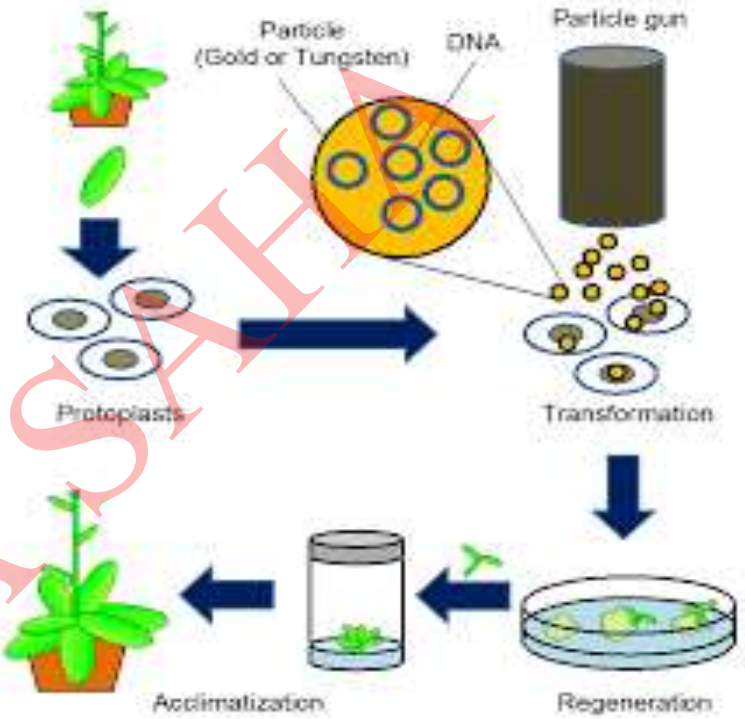
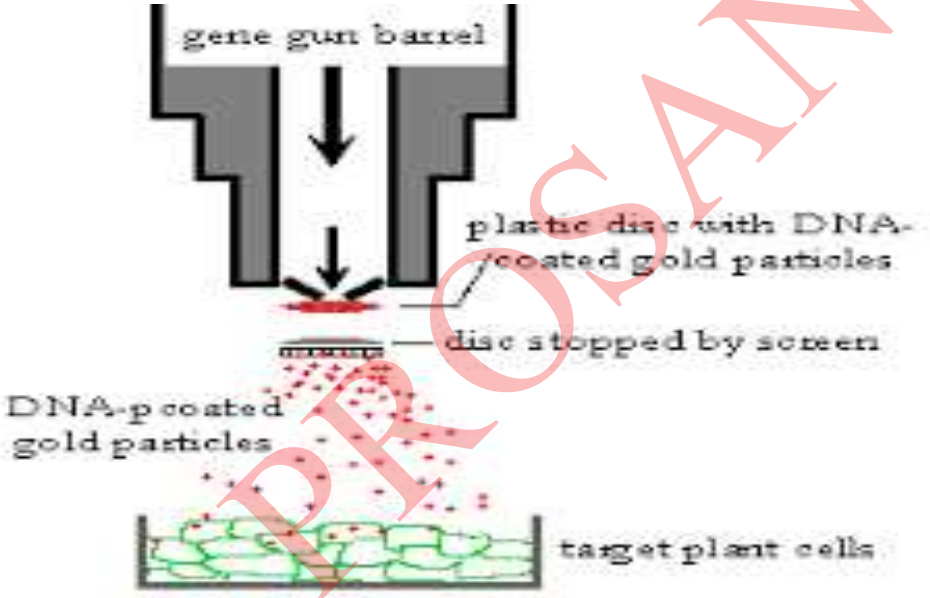
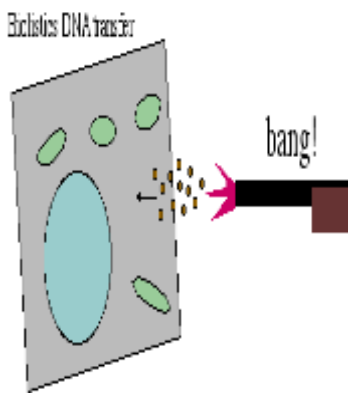


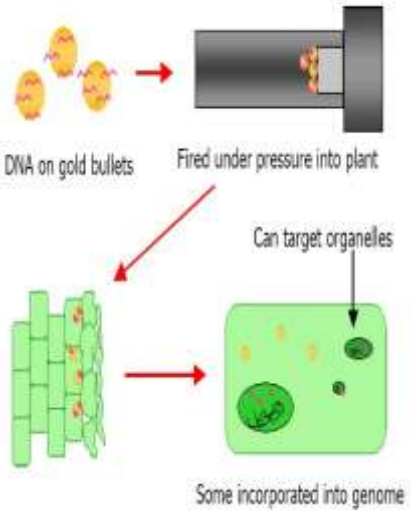
Figure 4.15: Liposome mediated method of Gene Transfer

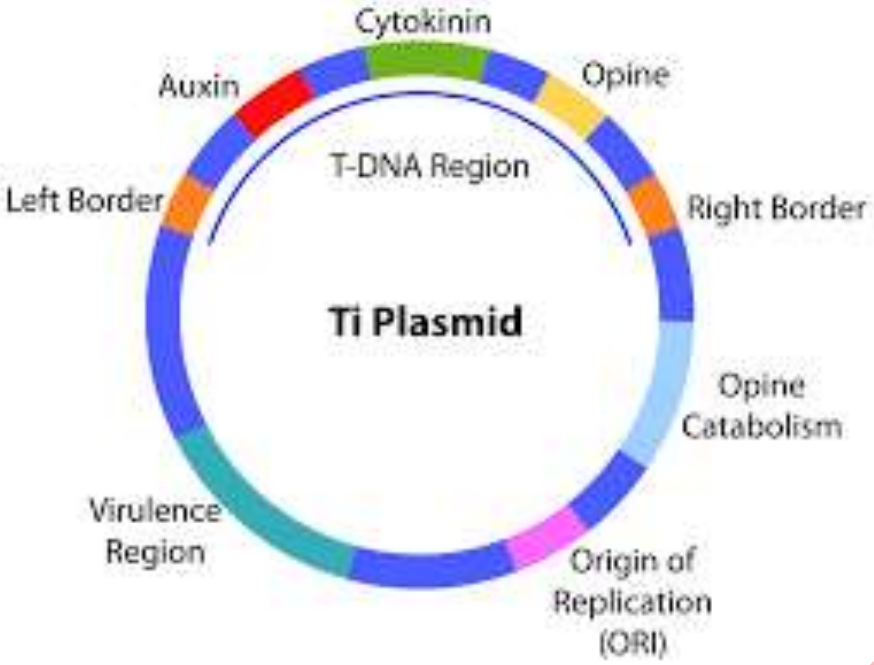


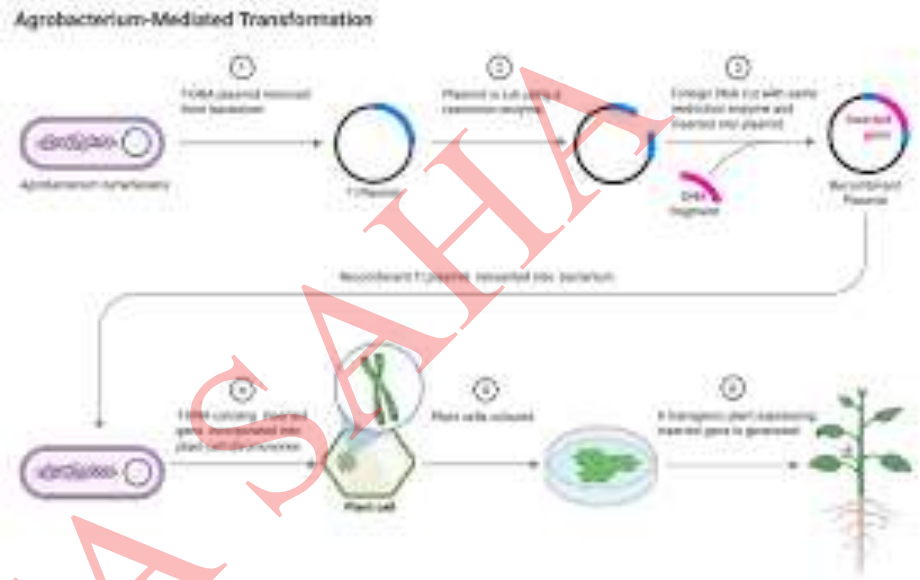


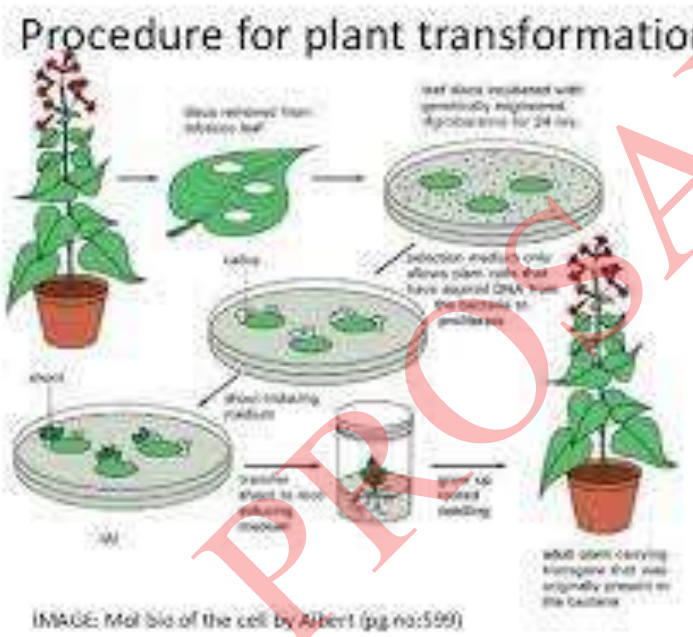


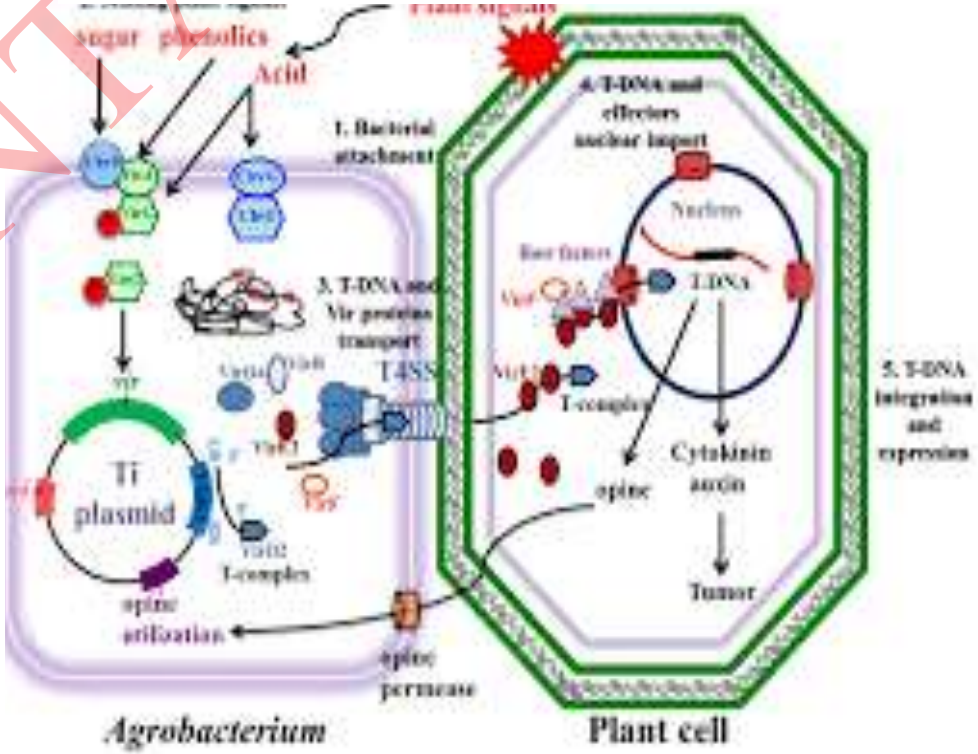




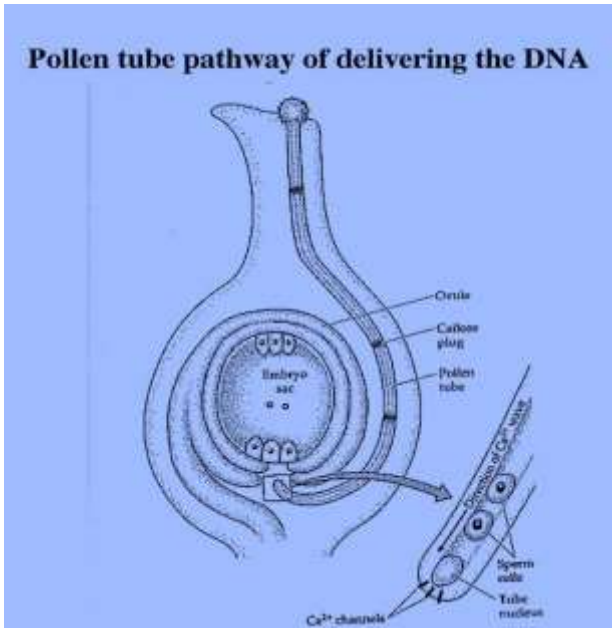








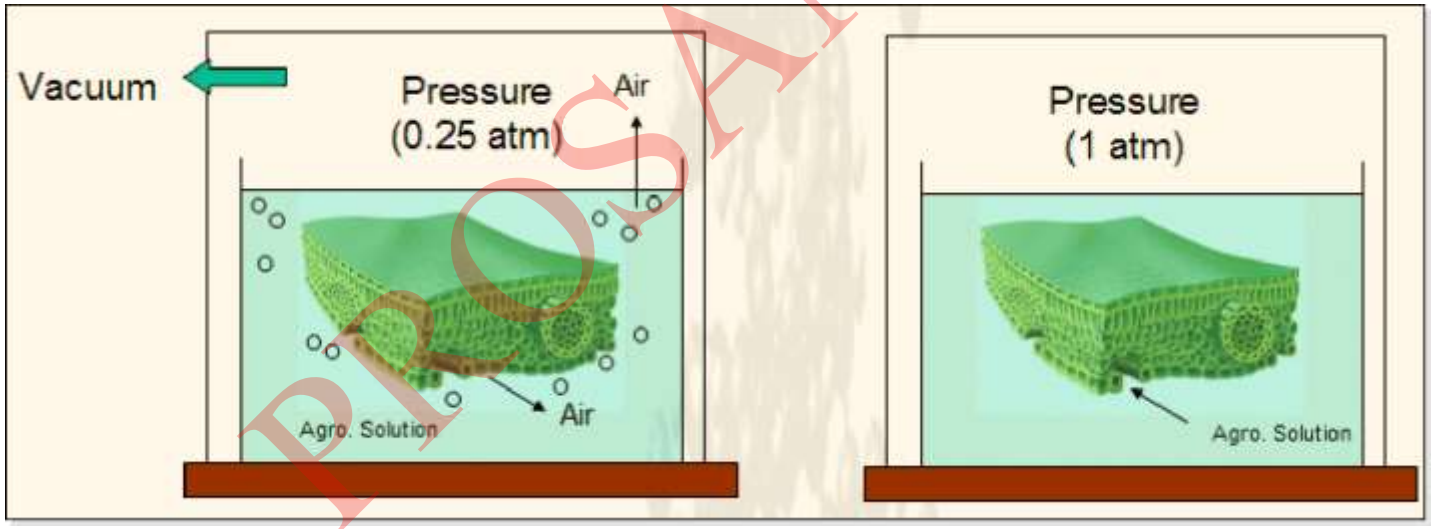
Non-tissue culture based



- Meristem transformation
- Floral dip method
- Pollen transformation

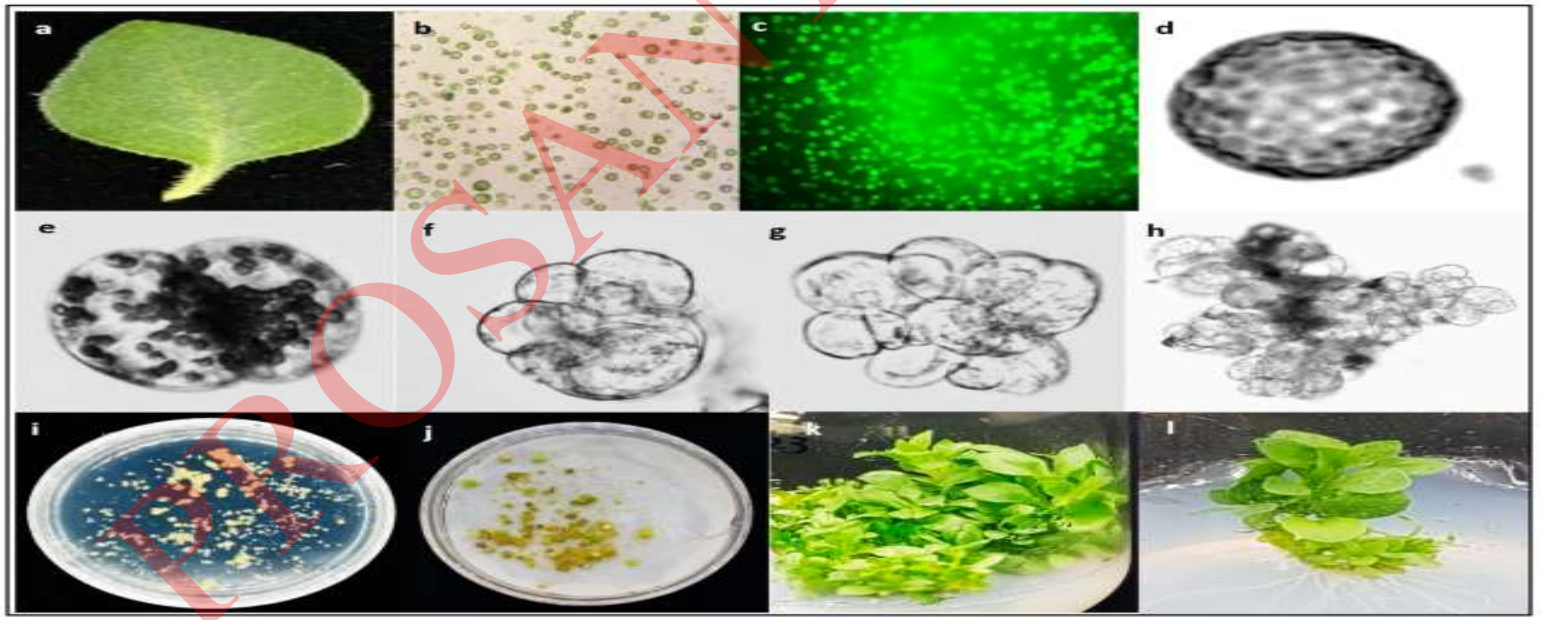
Floral Dip

- Simple submersion of plant into bacterium suspension
- No vacuum is needed
- Conducted with plants grown until just flowering
- Progeny seeds are harvested and germinated using selective antibiotic

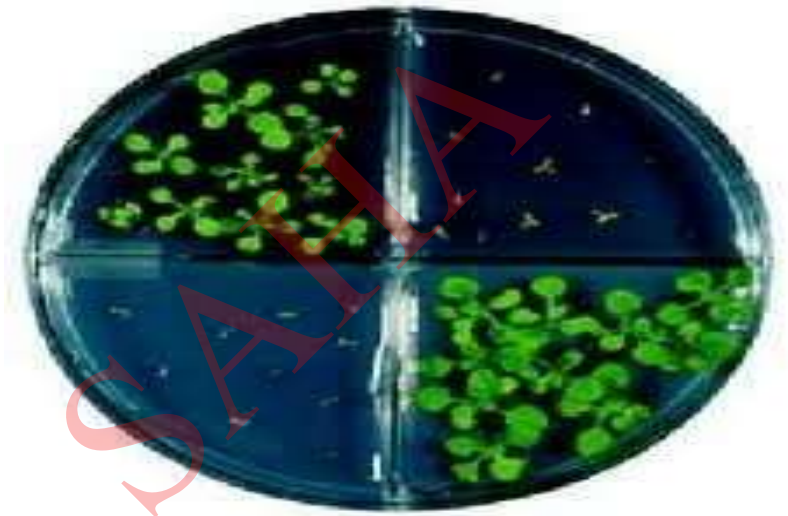


Vacuum Infiltration

Protoplast Fusion

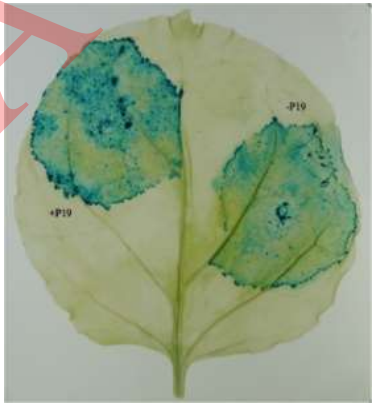


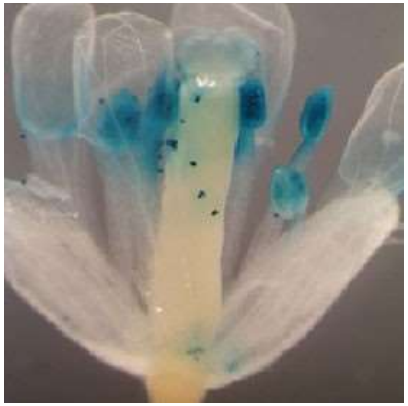
Screening Technique



There are many thousands of cells in a leaf disc or callus clump - only a proportion of these will have taken up the DNA

therefore can get hundreds of plants back - maybe only 1% will be transformed

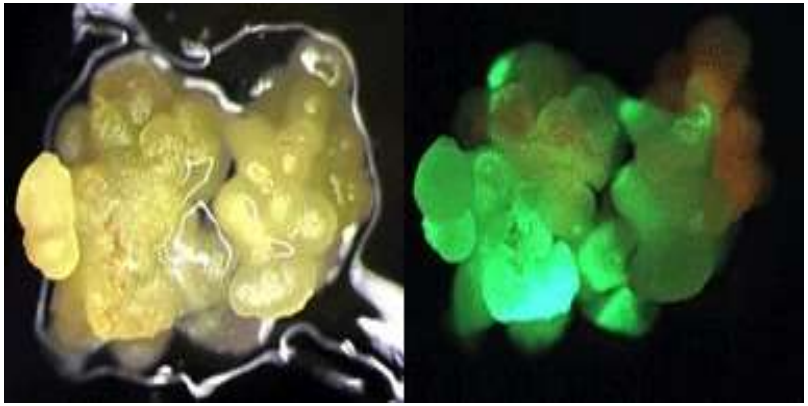




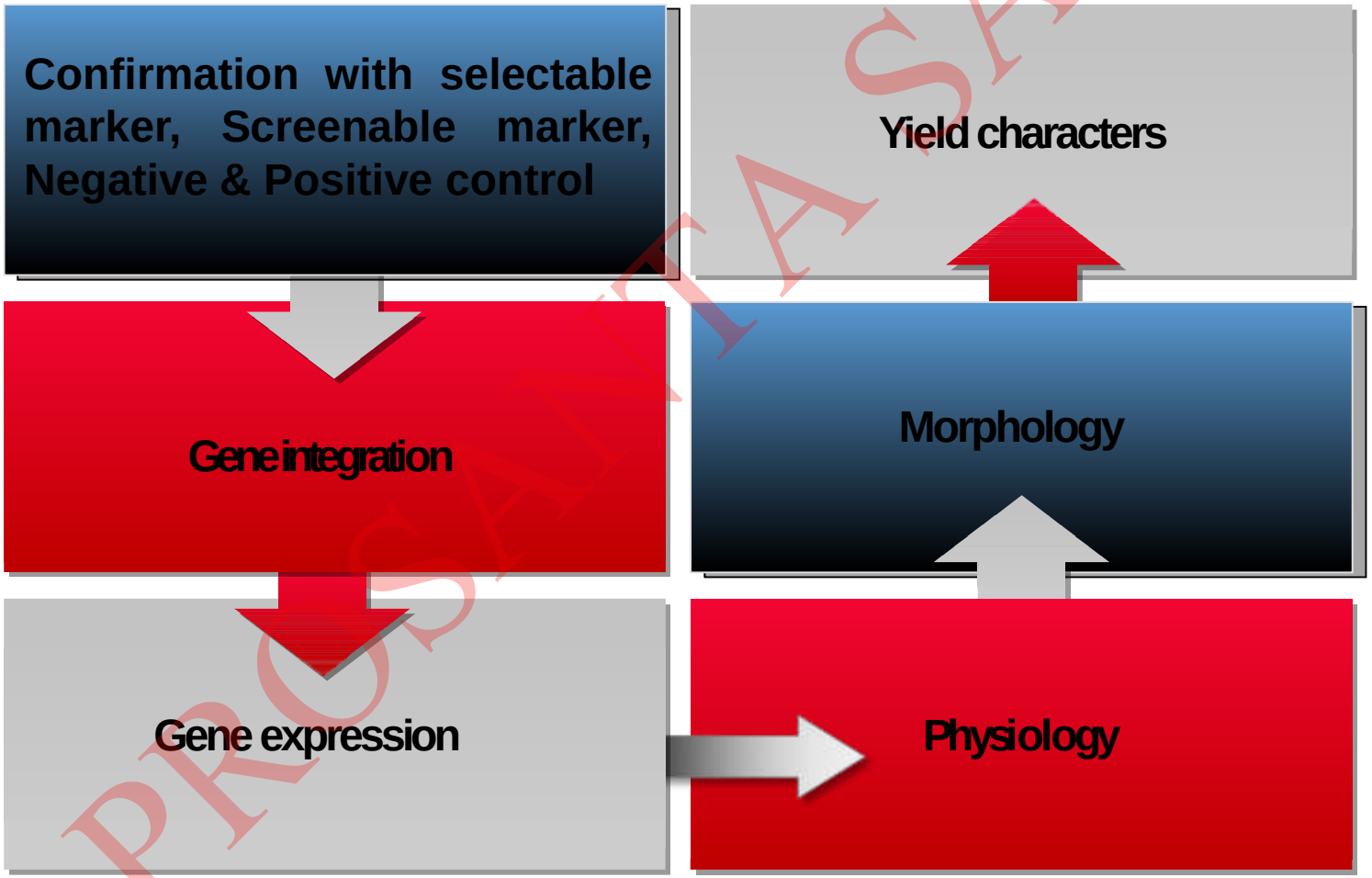
How do we know which plants have taken up the DNA?

Could test each plant - slow, costly

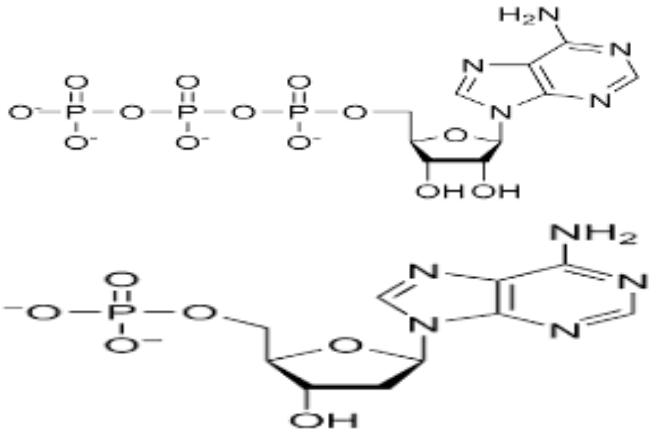
Or use **reporter** genes & **selectable marker** genes

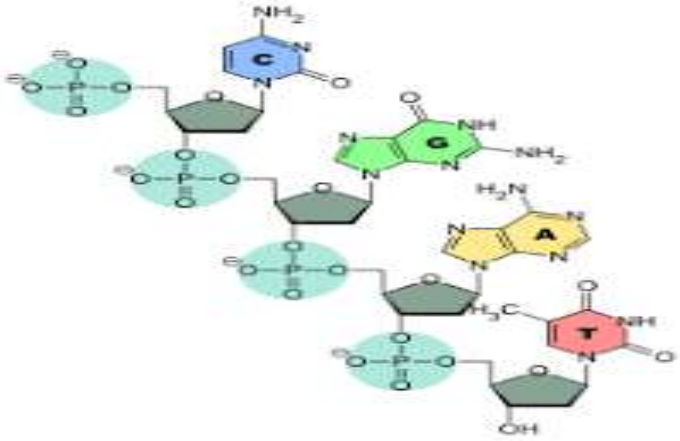


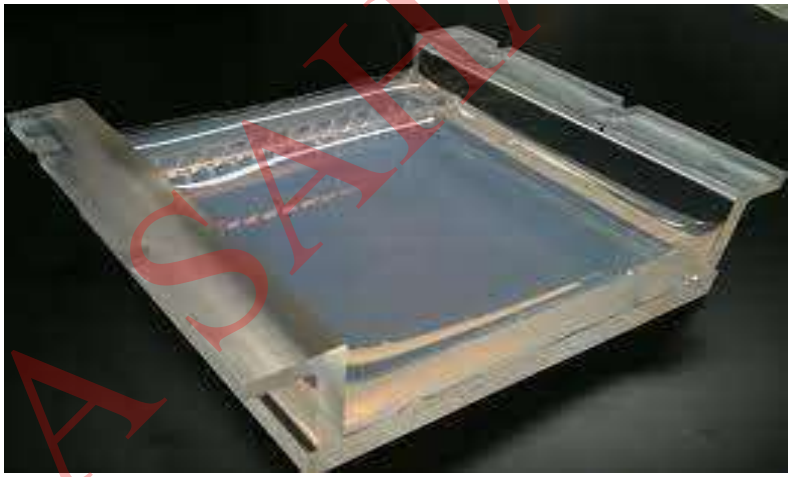
Analysis of T_0 plants

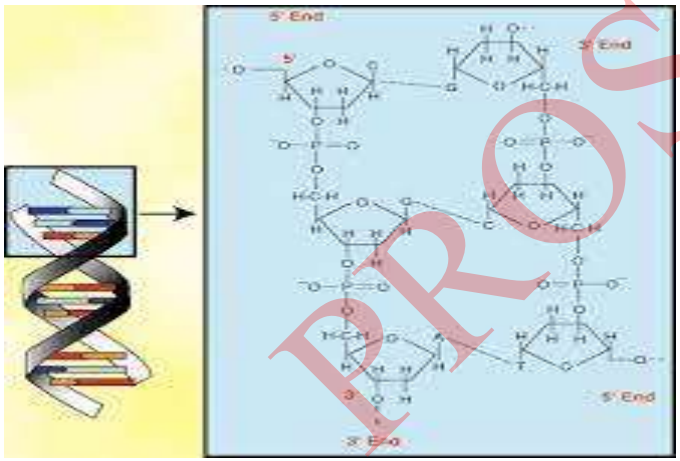


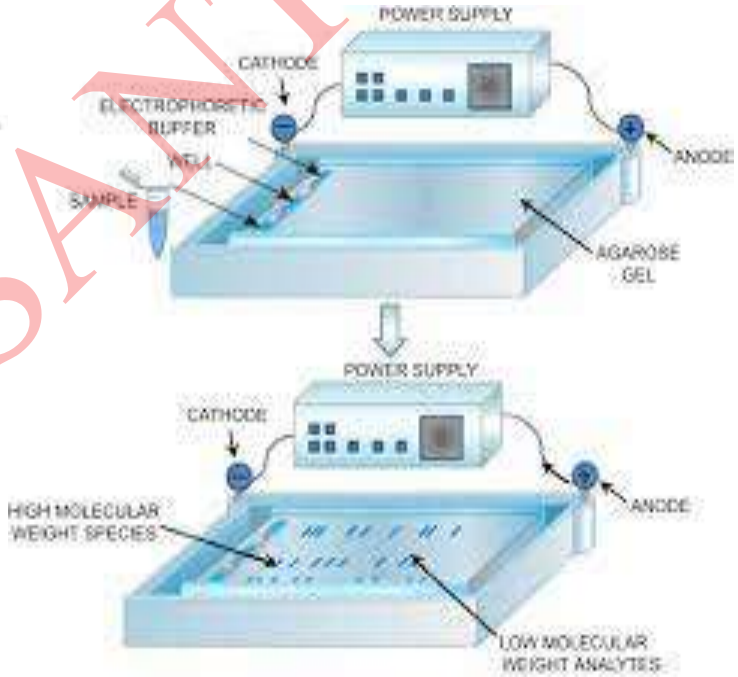
Agarose Gel Electrophoresis)

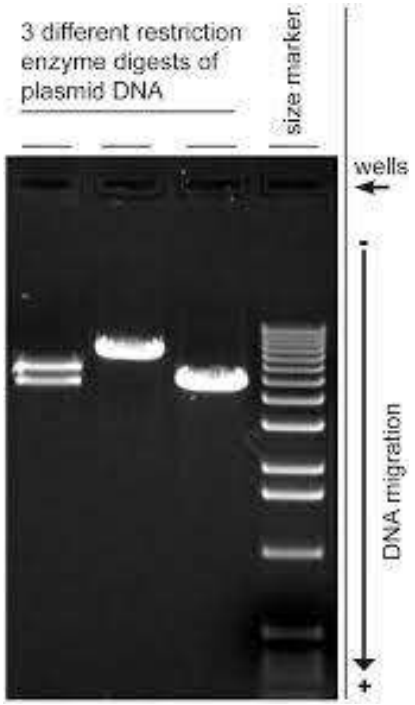


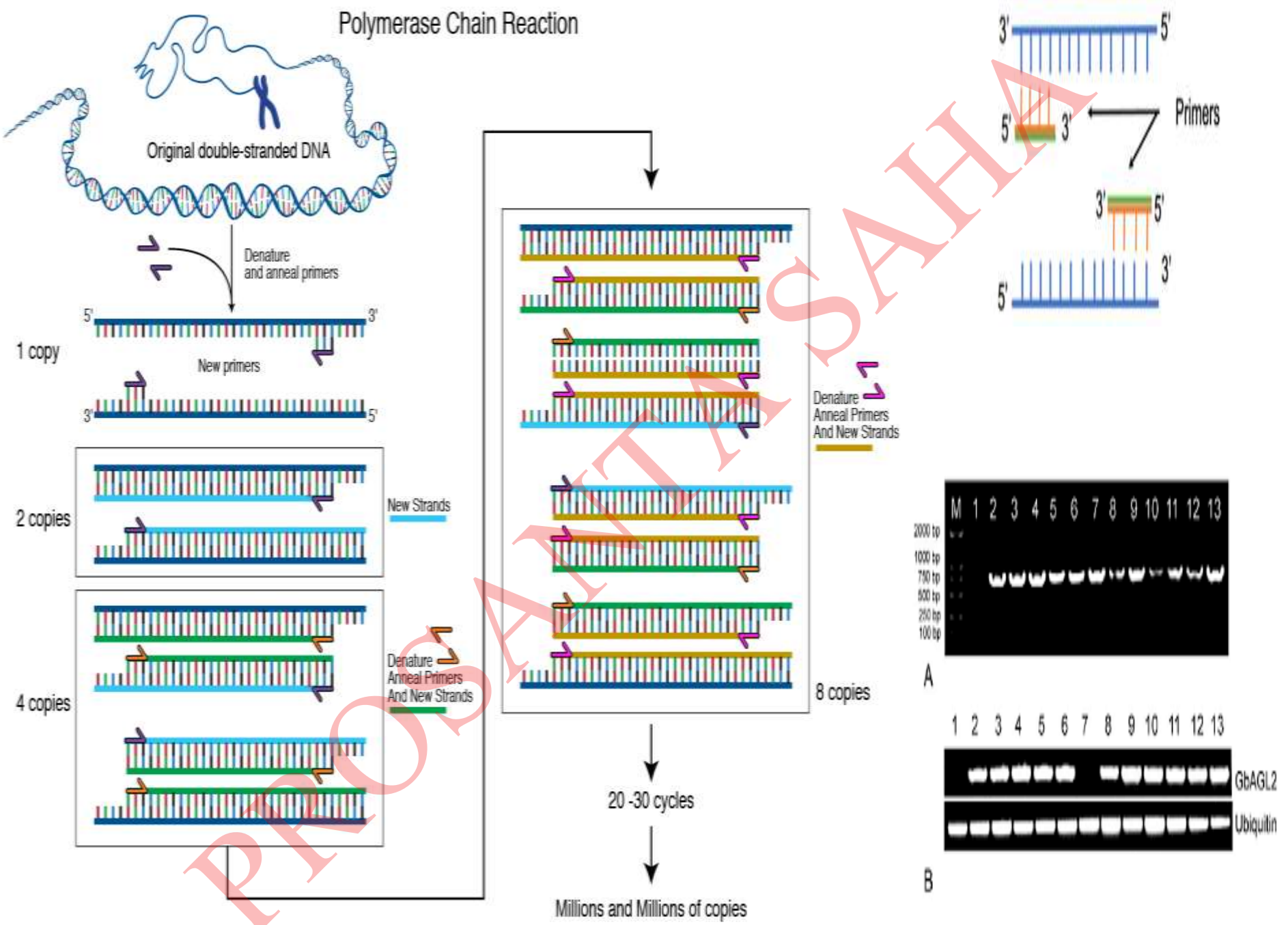


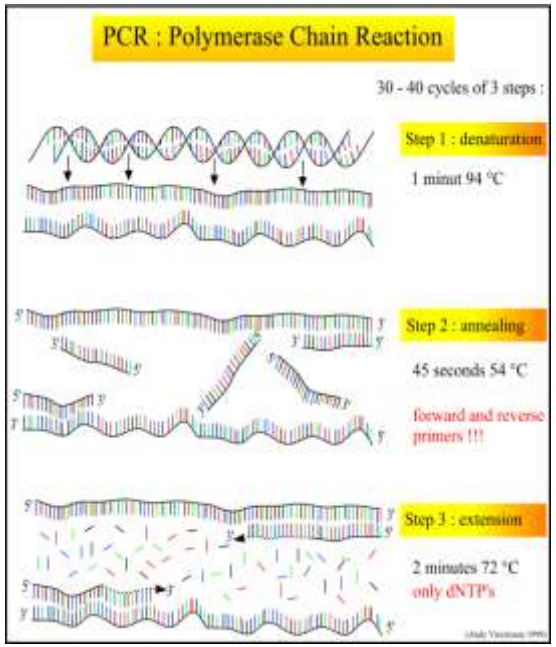


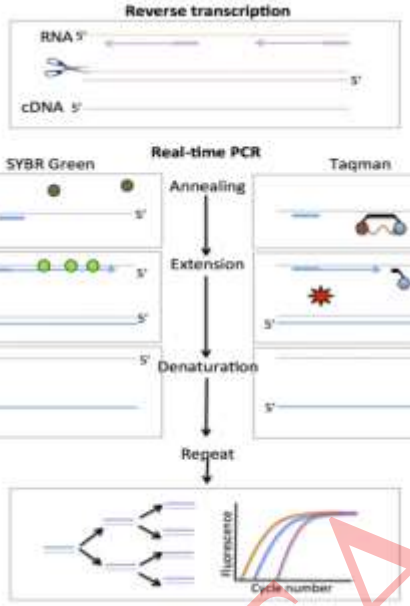


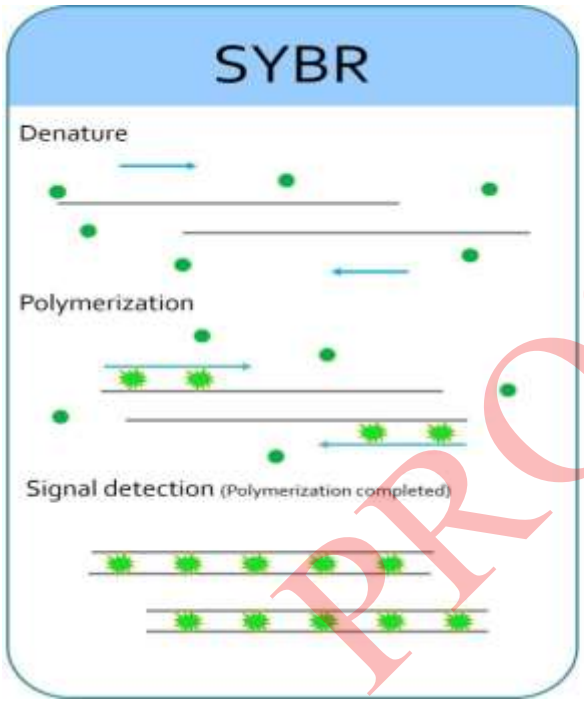


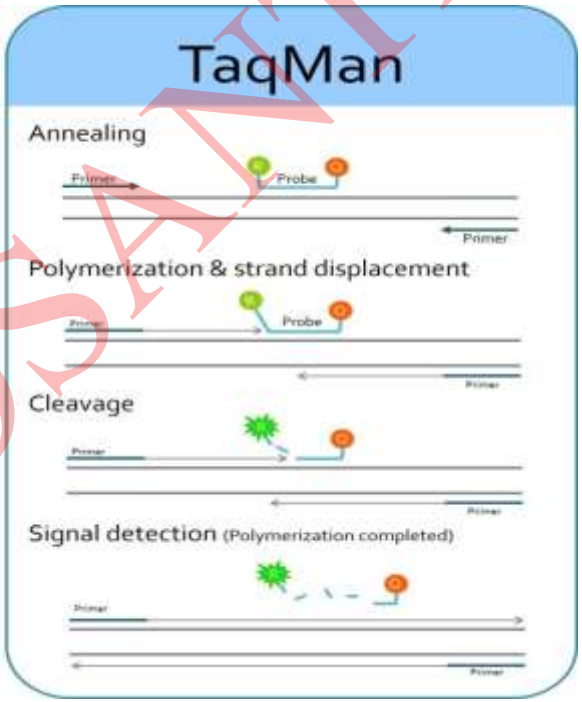


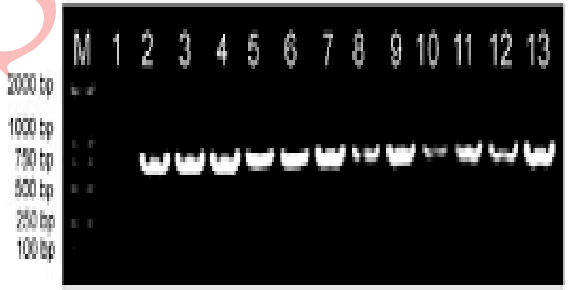












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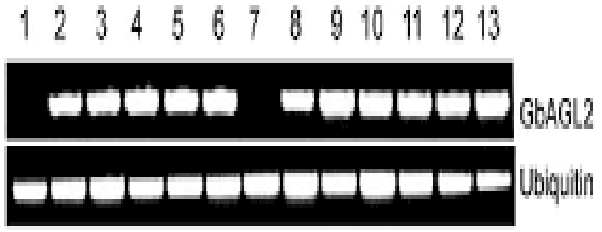
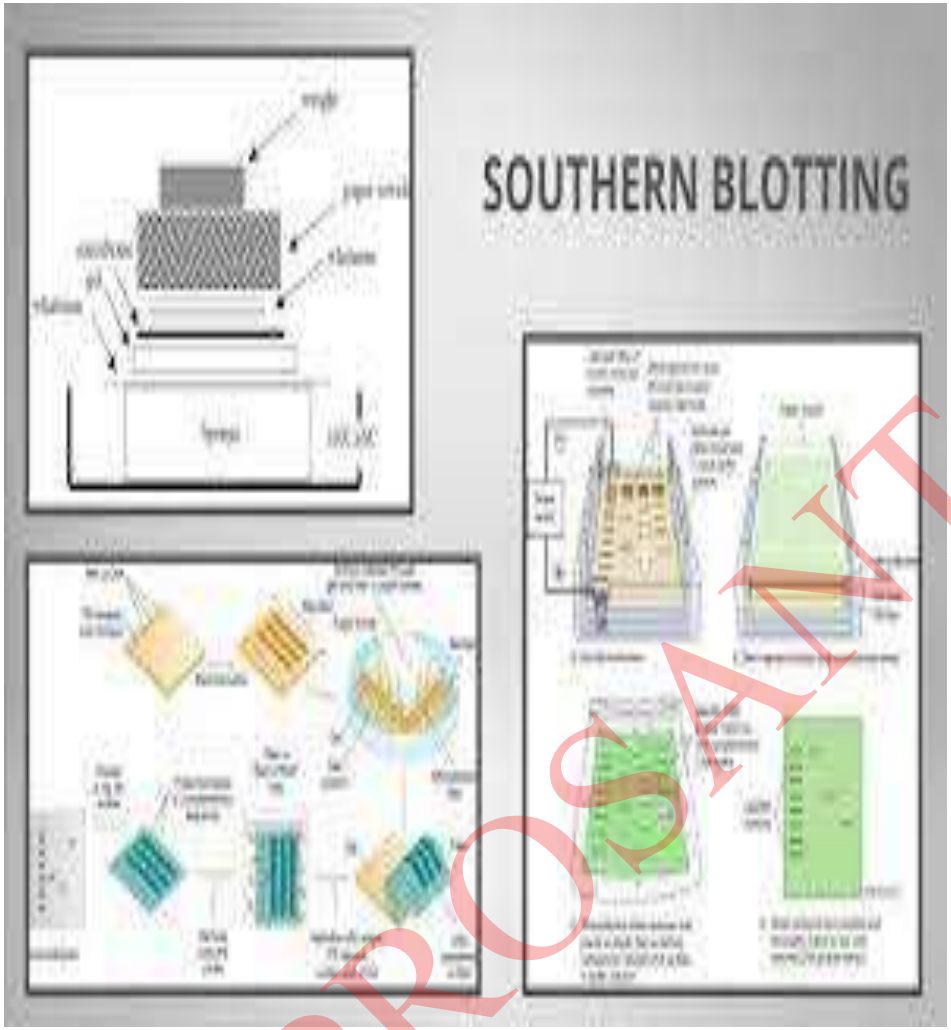
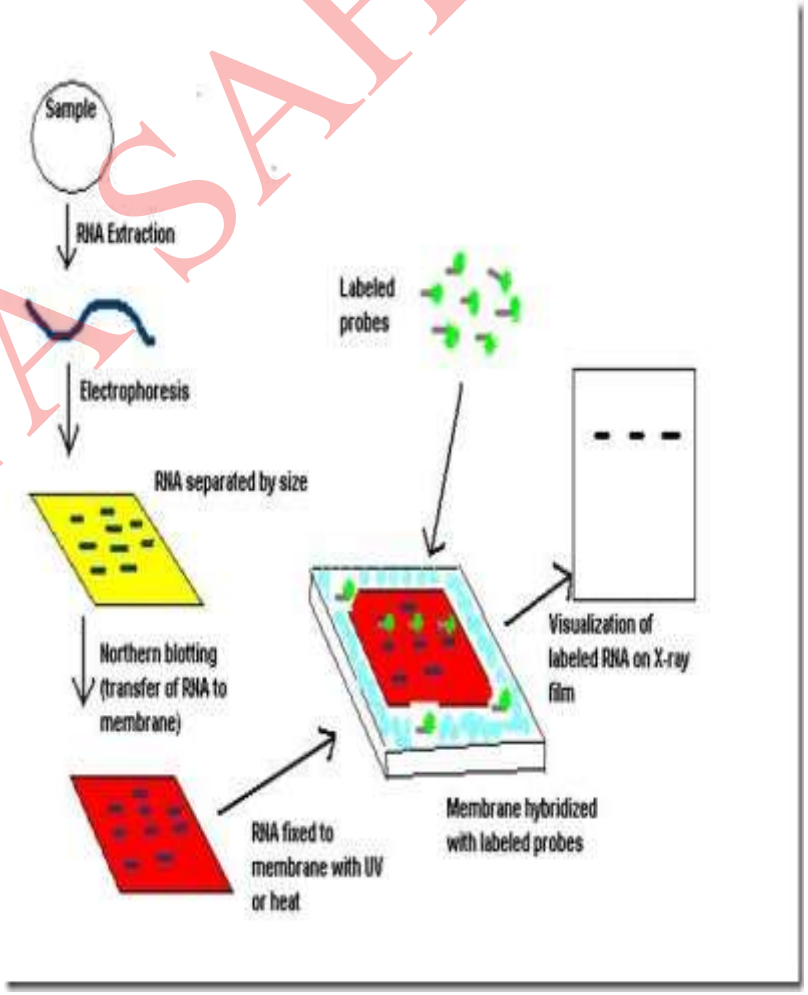
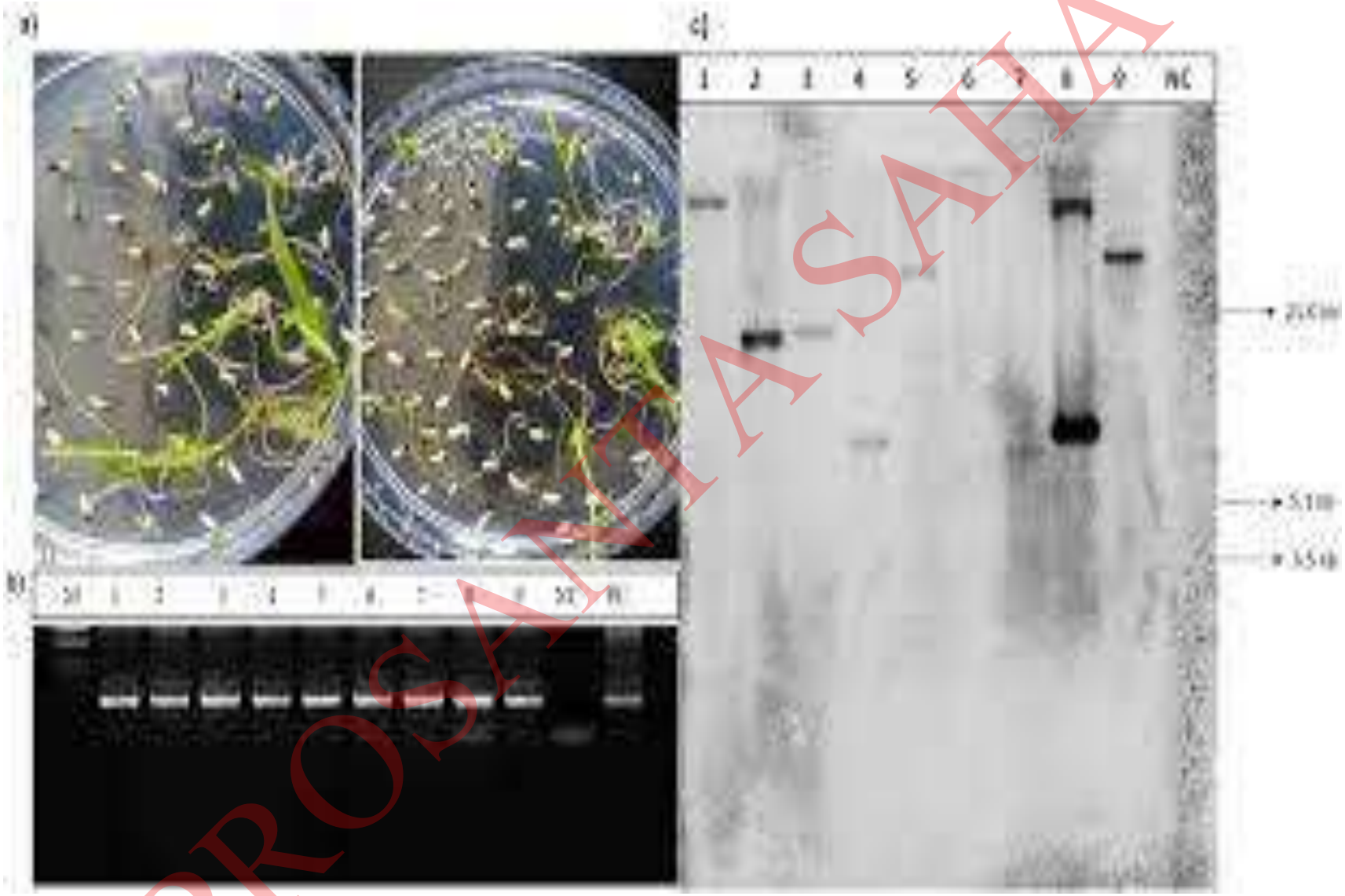


B

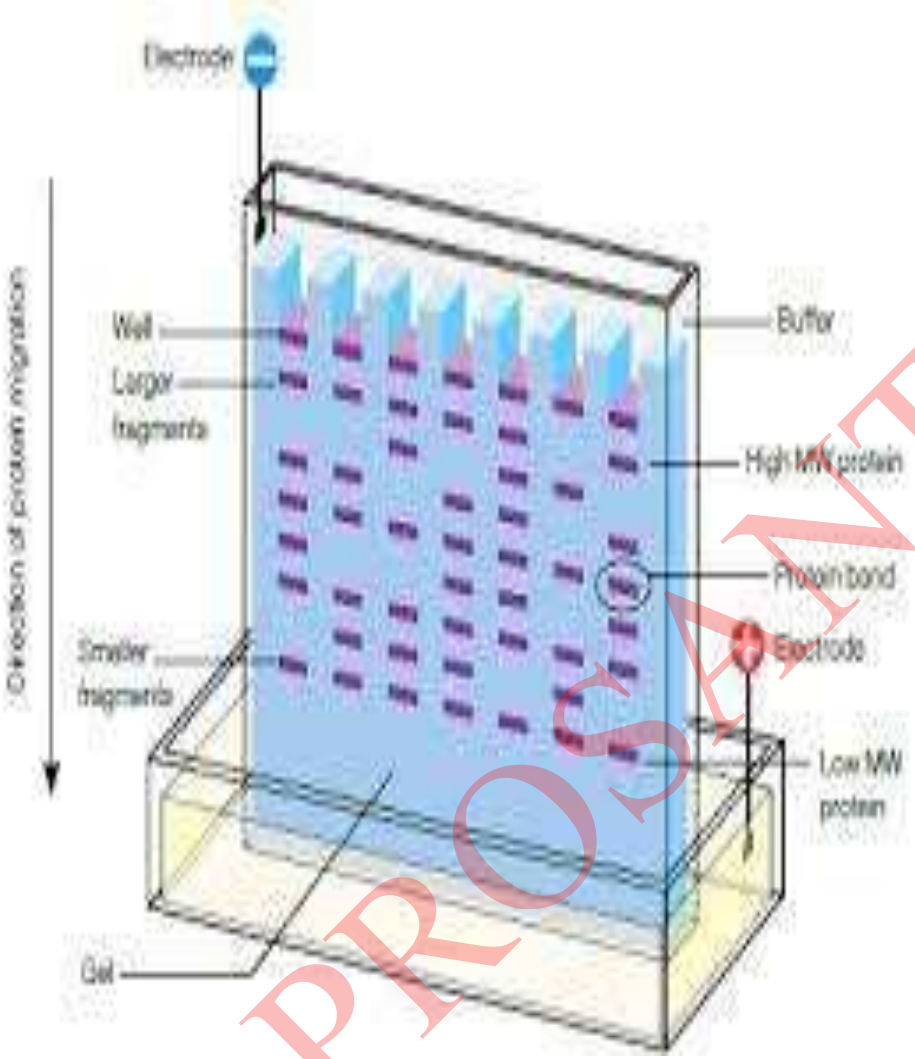
Southern and Northern Blotting techniques

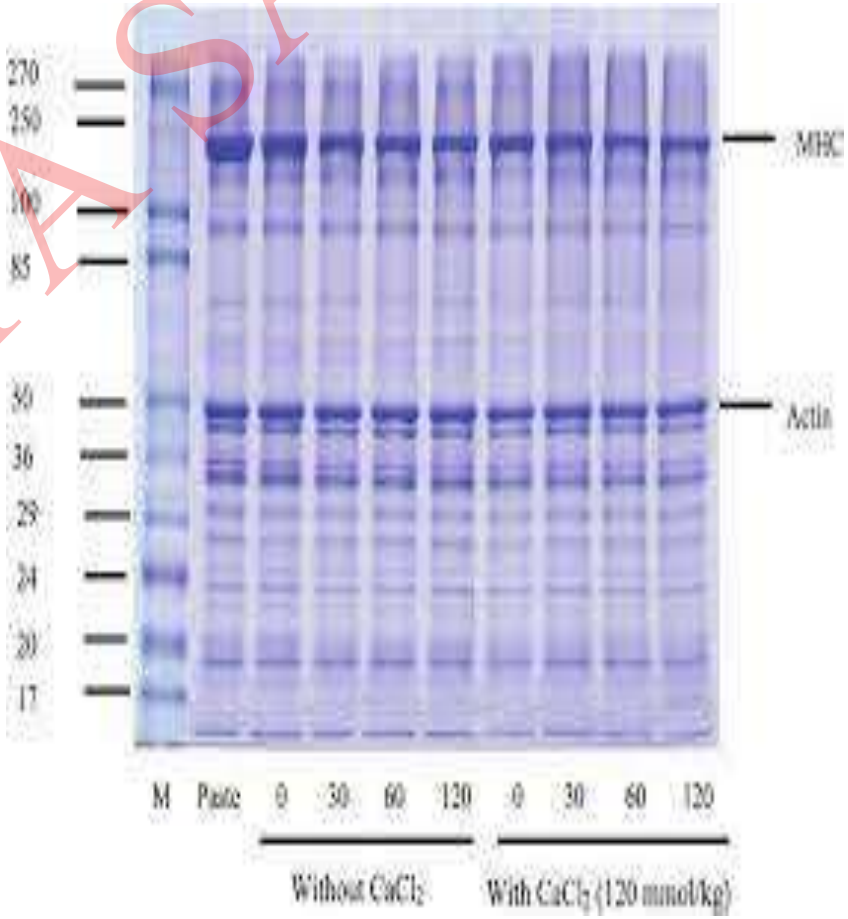




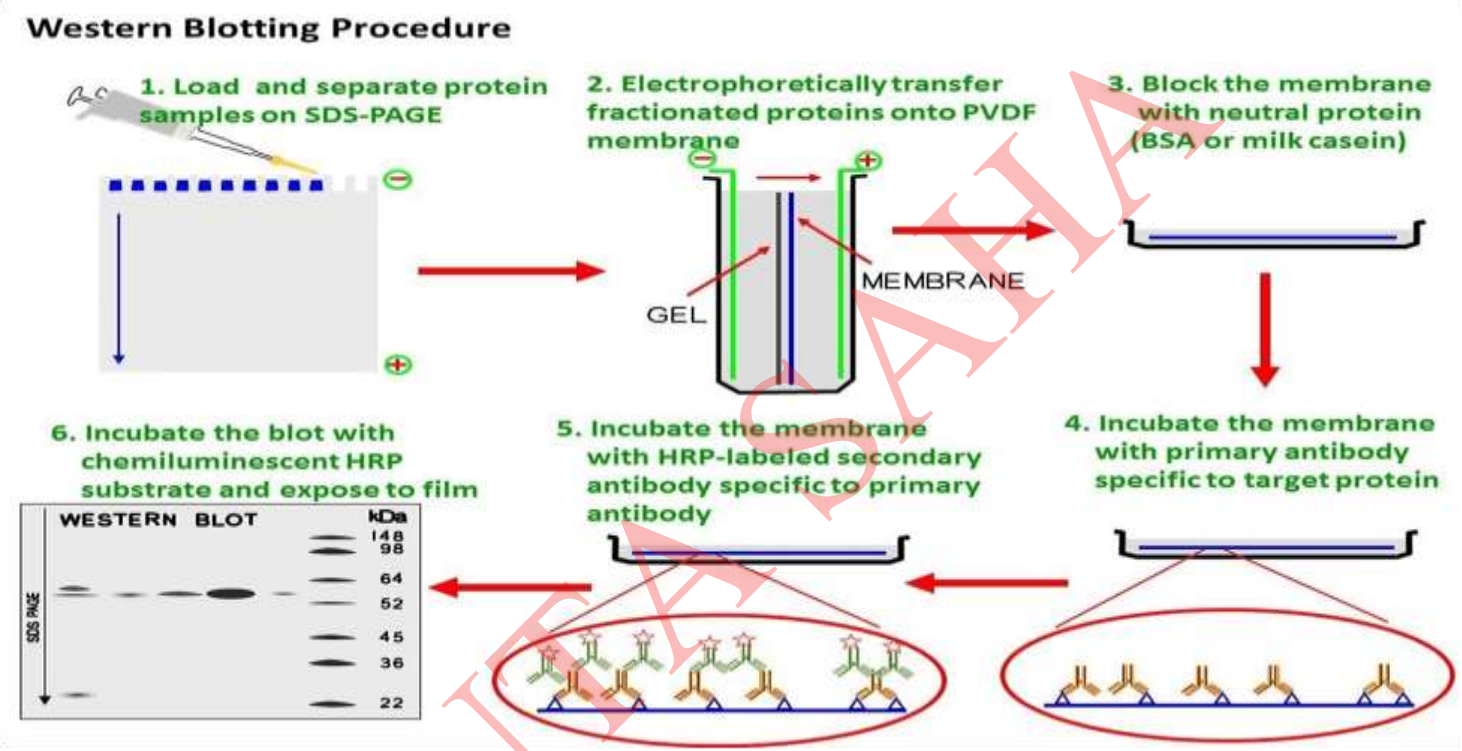


PAGE (Polyacrylamide Gel electrophoresis)





Western Blotting



	Southern Blot	Northern Blot	Western Blot
Target molecule	DNA	RNA	Protein
Sample preparation	DNA extraction enzymatic digestion	RNA isolation	Protein extraction
Separation	Electrophoresis	Electrophoresis	Electrophoresis
Membrane material	Nylon	Nylon	Nitrocellulose or PVDF
Probe	Nucleic acid probe with sequence homologous to target	RNA, DNA, or oligodeoxynucleotide	Primary antibody
Probe label	Radiolabel, enzyme	Radiolabel, enzyme	Enzyme
Detection methods	X-ray film, chemiluminescence	X-ray film, chemiluminescence	Film, cooled CCD, camera, LED, or infrared imaging system

Table 1: Comparing Southern, Northern, and Western Blots.

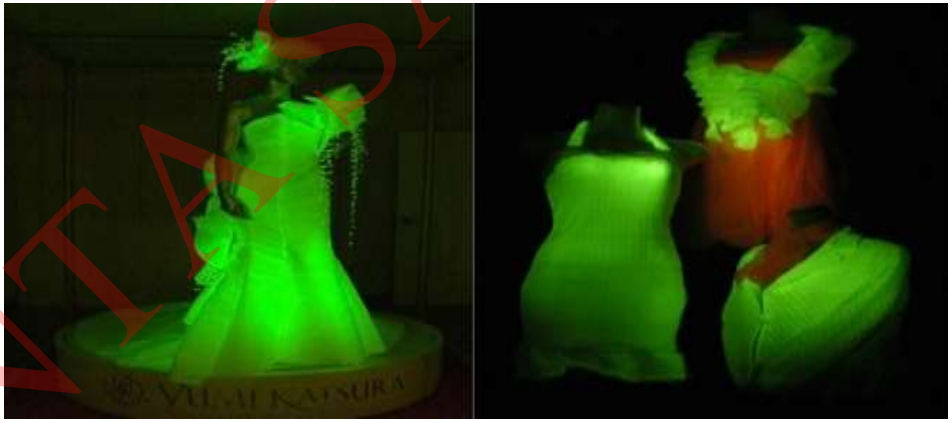
APPLICATIONS

transfer of exogenous genes

- Pathogen resistance
- Herbicide resistance
- Bioreactors/molecular farming
- Delivery systems
- Plant improvement

manipulation of endogenous genes

- Gene silencing/ downregulation



TRENDS in Plant Science

Examples of Genetically modified plants

Table 17.2 Plants that have been genetically transformed

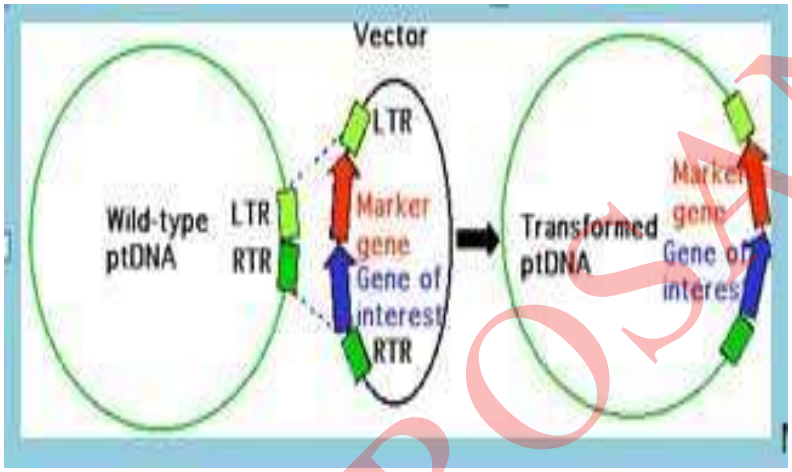
Alfalfa	Carnation	Kiwi fruit	Papaya	Potato	Sunflower
Apple	Carrot	Lettuce	Pea	Red fescue	Sweet potato
Arabidopsis	Corn	Licorice	Peanut	Rice	Tall fescue
Asparagus	Cotton	Lily	Pear	Rye	Tobacco
Banana	Cranberry	Lotus	Pearl millet	Sorghum	Tomato
Barley	Cucumber	Norway spruce	Peony	Soybean	Wheat
Bean	Eggplant	Oat	Petunia	Strawberry	White spruce
Cabbage	Flax	Orchard grass	Plantain	Sugar beet	
Canola	Grape	Orchid	Poplar	Sugarcane	

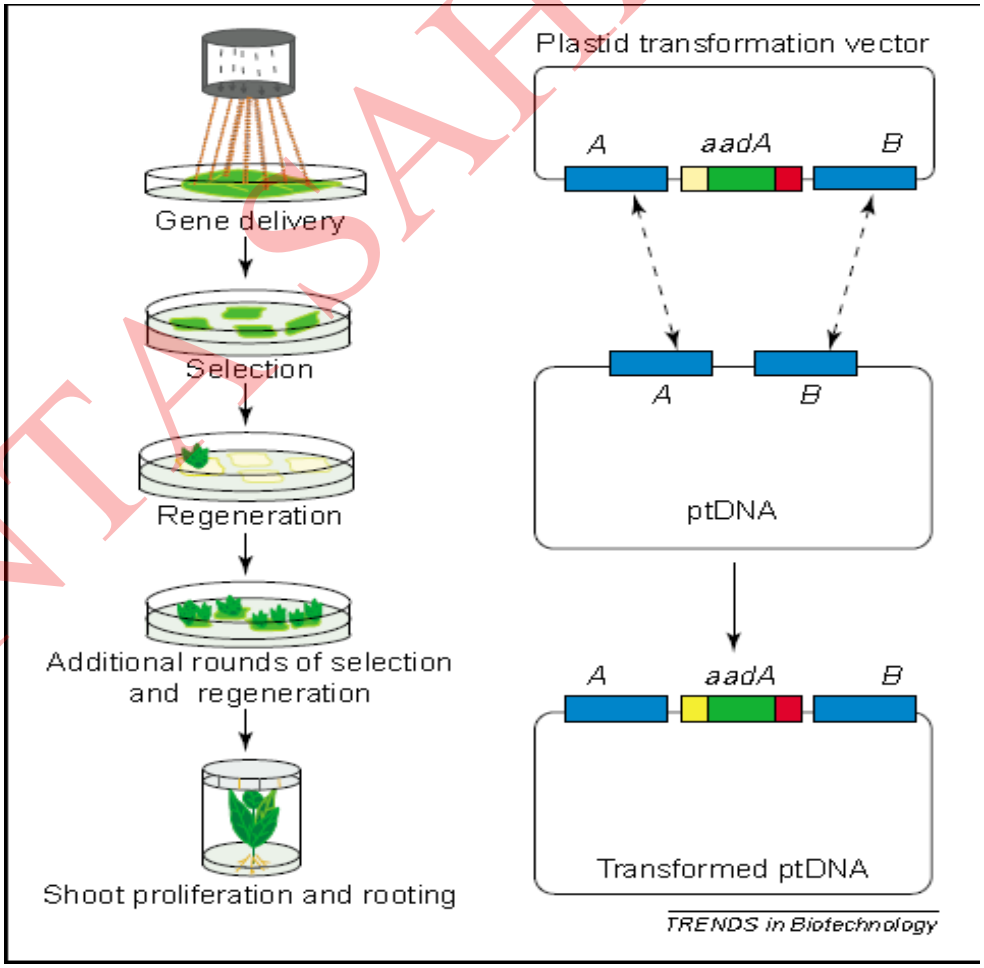
Chloroplast transformation requires:

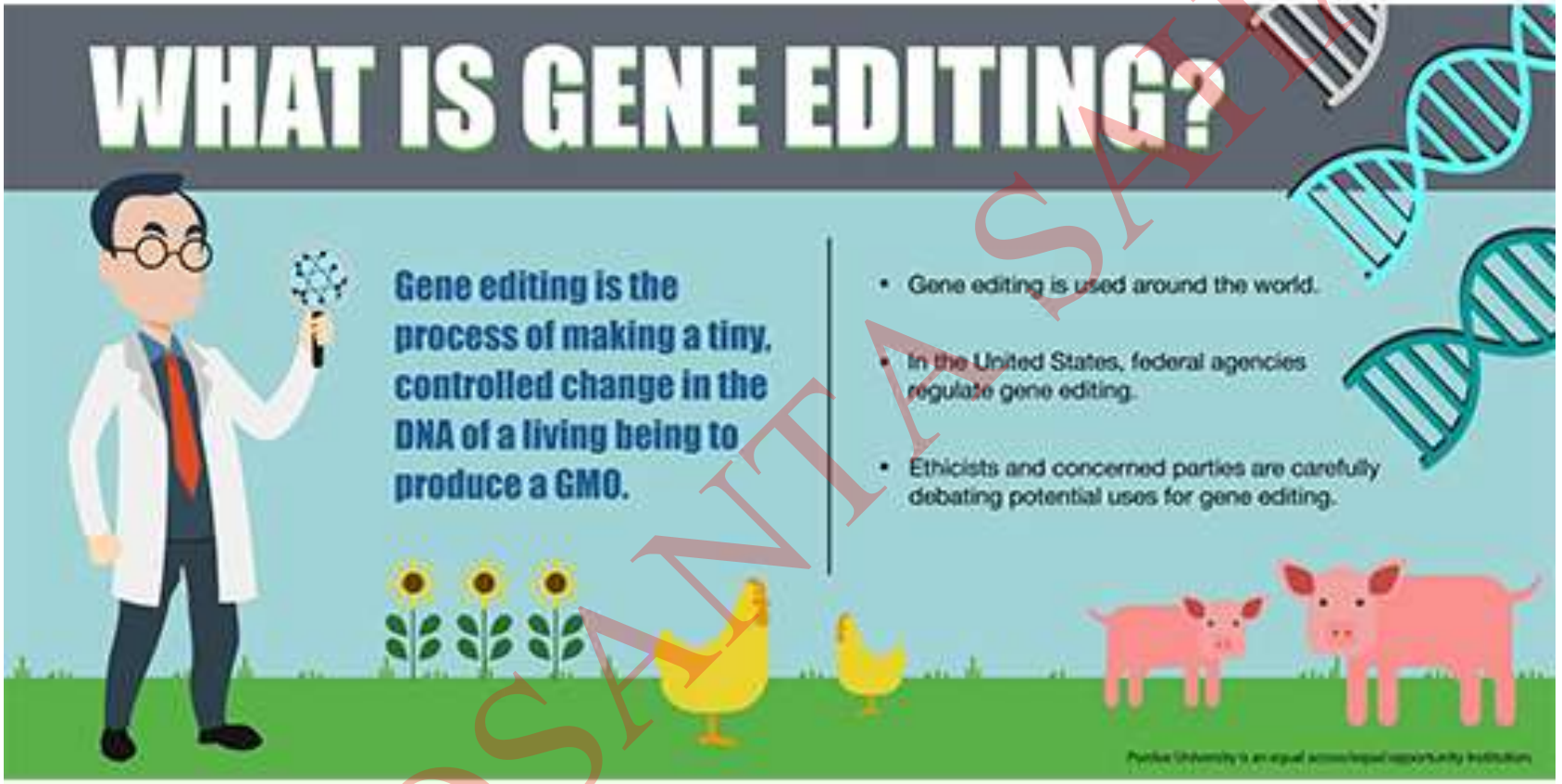
1. **A chloroplast specific expression vector.**

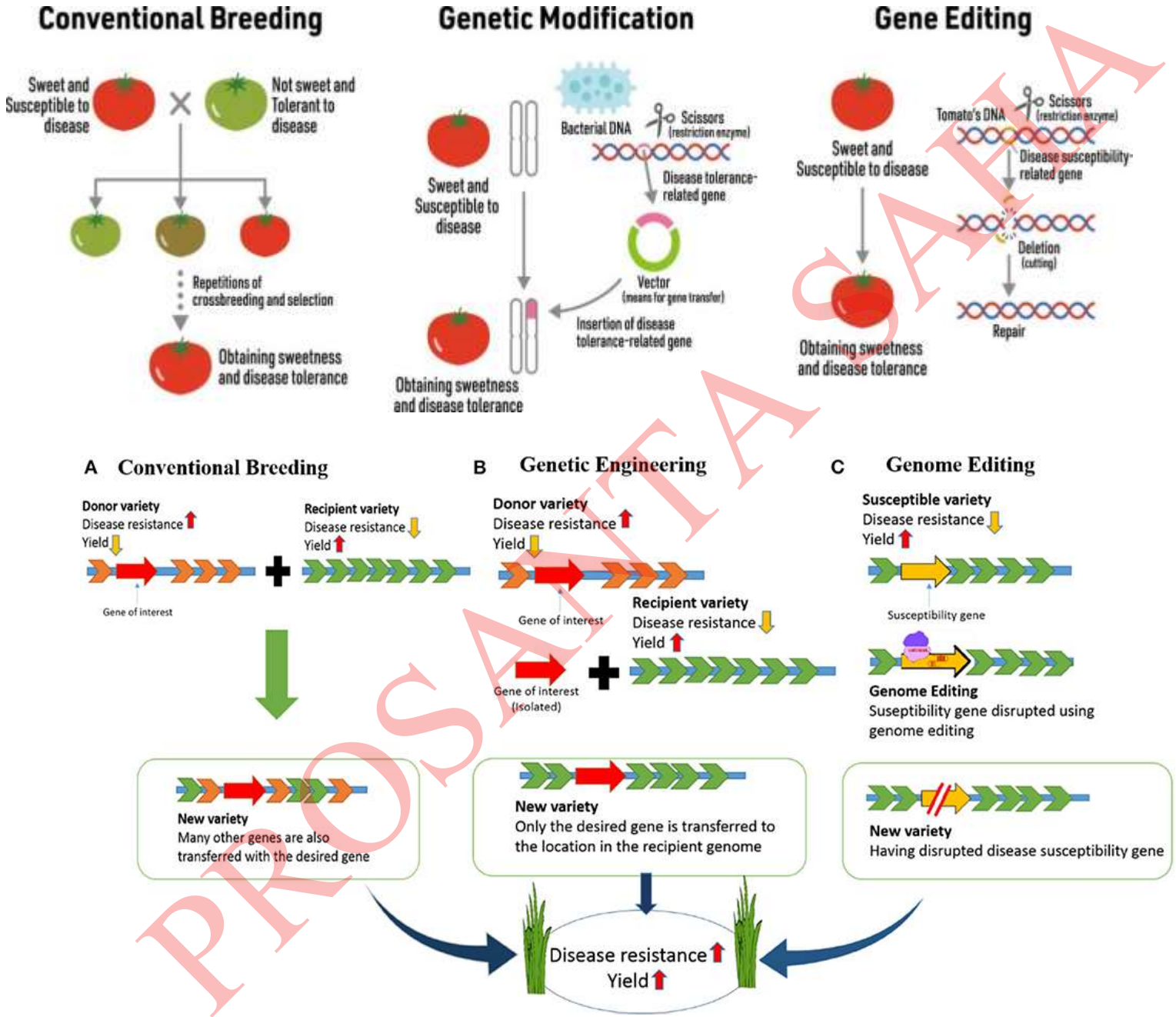
2. **A method for DNA delivery through a double membrane of the chloroplast.**

3. **An efficient selection for the transplastome.**





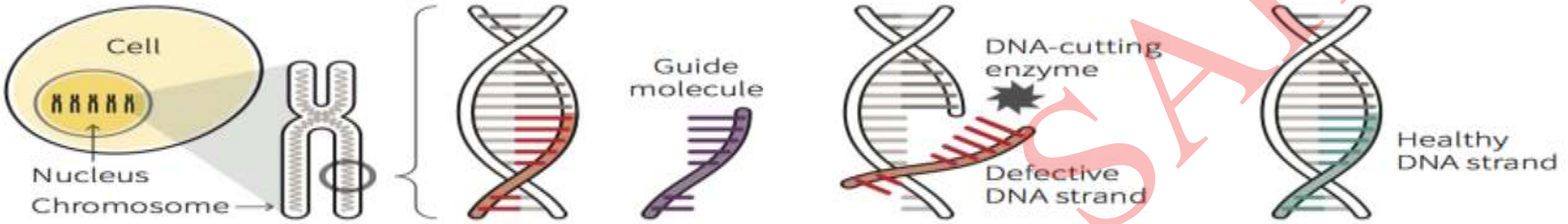




DNA editing

A DNA editing technique, called CRISPR/Cas9, works like a biological version of a word-processing programme's "find and replace" function.

HOW THE TECHNIQUE WORKS



A cell is transfected with an enzyme complex containing:
- Guide molecule
- Healthy DNA copy
- DNA-cutting enzyme

A specially designed synthetic guide molecule finds the target DNA strand.

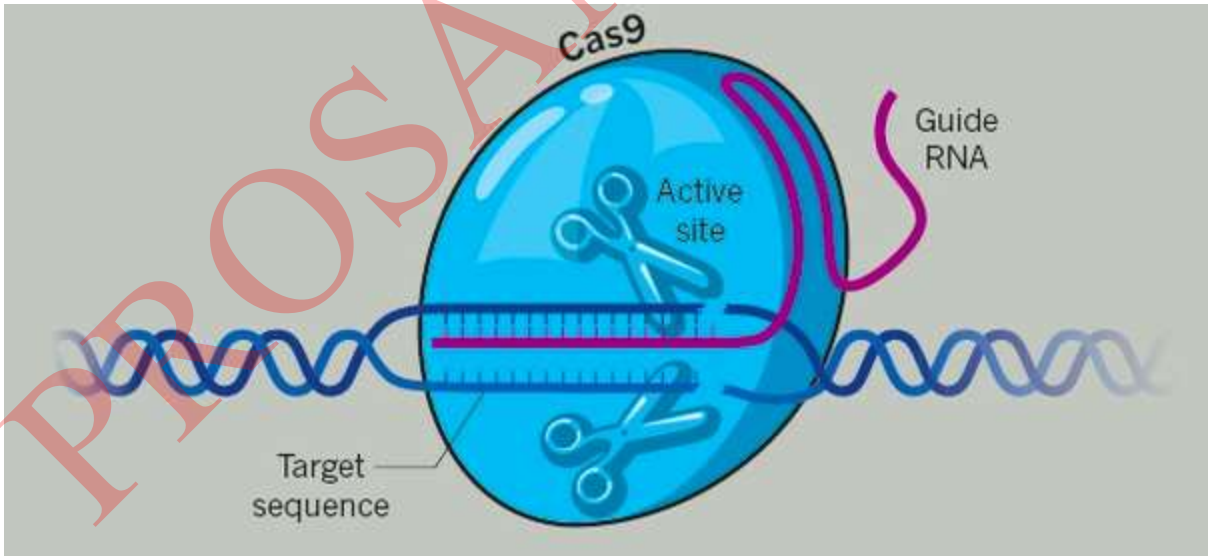
An enzyme cuts off the target DNA strand.

The defective DNA strand is replaced with a healthy copy.

Sources: Reuters; Nature; Massachusetts Institute of Technology

W. Foo, 24/04/2015

REUTERS



CONCLUSION

Plant breeding techniques result in stable introgression of genes into the genome, but many undesired genes also get transferred..

Alternative is application of plant biotechnology for crop improvement...

Recombinant DNA Technology (RDT) to prepare a construct containing gene of interest and marker sequences under suitable promoters....

Plant tissue culture for *in vitro* regeneration is essential for genetic transformation

Genetic transformation followed by selection result in transfer of only desired genes, but integration into geneome is at random location..

Direct method and Vector mediated methods of genetic transformation are operational...

Tissue culture independent methods are also available...

Genome editing techniques modify the genome at only desired locations (Targeted mutation)... And thereby modify the receipient genome to minimum level.....

Thank you!