Applications of recombinant DNA technology in Health, Agriculture,Environment, Industry

PRESENTED TO:

DR.SUMAIRA RASOOL

Introduction:

- Basic principle.
- Highly specific genetic manipulation of genetic material.
- Gene transfer between unrelated species.
- Applications in health, agriculture, environment, industry.

Application of rDNA technology in health:

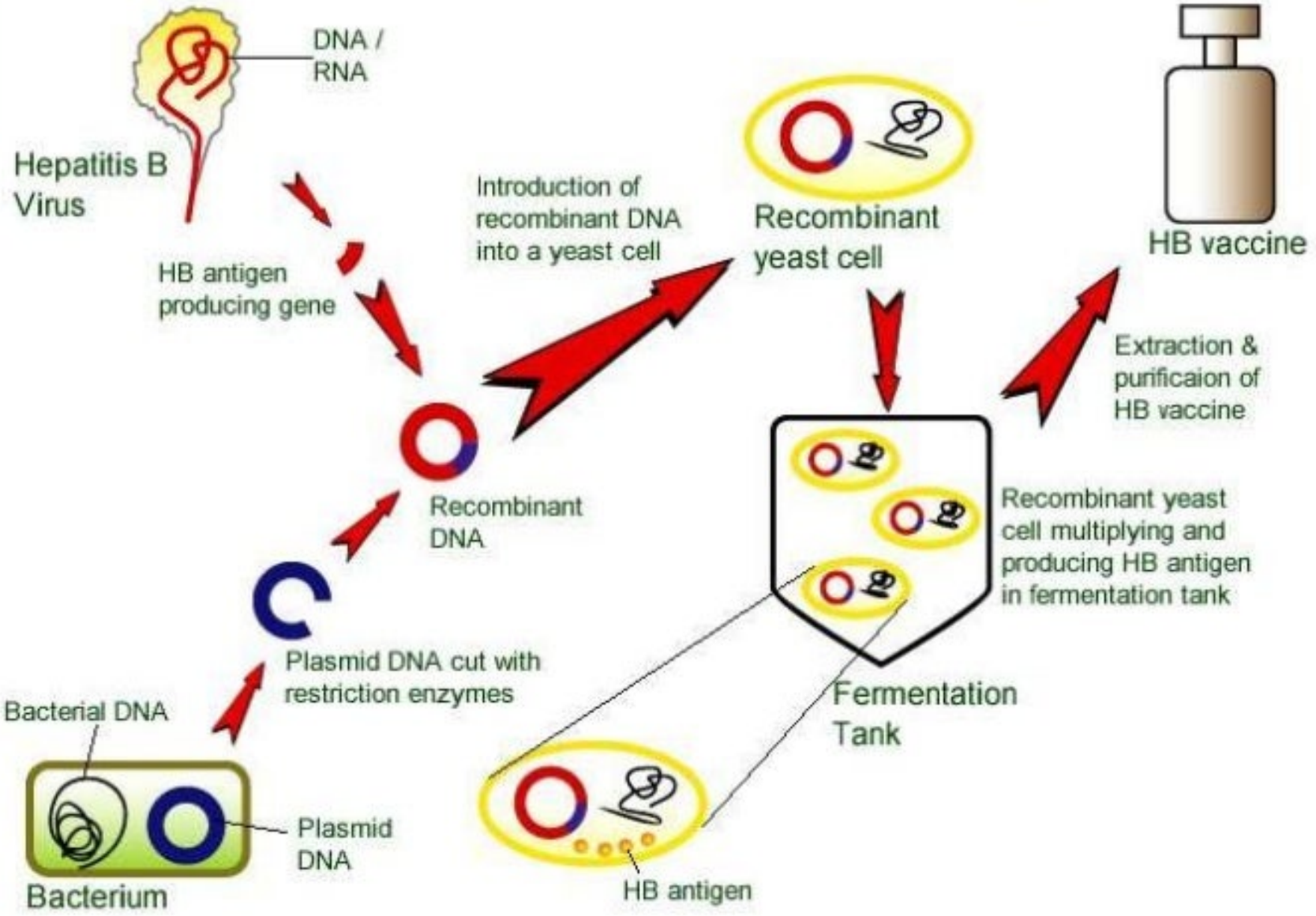
Applications in health:

- rDna technology Wide spectrum in improving health.
- Treat defected gene or introduce new one.
- Applications,laboratory test and parental diagnosis of genetic disease

Production of vaccine:

- Introduce live attenuated .
- Acquired immunity.
- Rdna technology can be use to clone gene for protective antigen protein.
- Hepatitis B vaccine (rDNA) , influenza ,HIV and mouth and foot disease.

Production of Recombinant HB Vaccine

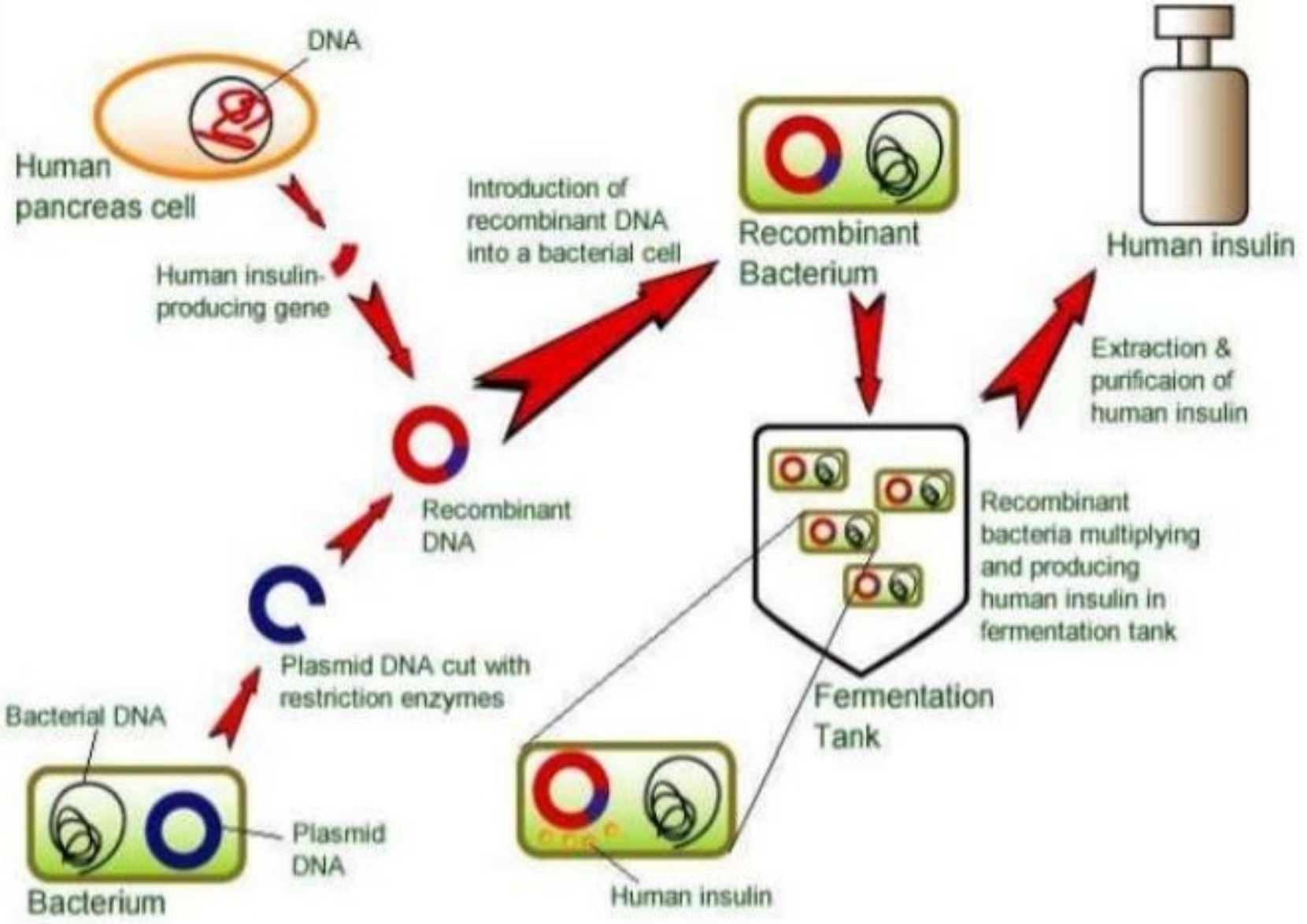


Commercial and pharmaceutical products:

Insulin:

- Insulin(hormone) controls glucose level in humans.
- By rDna , done cloning of human insulin gene and put in E.coli.
- availability of insulin.
- devoid of getting by-products by animal slaughtering.

Human Insulin Production



Human growth hormone:

HGH is homing polypeptide .

- 121 amino acids, 2 to 115 Dalton molecular weight.
- role in growth , regeneration or differentiation.
- E.g

dwarfism treating by injecting these.

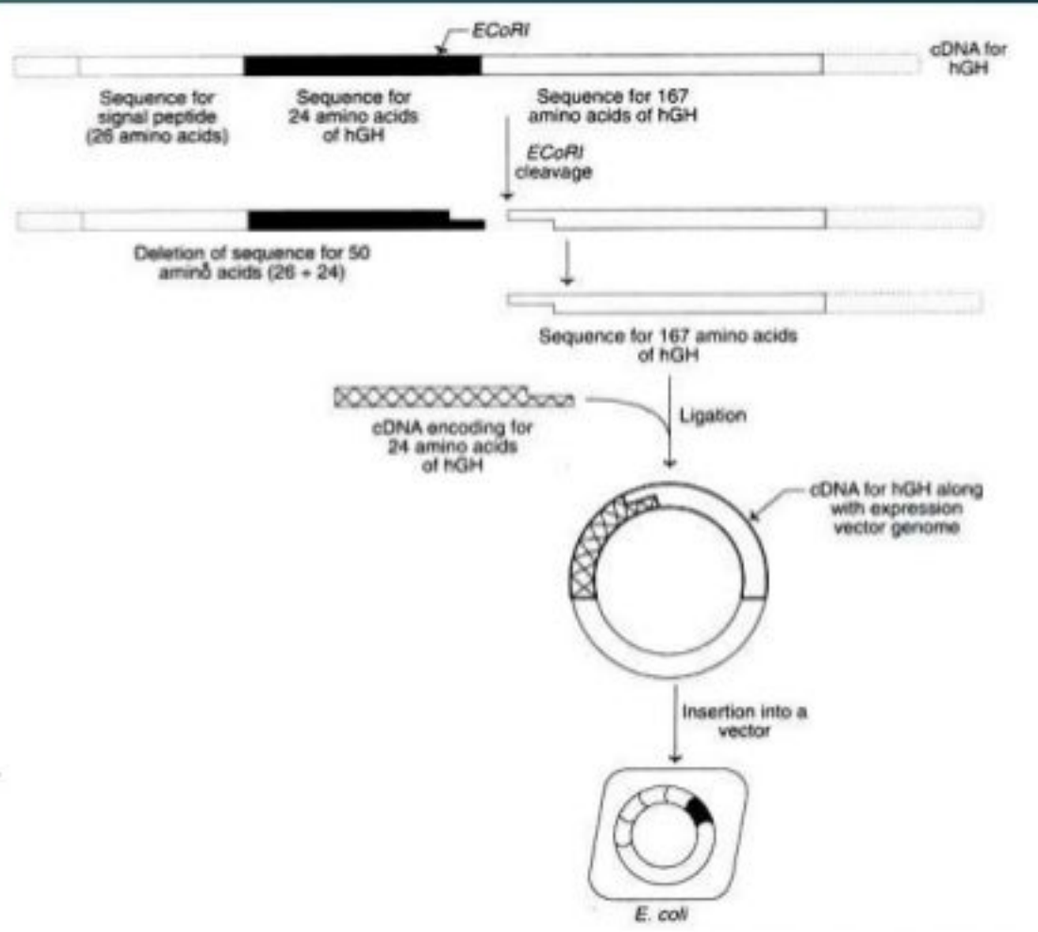


Fig. 15.2 : The production of recombinant human growth hormone (cDNA–Complementary DNA, hGH–Human growth hormone)

Interferon:

- Interferon are group of proteins that interfere with viral multiplication or replication.
- By rDna,capable of making interferon.
- Alpha component of which have role in curing lymphoma and myelogenous leukemia.

Antibiotics:

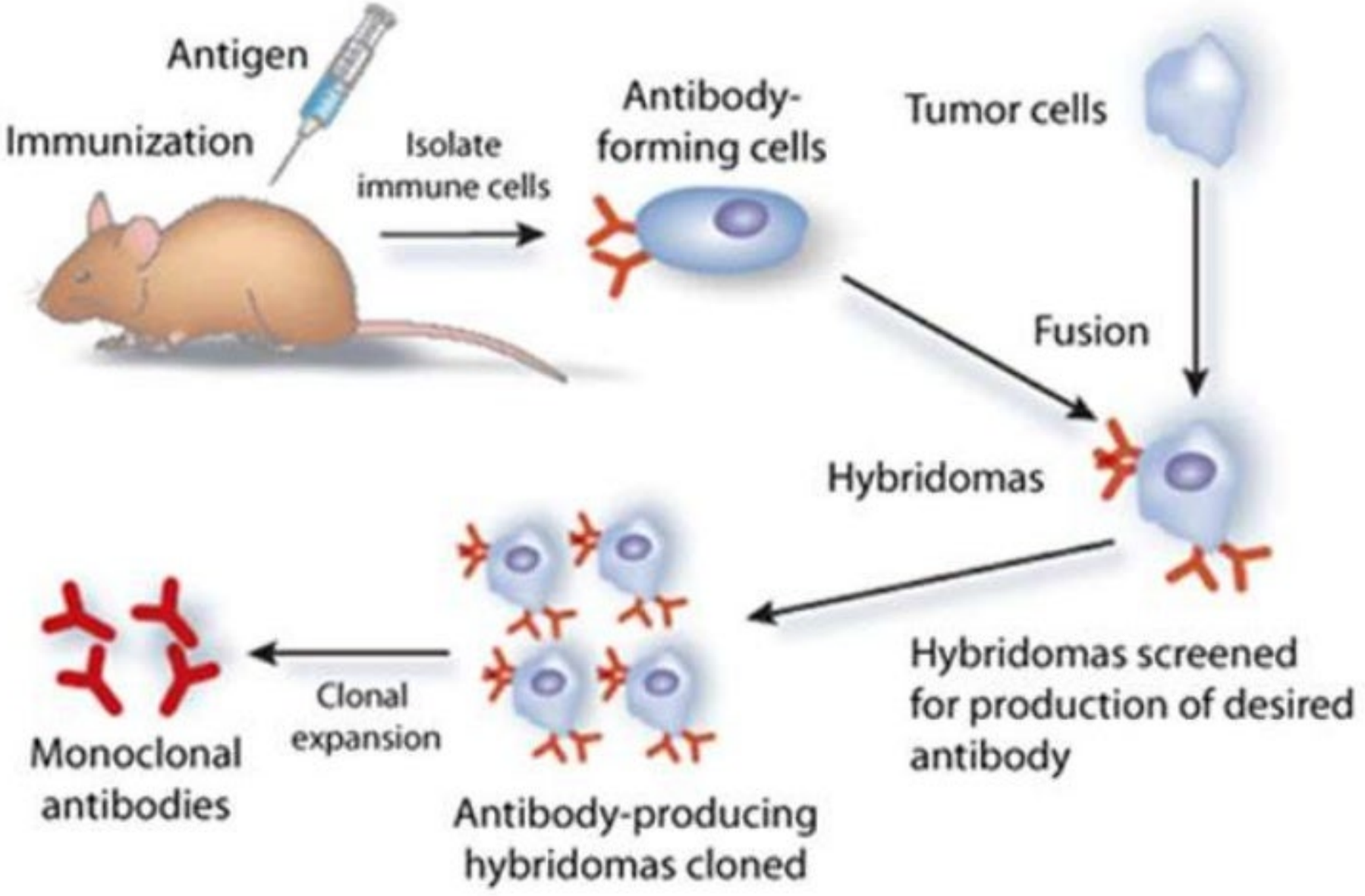
- Artificially prepared antibiotics also available.
- They denature harmful living pathogens.
- pencillin in 1928.

Monoclonal antibodies:

- Antibodies are specific proteins produced by the immune system in response to presence of a specific antigen.
- Monoclonal antibodies produce from single clone of antigen. That's why are monospecific in nature.
- Production through **hybridoma technology**.

Applications:

- mAb are used for diagnosis of disease, Pregnancy and Treatment of cancer.


Antigen
Immunization
Isolate immune cells
Antibody-forming cells
Tumor cells
Fusion
Hybridomas
Hybridomas screened for production of desired antibody
Antibody-producing hybridomas cloned
Clonal expansion
Monoclonal antibodies

Molecular diagnosis of diseases:

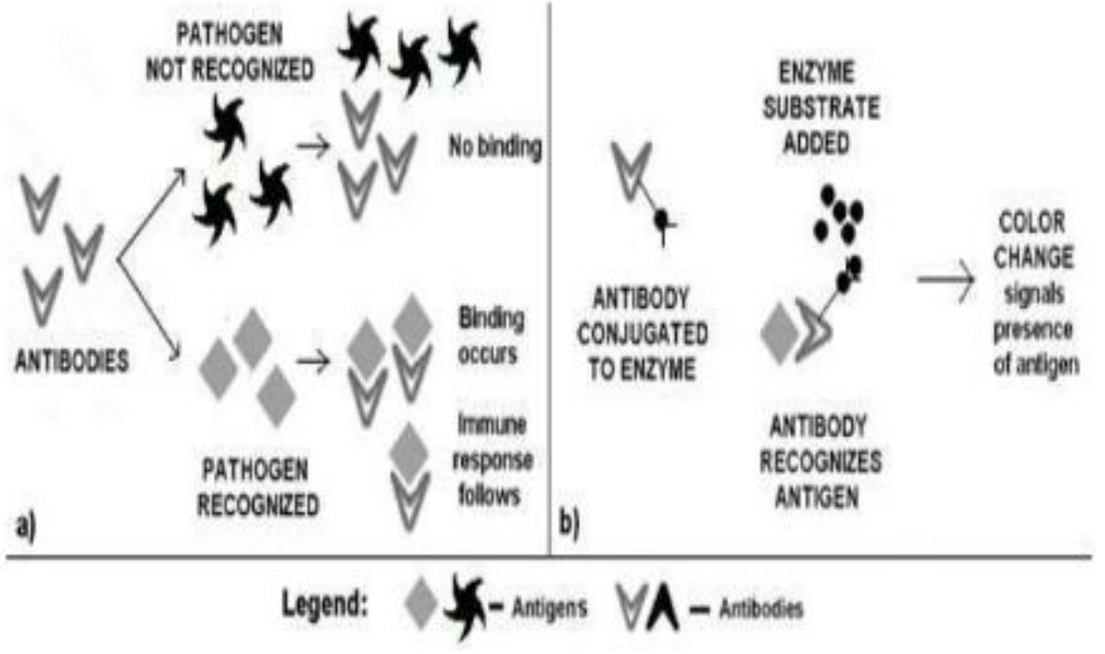
- Infectious diseases diagnosis mainly depends upon isolation and identification of pathogens, which may take several days.
- Development of **diagnostic kits** to identify pathogenic organisms by knowing the organism-specific DNA sequence has provided rapid, specific and correct diagnosis.
- Various diagnostic kits have been developed for AIDS, cancer, foot and mouth diseases, tuberculosis, etc.

- Based on ELISA and PCR principles.

For example:

in HIV patients, diagnosis conduct by:

- Antibody test uses a recombinant HIV protein to measure antibodies in the body that proliferate when there is a HIV infection.
- DNA test uses reverse transcription polymerase chain reaction (RT-PCR) to detect presence of HIV genetic material. This technique was developed using rDNA of molecules and analyzing the genome sequences.



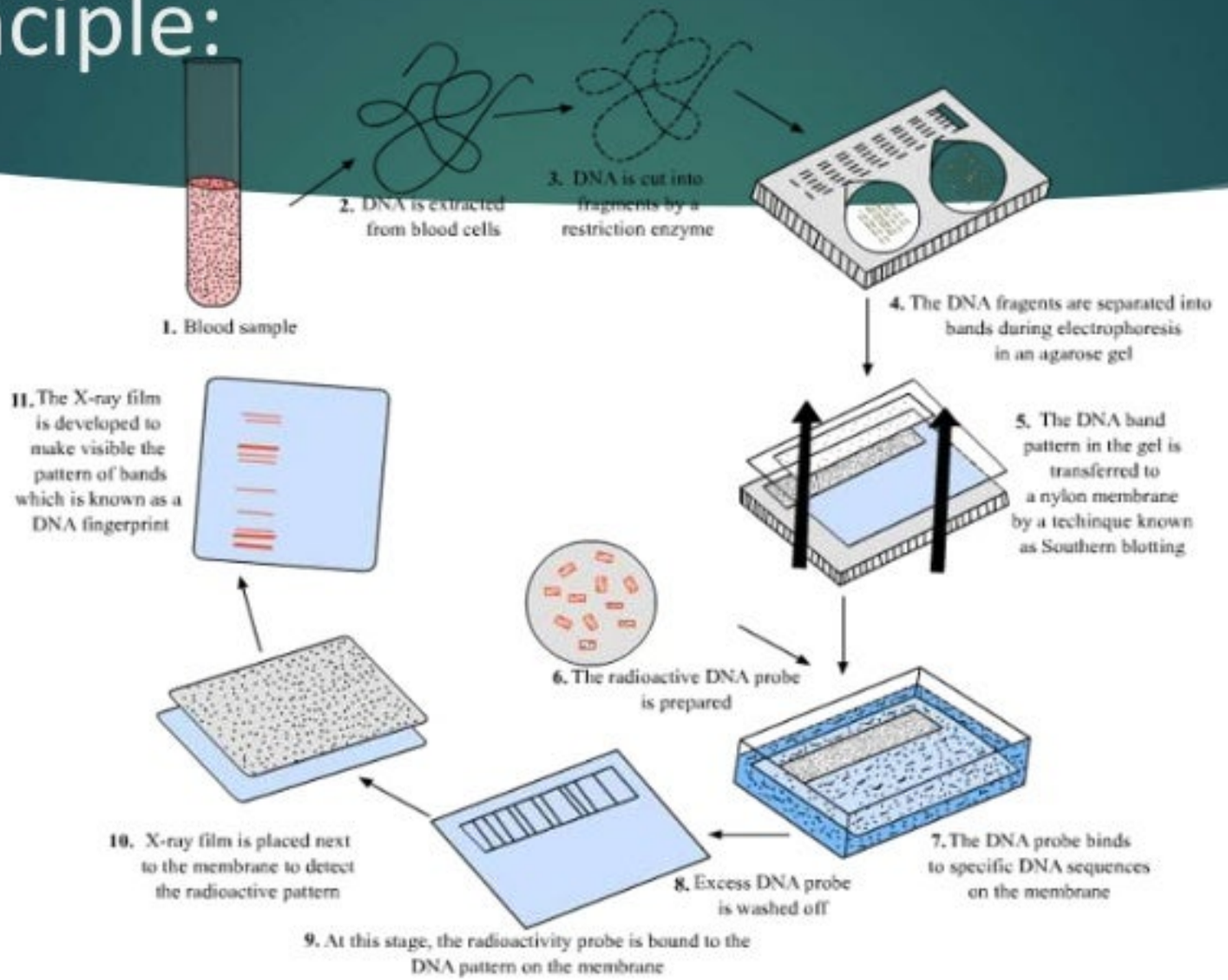
DNA Fingerprinting:

- **Dr. Alec Jeffreys** developed DNA fingerprinting technique.
- Every person have its unique finger patterns that differs from other individual. There is possibility to alter these patterns but specific principle is unknown.
- Finger prints are detected on the basis of number of highly polymorphic genes i.e. VNTR's.

Applications:

- Used in criminal identification.
- For child parentage establishment.
- Helpful for deduction of racial group.

Principle:



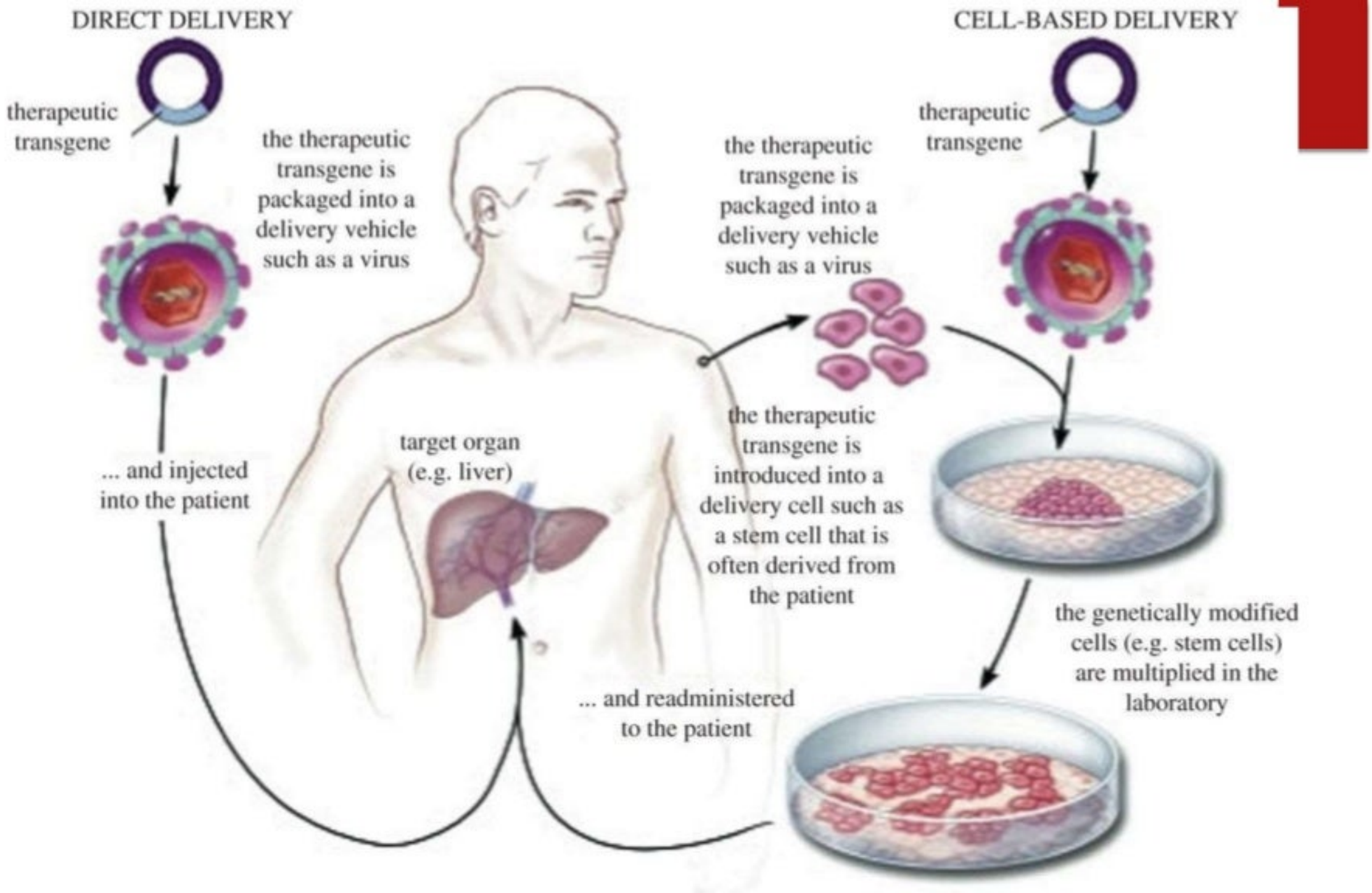
Steps in DNA fingerprinting techinque

Gene Therapy:

- injects functional genes into a cell to replace missing or defective genes in order to correct genetic disorders.
- A gene that is inserted directly into a cell usually does not function. Instead, a carrier called a **vector** is genetically engineered to deliver the gene.
- Gene therapy may be done in-vivo or e-vivo.

Health Risks:

toxicity, inflammation, and cancer.


DIRECT DELIVERY
CELL-BASED DELIVERY
therapeutic transgene
the therapeutic transgene is packaged into a delivery vehicle such as a virus
... and injected into the patient
target organ (e.g. liver)
... and readministered to the patient
therapeutic transgene
the therapeutic transgene is packaged into a delivery vehicle such as a virus
the therapeutic transgene is introduced into a delivery cell such as a stem cell that is often derived from the patient
the genetically modified cells (e.g. stem cells) are multiplied in the laboratory

Applications of recombinant DNA technology in environment

Importance

- We can use recombinant DNA technology in environment to cleanup the environment
- Measure the presence of hazardous compounds

Applications of recombinant DNA technology in environment

By recombinant DNA technology we can remediate environmental pollutants

- Some harmful chemicals that are not digested and degrade. They are partially degradable e.g. TNT chemical.
- In this nitrogen is reacted with oxygen and super oxide is formed that is toxic.
- knock out the monodehydroascorbate reductase gene that will create resistant against TNT

- Arsenic particles are toxic and they are present in the soil that is harmful for plants.
- PvACR3 gene is inserted in Arabidopsis.
- PvACR3 gene expression will give rise to generate tolerance plant to arsenic.
- In Arabidopsis thaliana
- Reductase enzyme is present

Reduce the arsenic

Phytochelatins resist the movement of arsenic in root cells and phloem cells.

- Waste product of agriculture have cellulose that do not easily decompose.
- By using recombinant DNA technology we can convert cellulose to sugar for break down of cellulose.
- Cellulase enzyme gene ⟹ vector
- Incorporate to the bacteria.
- Expression of this enzyme will enhance to degrade the waste that contain cellulose.

- By Recombinant DNA technology plastic degradation can be enhanced by genetically modified organisms.
- Degrade oil spills or organic waste.
- Genetically modified strain of Pseudomonas putida able to degrade chemicals in oil spills.
- used in development of bioindicators
- bacteria have been genetically modified as 'bioluminescors' that give off light in response to several chemical pollutants.

- These are being used to measure the presence of some hazardous chemicals in the environment.
- Other genetic sensors that can be used to detect various chemical contaminants are also undergoing trials
- and include sensors that can be used to track how pollutants are naturally degrading in ground water.

For example

- mercury resistance gene (mer) or the toluene degradation (tol) gene is linked to genes that code for bioluminescence within living bacterial cells,
- the biosensor cells can signal extremely low levels of inorganic mercury or toluene that are present in contaminated waters and soils
- emitting visible light,
- measured with fiber-optic fluro meters.

Applications of recombinant DNA technology in agriculture

Importance

- Recombinant DNA technology can be used for insertion of genes in plants not only from related plant species, but also from unrelated species such as microorganisms.

Importance

- used for the production of transgenic plants with:
- higher yield
- nutritional values.
- increased resistance to stress and pests.

Applications :-

- Development of plant having improved yield.
- Development of stress tolerant plant.
- Transgenic plant as a source of biopharmaceuticals.

Plants with improved yield:-

- Genes are inserted into plants to increase their yield.
- Researchers at Japan's National Institute of Agrobiological Resources added maize photosynthesis genes to rice.
- Increased yields by 30 percent.

continue,..

- Mexican scientists have genetically modified plants to secrete citric acid from their roots.

- In response to the slight increase in acidity, minerals bound to soil particles and are released and made available to the plant.

Stress tolerant plants:-

- Plant resistance to environmental stress:-

- rDNA technology is used to develop crops that can tolerate abiotic stress.
- Genetically modified tomato and canola plants that tolerate salt levels 300 percent greater than normal.

Herbicide resistant plants:-

- Roundup is an herbicide but it kills almost all species of plants.
- using rDNA technology, modified EPSP synthase gene (that produced enzymes that were still functional but were not inhibited by glyphosate) have

  been introduced into crop plants
- such as cotton and soyabean.

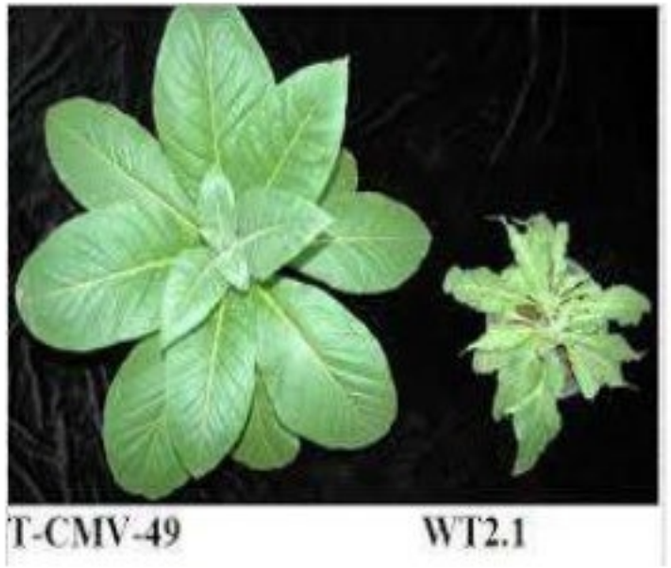
- **Insect resistance plants:-**
- Cry genes (popularly known as Bt genes) from a bacterium Bacillus thuringiensis are isolated.
- Then plant is modified using this gene.

  e.g cotton, rice, maize, potato, brinjal, cauliflower, cabbage etc.) with Bt genes have been developed.

Disease resistance plants:-

- Plants are modified to produce resistance against diseases.

e.g tobacco was first modified to produce resistance against tobacco mosaic virus.



Production of biopharmaceuticals:-

- rDNA used to produce a plant that will generate a seed that expresses a desired therapeutic protein.
- Then seed stock is use for producing the desired protein.
- The desired protein can be extracted from the seed.
- E.g In corn biopharmaceuticals are produced.

- **Edible vaccines:-**
- The genes encoding antigenic proteins can be isolated from the pathogens.
- Expressed in plants producing antigens can be eaten for vaccination/immunization (edible vaccines).

E.g In banana and tomato edible vaccines are made.

Production of secondary metabolites:-

- Arabidopsis plant is modified.
- polyhydroxy butyrate is released by Arabidopsis.
- PHB is a biodegradable plastic.

Application of recombinant DNA technology in industry

- Traditional industrial microbiology is merged with molecular biology to yield improved recombinant processes for the industrial production.

- primary and secondary metabolites, protein biopharmaceuticals and industrial enzymes are formed for industrial usage

Primary metabolites

- important primary metabolites like amino acids, nucleotides, vitamins, solvents and organic acids.
- their production is related to energy production and substrate utilization
- essential for growth

vitamines

- **Vitamin B_{12}** is produced commercially by direct fermentation utilizing the fungus Ashbya gossypii.
- vitamin B_{12} is produced by a direct fermentation utilizing streptomyces species such as Streptomyces griseus

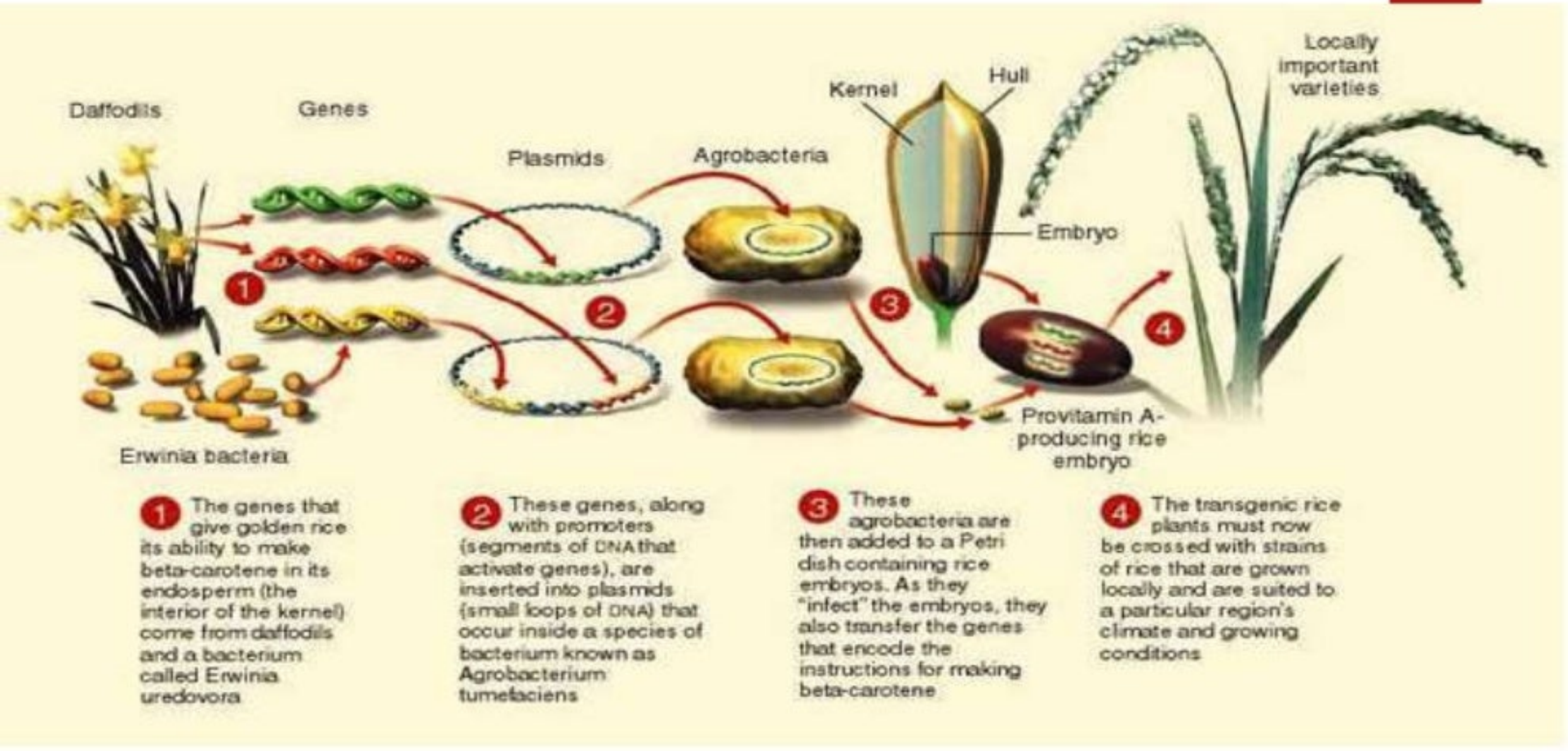
- Vitamin C is produced by utilizing Gluconobacter oxydans.

- P-carotene is produced by members of choanephoraceae family of phycomycetes.

Amino acids

- E: coli and cloning vector pBR322 were used to increase the genes for the production of amino acids e.g. glutamic acid, lysine, phenylalanine, valine.

- amino acids, L-glutamate (MSG) and L-lysine, mostly used as feed and food additives.


Daffodils
Genes
Plasmids
Agrobacteria
Kernel
Hull
Locally important varieties
Embryo
Erwinia bacteria
Provitamin A-producing rice embryo
1 The genes that give golden rice its ability to make beta-carotene in its endosperm (the interior of the kernel) come from daffodils and a bacterium called Erwinia uredovora
2 These genes, along with promoters (segments of DNA that activate genes), are inserted into plasmids (small loops of DNA) that occur inside a species of bacterium known as Agrobacterium tumefaciens
3 These agrobacteria are then added to a Petri dish containing rice embryos. As they "infect" the embryos, they also transfer the genes that encode the instructions for making beta-carotene
4 The transgenic rice plants must now be crossed with strains of rice that are grown locally and are suited to a particular region's climate and growing conditions

- Enzymes are biological molecules that catalyze increase the rates of chemical reactions
- In recombinant enzymes playing vital role in food industry
- amylases from fungi and plants for making high-fructose corn syrup
- Proteases are used by biscuit manufacturers to lower the protein level of flour

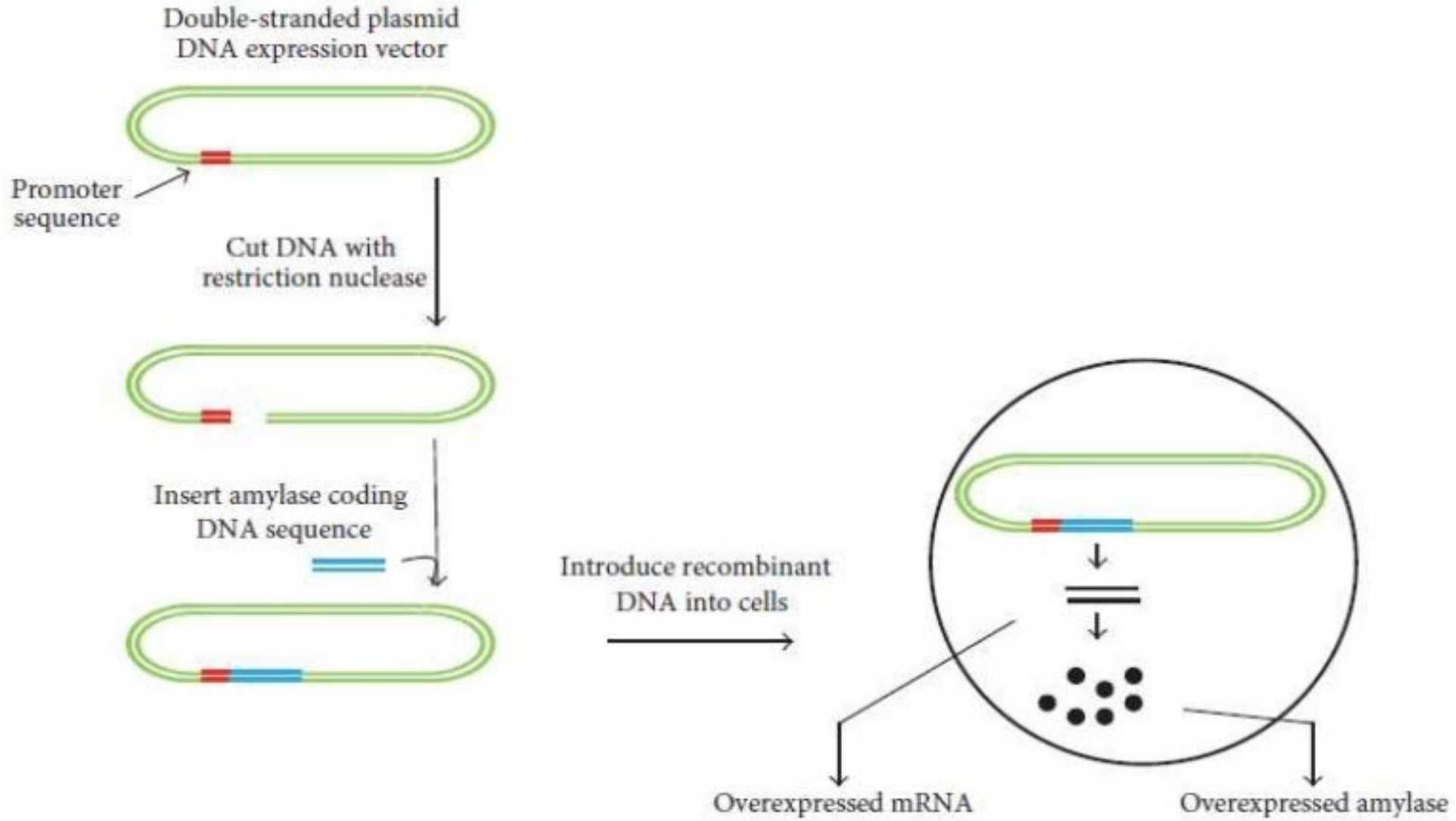
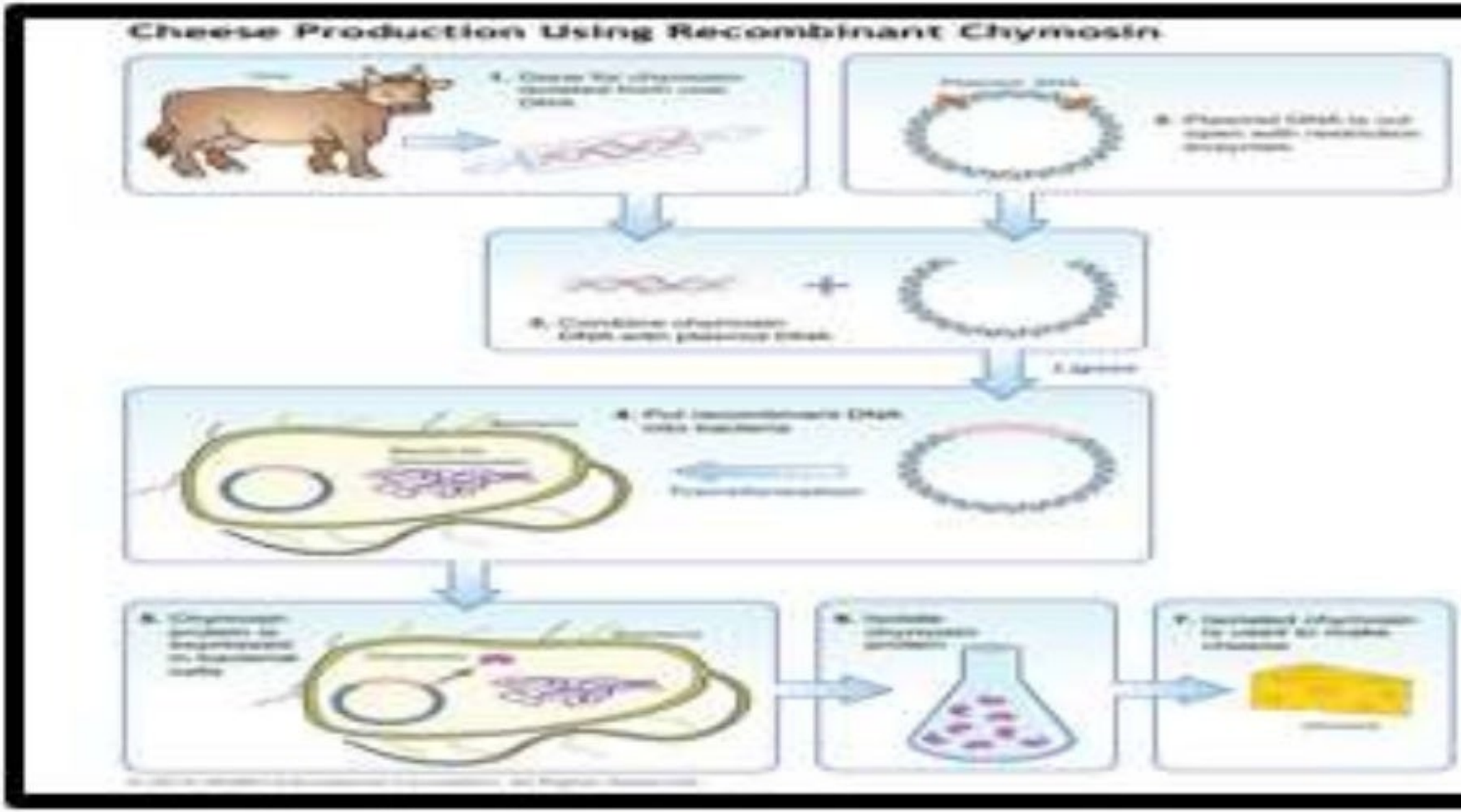


FIGURE 5: Recombinant DNA technology for amylase production. The steps involve selection of an efficient amylase gene, insertion of the gene into an appropriate vector system, transformation into an efficient bacterial system to produce a higher amount of recombinant mRNA, and overproduction of amylase from the bacterial system.

- **C**hymosin derived from “rennet” used to to manufacture of cheese
- amino acids, and peptides that are used by yeast for fermentation
- proteases split the polysaccharides and proteins in the malt

- prochymosin genes are isolated from young calves
- then they are transferred to plasmid and this plasmid is then introduced into microorganisms.
- On expression prochymosin is activated into Chymosin. Producing 100 % pure Chymosin

- first step is the milk clotting process in cheese making in which k-caeinolytic enzymes contribute to micelle precipitation and because of its specificity toward k –casein it the best enzyme for this purpose.


Cheese Production Using Recombinant Chymosin

- biofuel industry, cellulases used to break down cellulose into sugars that can be fermented.
- In molecular biology, restriction enzymes, DNA ligase, and polymerases used to manipulate DNA in genetic engineering, important in pharmacology, agriculture and medicine
- Use in forensic science.

THANK YOU