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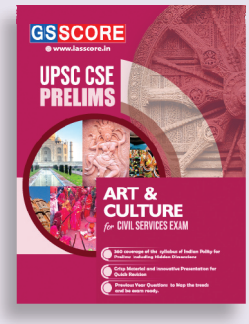

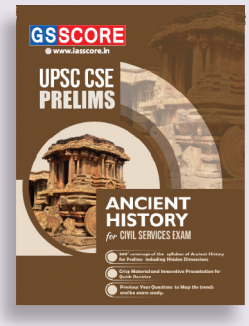

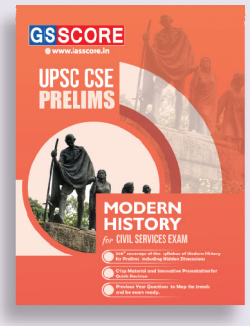

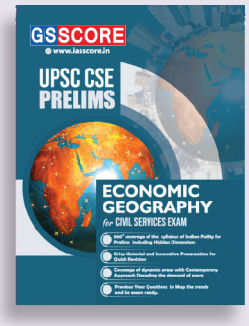

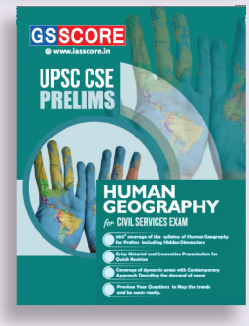

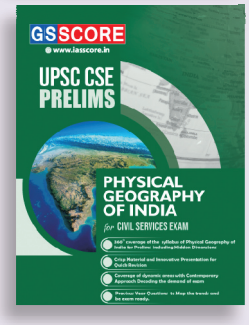

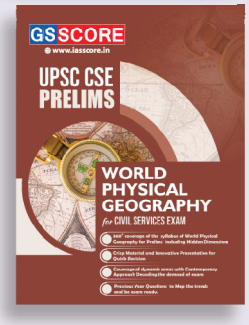

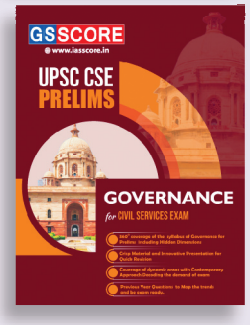

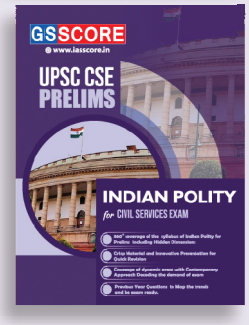

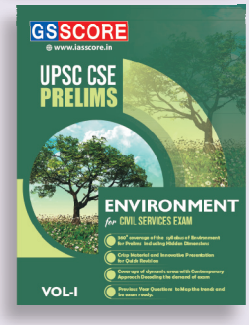

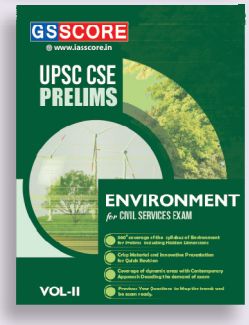

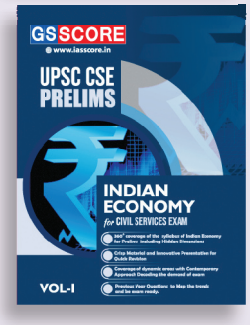

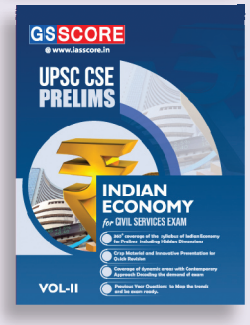

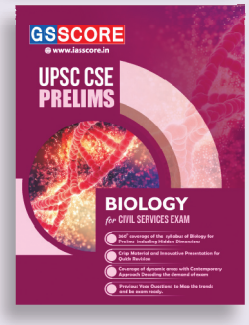

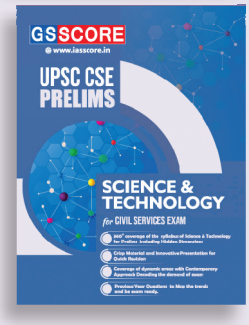

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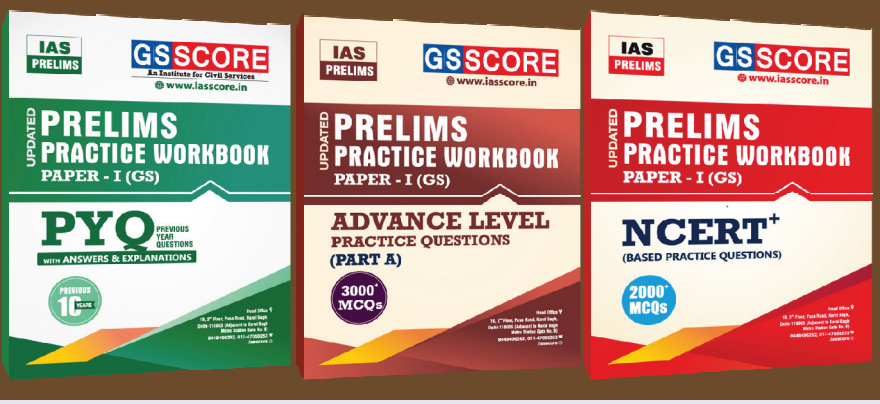

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Preface

In the last 5 years, Science & Technology has created a niche for itself in the civil services examination. Every year 7-8 questions appear from this section in Prelims which comprises of both conceptual and factual questions. One needs to be thorough with both the facts as well as the concepts. To make the learning process easy this book has been made.

This book comprises of two parts where part 1 deals with topics such as Science and Social Transformation, Bird's Eye View of India's Approach to Science, Science Related Legislations in India, Initiatives in Scientific Research, Biotechnology, Information Technology, Supercomputer & Its Applications, Television Technologies, Nanotechnology & Its Applications, Transportation Technologies, etc. All these topics and their subtopics have been comprehensively covered under the book.

The book has been written with the point of view of comprehensive coverage of the entire syllabus of Science and Technology. One of the distinguishing characteristics of the book is that it covers a wide canvas of Science and Technology. The text of the book has been written in a lucid, cogent, and convincing style. This will ease their preparation and provides consolidated and complete UPSC notes at one place. We have also assimilated previous Year questions in the book in order to keep the preparation as per the UPSC framework.

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CHAPTER
1

BIOTECHNOLOGY AND ITS APPLICATION

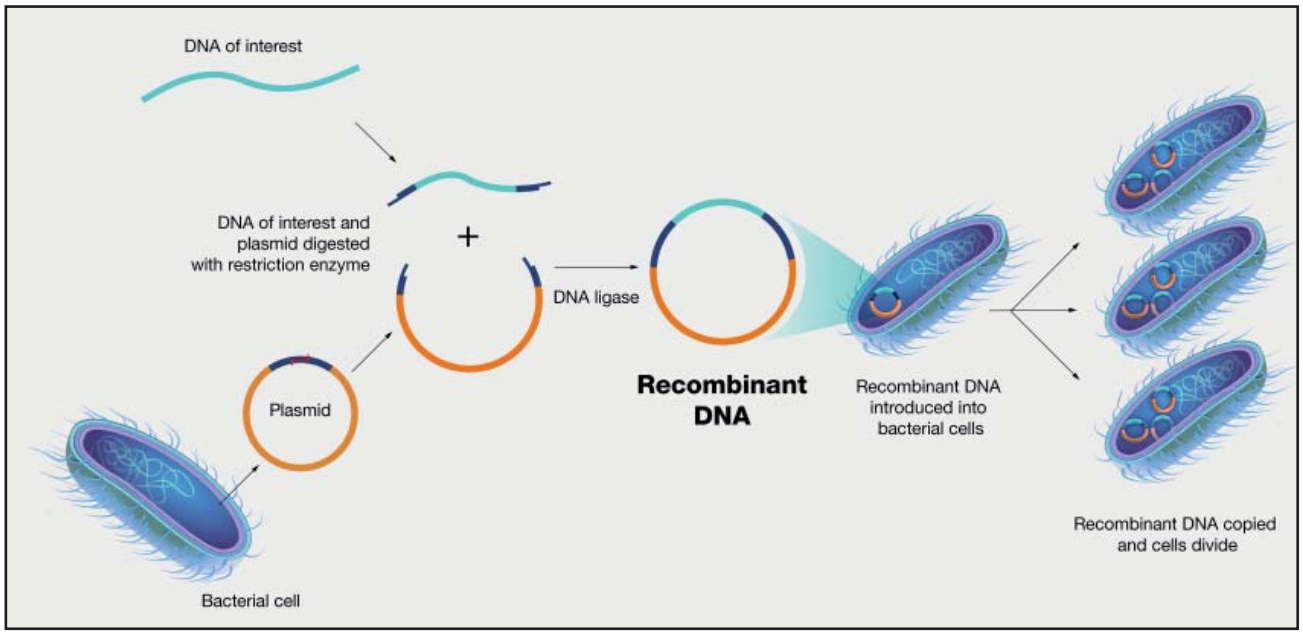
Biotechnology deals with techniques of using **live organisms or enzymes** from organisms to produce products and **processes useful to humans and environment**. It is the integration of **natural science** and **organisms, cells,** parts thereof, and **molecular analogues** for various products and services.

■ Principles of Biotechnology

Among many, the **two core techniques** that enabled birth of modern biotechnology are:

- **Genetic engineering:** Techniques to alter the chemistry of genetic material **(DNA and RNA),** to introduce these into host organisms and thus change the **phenotype** of the **host organism.**
- **Bioprocess engineering:** Maintenance of **sterile (microbial contamination-free)** ambience in chemical engineering processes to enable growth of only the desired **microbe/eukaryotic cell** inlarge quantities for the manufacture of **biotechnological products** like **antibiotics, vaccines,** enzymes, etc.

■ Recombinant DNA technology (Genetic Engineering)



Genetic engineering, also called **recombinant DNA technology,** involves the group of techniques used to **cut up and join** together genetic material, **especially DNA** from **different biological species**, and **to**

introduce the resulting **hybrid DNA** into an organism in order to form **new combinations** of heritable **genetic material**.

The recombinant DNA technology emerged with the discovery of **restriction enzymes** in the year **1968** by Swiss **microbiologist Werner Arber**.

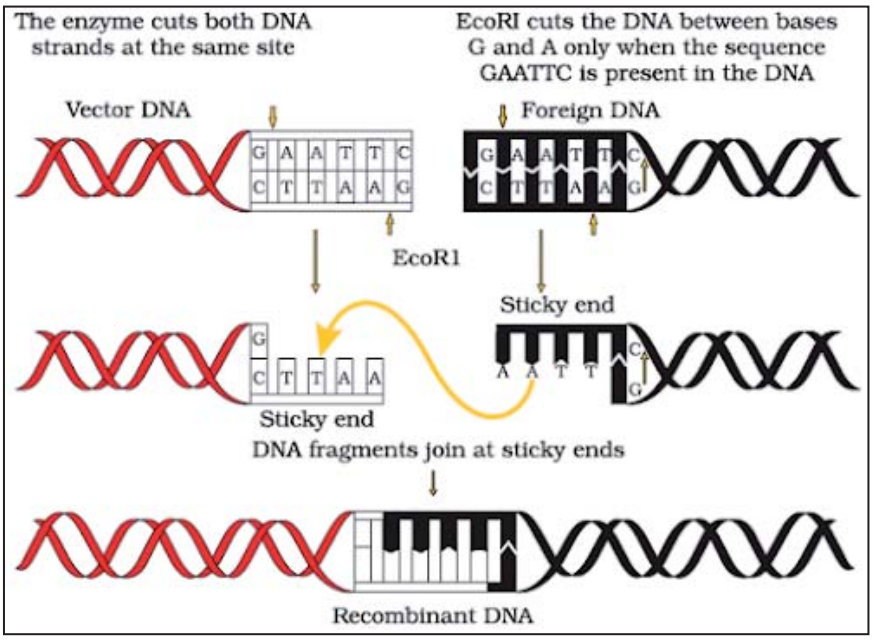
The techniques of genetic engineering which include creation of recombinant DNA, use of **genecloning** and **gene transfer,** overcome the limitation of **Traditional Hybridization** (inclusion and multiplication of **undesirable genes** along with the desired genes) and allows us to isolate and introduce only one or a **set of desirable genes** without introducing undesirable genes into the target organism.

There are **three** basic steps in **genetically modifying** an organism — (i) **identification of DNA** with desirable genes; (ii) introduction of the **identified DNA** into the **host**; (iii) maintenance of **introduced DNA** in the **host and transfer** of the **DNA to its progeny**.

Tools of Recombinant DNA Technology

■ Restriction Enzymes:

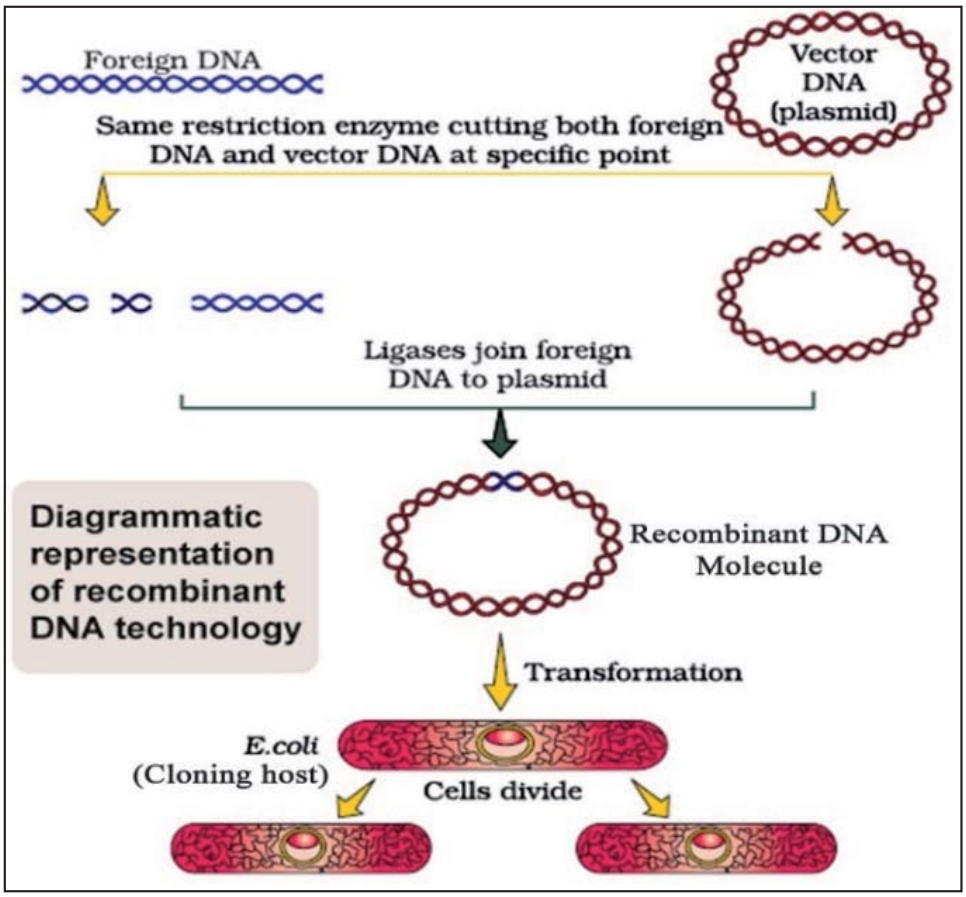
- **Restriction enzymes** belong to a larger class of enzymes called **nucleases.**
- These are of **two** kinds; **exonucleases** and **endonucleases.**
- **Exonucleases** remove **nucleotides** from the **ends of the DNA** whereas, **endonucleases make cuts** at specific positions within the DNA.
- Each restriction **endonuclease** functions by **'inspecting'** the length of a **DNA sequence.**
  - Once it finds its **specific recognition sequence**, it will bind to the DNA and cut each of the two strands of the **double helix** at specific points in their **sugar –phosphate backbones.**
  - Each restriction endonuclease recognizes a specific **palindromic nucleotide sequences** in the DNA.



Palindromes are groups of letters that form the same words when read both forward and backward, e.g., "MALAYALAM". As against a word-palindrome where the same word is read in both directions, the palindrome in DNA is a sequence of base pairs that reads same on the two strands when orientation of reading is kept the same. For example, the following sequences reads the same on the two strands in 5' à 3' direction. This is also true if read in the 3' à 5' direction.

5′ —— GAATTC —— 3′
3′ —— CTTAAG —— 5′

- Restriction enzymes cut the **strand of DNA** a little away from the centre of the **palindrome sites,** but between the same two bases on the opposite strands.
- This leaves single stranded portions at the ends.
- There are overhanging stretches called sticky ends on each strand.
- These are named so because they form **hydrogen bonds** with their complementary cut counterparts.
- This stickiness of the ends facilitates the action of the enzyme **DNA ligase.**



Gel electrophoresis: The cutting of DNA by restriction endonucleases results in the fragments of DNA. These fragments can be separated by a technique known as gel **electrophoresis.**

■ Cloning Vectors:

- A vector, as related to molecular biology, is a DNA molecule (often bacterial plasmid or virus) that can be used as a vehicle to carry a particular DNA segment into a host cell as part of acloning or recombinant DNA technique.
- The vector typically assists in replicating and/orexpressing the inserted DNA sequence inside the host cell.
- Vectors used at present, areengineered in such a way that they help easy linking of foreign DNA and selection of recombinants from non-recombinants.

The following are the features that are required to facilitate cloning into a vector:-

■ Origin of replication (ori) :

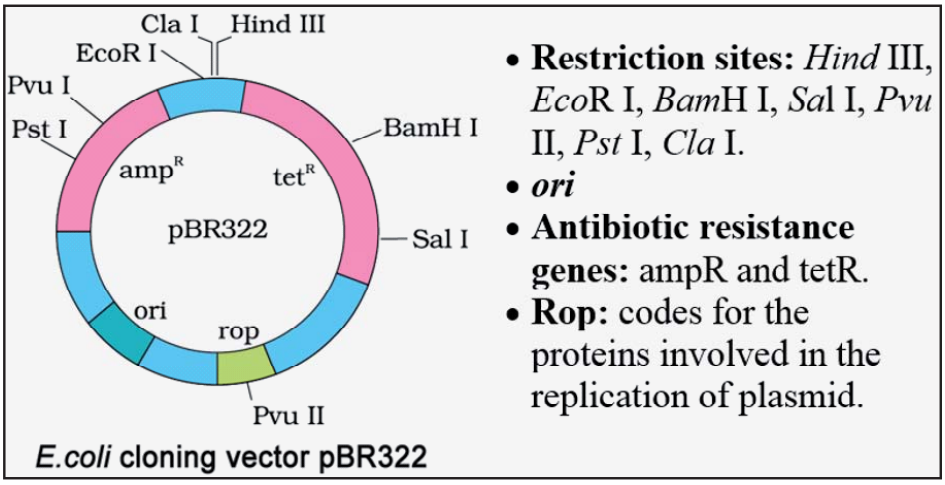
- This is a sequence from where replication starts and any piece of DNA when linked to this sequence can be made to replicate within the host cells.
- This is a sequence from where replication starts and any piece of DNA when linked to this sequence can be made to replicate within the host cells.

■ Selectable marker :

- In addition to 'ori', the vector requires a selectable marker, which helps in identifying and eliminating non transformants and selectively permitting the growth of the transformants.
- Normally, the genes encoding resistance to antibiotics such as ampicillin, chloramphenicol, tetracycline or kanamycin, etc., are considered useful selectable markers for E. coli.

■ Cloning sites:

- In order to link the alien DNA, the vector needs to have very few, preferably single, recognition sites for the commonly used restriction enzymes.
- Presence of more than one recognition sites within the vector will generate several fragments, which will complicate the gene cloning
- The ligation of alien DNA is carried out at a restriction site present in one of the two antibiotic resistance genes.



E.coli cloning vector pBR322

Selection of recombinants:

Selection of recombinants due to inactivation of antibiotics is a cumbersome procedure because it requires simultaneous plating 0on two plates having different antibiotics. Therefore, alternative selectable markers have been developed which differentiate recombinants from non-recombinants on the basis of their ability to produce colour in the presence of a chromogenic substrate. In this, a recombinant DNA is inserted within the coding sequence of an enzyme, *β-galactosidase*. This results into inactivation of the gene for synthesis of this enzyme, which is referred to as insertion inactivation.

Vectors for cloning genes in plants and animals :

- *Agrobacterium tumifaciens*, a pathogen of several dicot plants is able to deliver a piece of DNA known as 'T-DNA' to transform normal plant cells into a tumor and direct these tumor cells to produce the chemicals required by the pathogen.

- Similarly, retroviruses in animals have the ability to transform normal cells into cancerous cells.
- The tumor inducing (Ti) plasmid *of Agrobacterium tumifaciens* has now been modified into a cloning vector which is no more pathogenic to the plants but is still able to use the mechanisms to deliver genes of our interest into a variety of plants.
- Similarly, retroviruses have also been disarmed and are now used to deliver desirable genes into animal cells.
- So, once a gene or a DNA fragment has been ligated into a suitable vector it is transferred into a bacterial, plant or animal host (where it multiplies).

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Q1: Recombinant DNA technology (Genetic Engineering) allows genes to be transferred:

1. across different species of plants
2. from animals to plants
3. from microorganisms to higher organisms

Select the correct answer using the codes given below:

(a) only 1 (b) 2 and 3 only

(c) 1 and 3 only (d) 1, 2 and 3

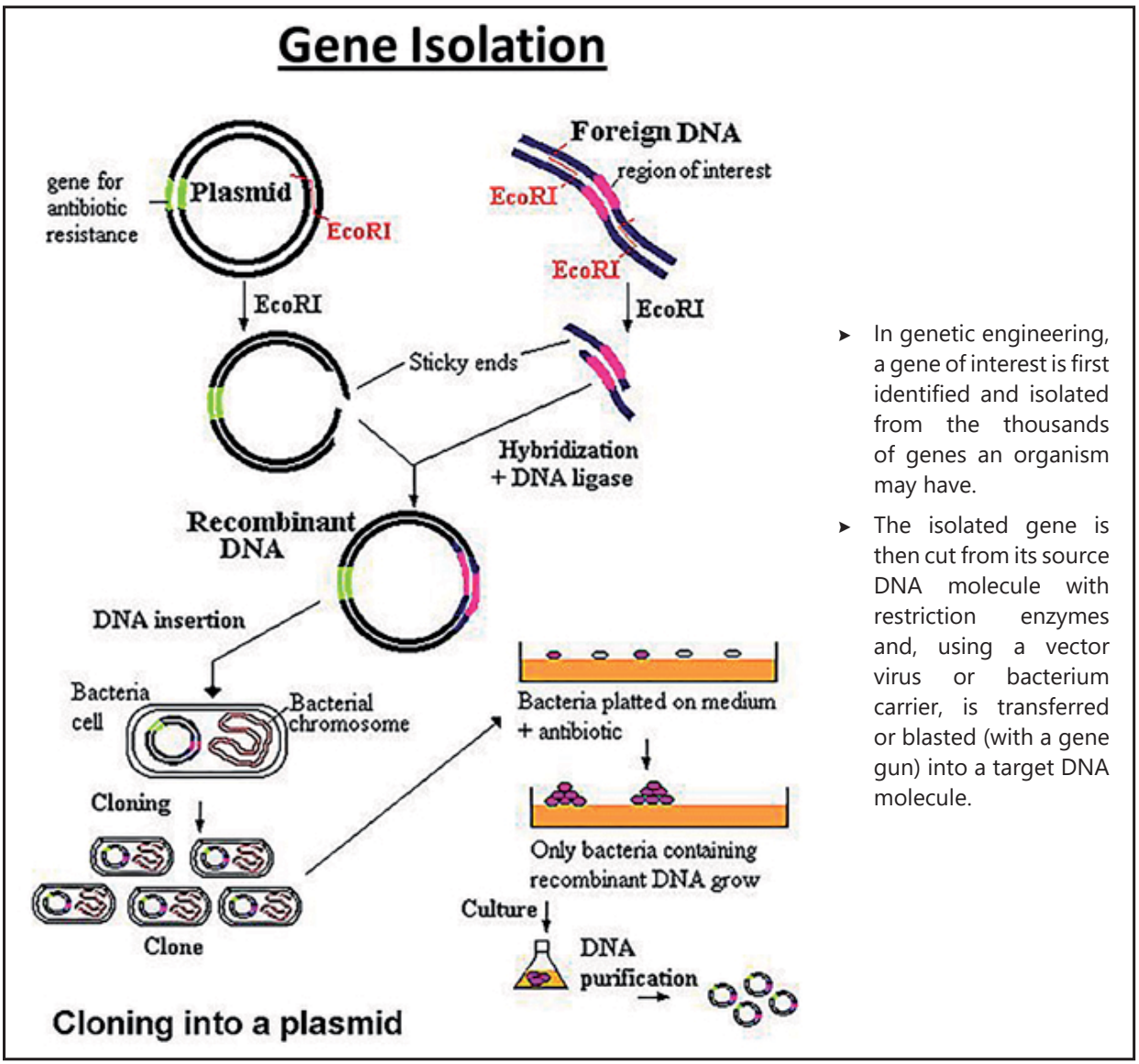
Correct Option: (d)

Processes of Recombinant DNA Technology

Recombinant DNA technology involves several steps in specific sequence such as isolation of DNA, fragmentation of DNA by restriction endonucleases, isolation of a desired DNA fragment, ligation of the DNA fragment into a vector, transferring the recombinant DNA into the host, culturing the host cells in a medium at large scale and extraction of the desired product.

■ Isolation of the Genetic Material (DNA)

- Nucleic acid is the genetic material of all organisms without exception.
- In majority of organisms this is deoxyribonucleic acid or DNA.
- In order to cut the DNA with restriction enzymes, it needs to be in pure form, free from other macro-molecules.
- Since the DNA is enclosed within the membranes, we have to break the cell open to release DNA along with other macromolecules such as RNA, proteins, polysaccharides and also lipids.
- Since the DNA is enclosed within the membranes, we have to break the cell open to release DNA along with other macromolecules such as RNA, proteins, polysaccharides and also lipids.
- The RNA can be removed by treatment with ribonuclease whereas proteins can be removed by treatment with protease.
- Other molecules can be removed by appropriate treatments and purified DNA ultimately precipitates out after the addition of chilled ethano l.

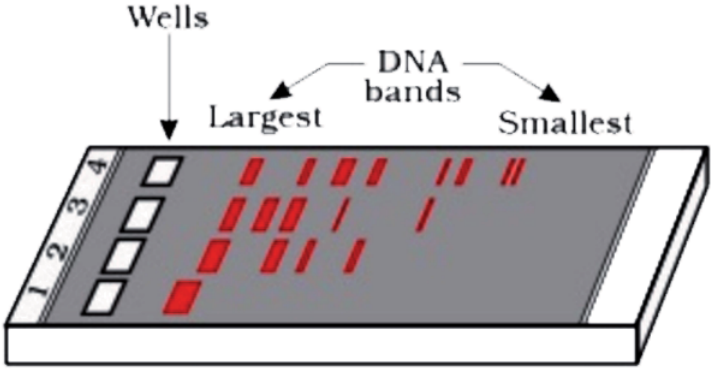


- In genetic engineering, a gene of interest is first identified and isolated from the thousands of genes an organism may have.
- The isolated gene is then cut from its source DNA molecule with restriction enzymes and, using a vector virus or bacterium carrier, is transferred or blasted (with a gene gun) into a target DNA molecule.

■ Cutting of DNA at Specific Locations:

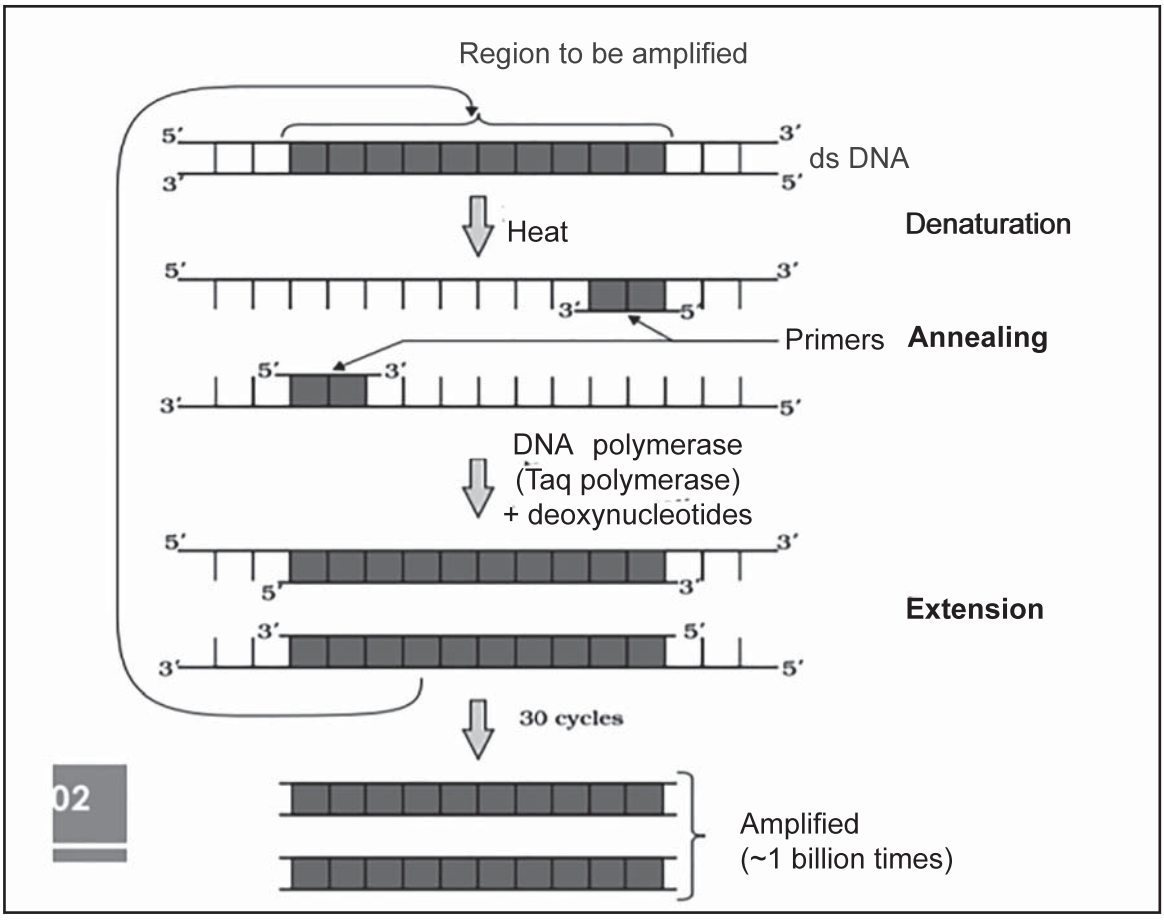
- Restriction enzyme digestions are performed by incubating purified DNA molecules with the restriction enzyme, at the optimal conditions for that specific enzyme.
- Agarose gel electrophoresis is employed to check the progression of a restriction enzyme digestion.
- DNA is a negatively charged molecule, hence it moves towards the positive electrode (anode).
- The process is repeated with the vector DNA also.
- The joining of DNA involves several processes.
- After having cut the source DNA as well as the vector DNA with a specific restriction enzyme, the cut out 'gene of interest' from the source DNA and the cut vector with space are mixed and ligase is added.
- This results in the preparation of recombinant DNA.

Given diagram showing a typical agarose gel electrophoresis showing migration of DNA fragments . The undigested DNA fragment will be present in the lane



■ Amplification of Gene of Interest using PCR:

- PCR stands for Polymerase Chain Reaction. In this reaction, multiple copies of the gene (or DNA) of interest is synthesised in vitro using two sets of primers (small chemically synthesised oligonucleotides that are complementary to the regions of DNA) and the enzyme DNA polymerase.



- The enzyme extends the primers using the nucleotides provided in the reaction and the genomic DNA as template.

- If the process of replication of DNA is repeated many times, the segment of DNA can be amplified to approximately billion times, i.e., 1 billion copies are made.
- Such repeated amplification is achieved by the use of a thermostable DNA polymerase (isolated from a bacterium, Thermus aquaticus), which remain active during the high temperature induced denaturation of double stranded DNA.
- The amplified fragment if desired can now be used to ligate with a vector for further cloning.

■ Introduction of the identified DNA into the host:

There are several methods of introducing the ligated DNA into recipient cells.

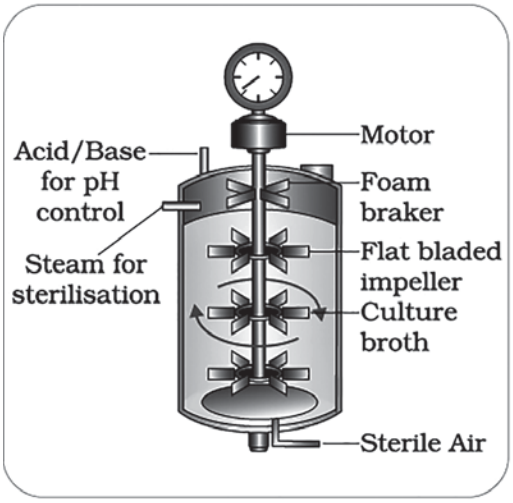
⊙ Biological Method

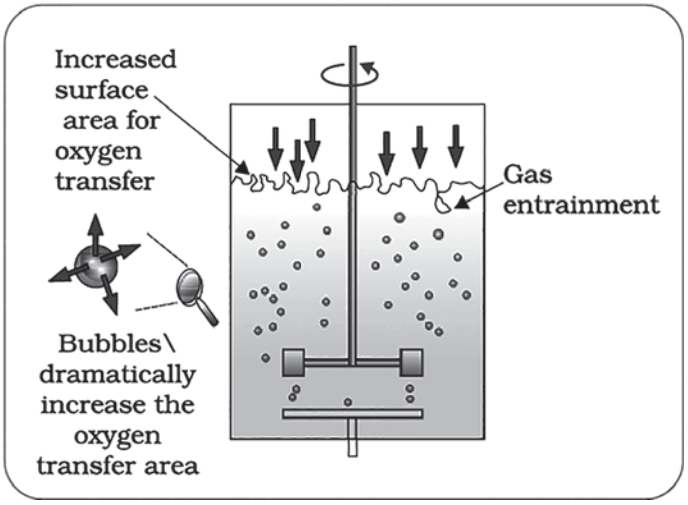
- **Using Bacteria (Bactofection)**
  - The genes located on the plasmids of the transformed bacterial strains are delivered and expressed into the cells. The gene delivery may be intracellular or extracellular. It has a potential to express various plasmid-encoded heterologous proteins (antigens, toxins, hormones, enzymes etc.) in different cell types. Strains that are invasive and having better cell to cell spread are more efficient.
- **Using Viruses (Transduction)**
  - This method involves the introduction of genes into host cell's genome using viruses as carriers. The viruses are used in gene transfer due to following features-
    - Efficiency of viruses to deliver their nucleic acid into cells
    - High level of replication and gene expression.
- The foreign gene is packaged into the virus particles to enter the host cell. The entry of virus particle containing the candidate gene sequences into the cell and then to the nuclear genome is a receptor-mediated process. The vector genome undergoes complex processes ending up with ds-DNA depending on the vector that can persist as an episome or integrate into the host genome followed by the expression of the candidate gene
- **Chemical method**
  - The commonly used methods of chemical transfection use the following:
    - Calcium phosphate
    - DEAE dextran
    - Cationic Lipid
    - Other polymers - poly-L-lysine (PLL), polyphosphoester, chitosan, dendrimers
- **Physical method (direct transfer)**
  - Various physical or mechanical methods are employed to overcome this and aid in gene transfer as listed below
    - Electroporation
    - Microinjection
    - Particle Bombardment (Gene Gun)
    - Sonoporation
    - Laser induced
    - Bead transfection

Bioprocess engineering (Obtaining the Foreign Gene Product)

- After having cloned the gene of interest and having optimised the conditions to induce the expression of the target protein, one has to consider producing it on a large scale.
- The cells harbouring cloned genes of interest may be grown on a small scale in the laboratory. The cultures may be used for extracting the desired protein and then purifying it by using different separation techniques.
- The cells can also be multiplied in a continuous culture system wherein the used medium is drained out from one side while fresh medium is added from the other to maintain the cells in their physiologically most active log/exponential phase. This type of culturing method produces a larger biomass leading to higher yields of desired protein.
- Small volume cultures cannot yield appreciable quantities of products. To produce in large quantities, the development of bioreactors, where large volumes (100-1000 litres) of culture can be processed, was required.

Bioreactors can be thought of as vessels in which raw materials are biologically converted into specific products, individual enzymes, etc., using microbial plant, animal or human cells.





- All these processes in which the large scale gene product (protein) are produced, comes under the ambit of bioprocess engineering which itself consists of two processes; upstream processing and downstream processing.

■ Upstream Processing

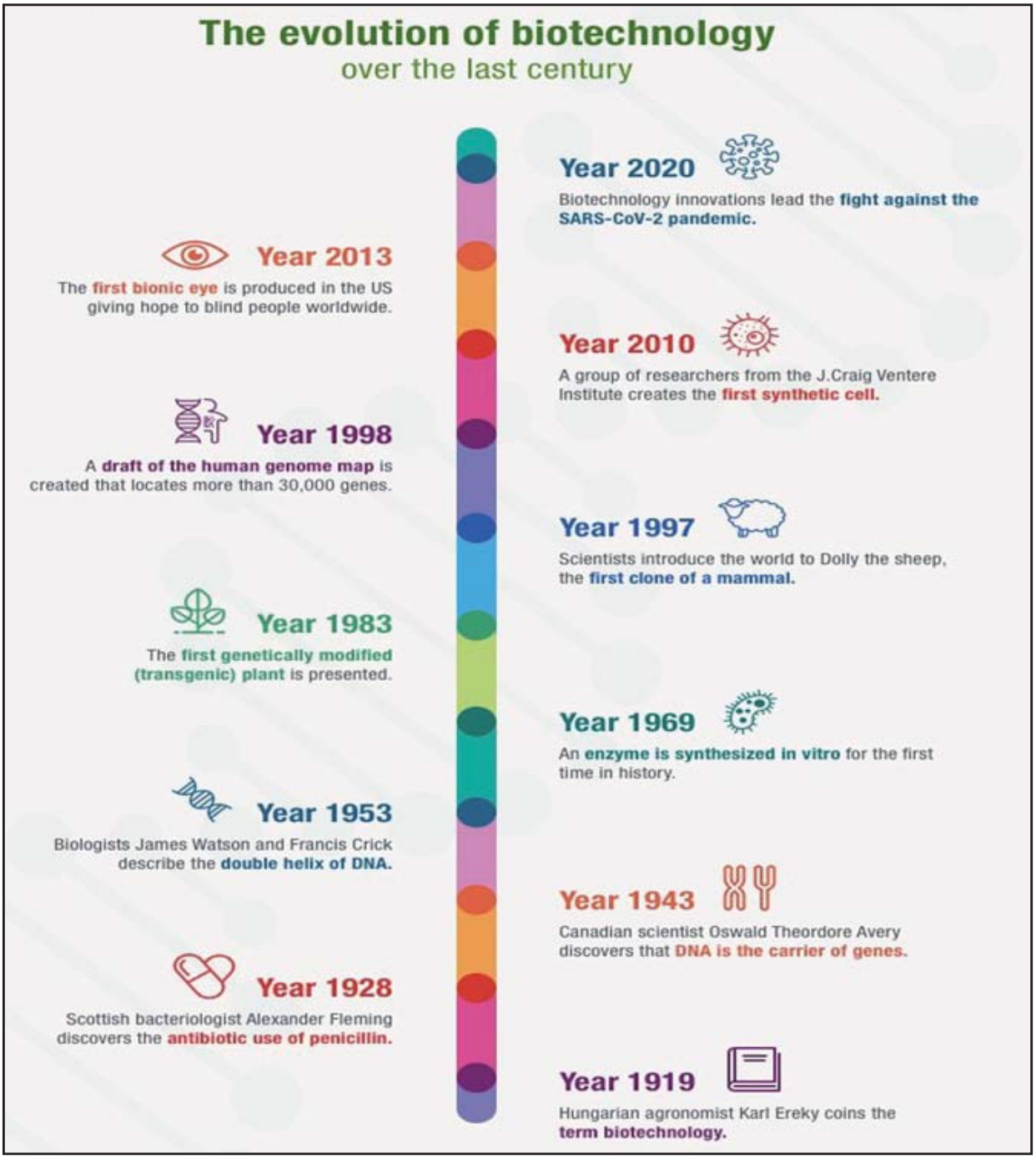
- The process by which optimal conditions are provided to achieve the desired product is called upstream processing. In this, optimum growth conditions such as temperature, pH, substrate, salts, vitamins, oxygen are provided and manipulated to gain the maximum efficiency. The entire upstream processing occurs in the bioreactors.

■ Downstream Processing

- After completion of the biosynthetic stage, the product has to be subjected through a series of processes before it is ready for marketing as a finished product.
- The processes include separation and purification, which are collectively referred to as downstream processing.

- The product has to be formulated with suitable preservatives.
- Such formulation has to undergo thorough clinical trials as in case of drugs.
- Strict quality control testing for each product is also required.
- The downstream processing and quality control testing vary from product to product.

■ Applications of Biotechnology



Agriculture

■ GM crops

- Agricultural biotechnology focuses on creating genetically modified plants to boost crop yields or add traits that provide those plants a competitive edge when grown in regions that place some kind of stress factor on the plant, namely weather, and pests.
- The most recent development in agriculture is genetically modified crops (GMO). These crops are the consequence of changes made to the genetic structure of the crops. The crops benefit from this alteration in a number of ways, Genetic modification has:
  - Made crops more tolerant to abiotic stresses (cold, drought, salt, heat).
  - Reduced reliance on chemical pesticides (pest-resistant crops).
  - Helped to reduce post-harvest losses.
  - Increased efficiency of mineral usage by plants (this prevents early exhaustion of fertility of soil).
  - Enhanced nutritional value of food, e.g., golden rice, i.e., Vitamin 'A' enriched rice.

In addition to these uses, GM has been used to create tailor-made plants to supply alternative resources to industries, in the form of starches, fuels and pharmaceuticals.

- Genetic modification involves the mutation, insertion, or deletion of genes.
- Bacillus thuringiensis (Bt) toxin gene has been cloned from the bacteria and been expressed in plants to provide resistance to insects without the need for insecticides; in effect created a bio-pesticide. Examples are Bt cotton, Bt corn, rice, tomato, potato and soyabean etc.
- Some strains of Bacillus thuringiensis produce proteins that kill certain insects such as lepidopterans (tobacco budworm, armyworm), coleopterans (beetles) and dipterans (flies, mosquitoes).
- B. thuringiensis forms protein crystals during a particular phase of their growth. These crystals contain a toxic insecticidal protein.

Why does this toxin not kill the Bacillus?

Actually, the **Bt toxin protein exist** as **inactive protoxins** but once an insect ingest the inactive toxin, it is converted into an active form of toxin due to the **alkaline pH** of the gut which **solubilise** the **crystals**. The activated toxin binds to the surface of **midgut epithelial cells** and creates pores that cause cell **swellingand lysis** and eventually cause death of the insect.

- Specific Bt toxin genes were isolated from Bacillus thuringiensis and incorporated into the several crop plants such as cotton:-
  - The choice of genes depends upon the crop and the targeted pest, as most Bt toxins are insect-group specific.
  - The toxin is coded by a gene cryIAc named cry.
  - There are a number of them, for example, the proteins encoded by the genes cryIAc and cryIIAb control the cotton bollworms that of cryIAb controls corn borer.
- **Major GM crops in India:**

Bt Brinjal	Janak and BSS-793
Bt cotton	Bollgard
GM mustard	Dhara Mustard Hybrid 11 (DMH 11)
GM tomato	*Flavr Savr*

■ Pest Resistant Plants

- A nematode Meloidegyne incognitia infects the roots of tobacco plants and causes a great reduction in yield.
- A novel strategy was adopted to prevent this infestation which was based on the process of RNA interference (RNAi).
- RNAi takes place in all eukaryotic organisms as a method of cellular defense.
- This method involves silencing of a specific mRNA due to a complementary dsRNA molecule that binds to and prevents translation of the mRNA (silencing).
- The source of this complementary RNA could be from an infection by viruses having RNA genomes or mobile genetic elements (transposons) that replicate via an RNA intermediate.
- Using **Agrobacterium vectors**, nematode-specific genes were introduced into the host plant.
- The introduction of DNA was such that it produced both sense and anti-sense RNA in the host cells.
- These two RNA's being complementary to each other formed a double stranded (dsRNA) that initiated RNAi and thus, silenced the specific mRNA of the nematode.
- The consequence was that the parasite could not survive in a transgenic host expressing specific interfering RNA.
- The transgenic plant therefore got itself protected from the parasite.

■ Biofertiliser Technologies

- A bio-fertilizer is a substance that contains living organisms that, when applied to seed, plant, surfaces, or soil, colonize the rhizosphere or the interior of the plants and promotes growth by increasing the supply or availability of primary nutrients to the host plants.
- Bio-fertilizers are eco-friendly and do not contain substances that harm the living soil.
- It acts indirectly helping the plants or the crops in proper stimulation through natural processes like nitrogen fixation, phosphorylation, enhancing the growth by the provision of the growing substances — for example, Rhizobium, Azotobacter, Azospirillum, Frankia, Blue-green algae.

■ Molecular Breeding

- Marker-assisted selection or molecular breeding is cutting edge technology among today's biotech companies.
- Plant breeders can use this technique to locate and assemble desirable traits to speed up the process of developing the new commercial hybrids.
- Unlike GMOs, new crop varieties produced by marker-assisted selection are spared the regulatory trials and the public opposition mainly because the plant's natural genetic boundaries are not crossed.

■ Micro propagation

- Micro propagation is one of the tools of tissue culture, used to increase the growing stock of required plant material rapidly.
- The propagated plants are generally disease resistant. It is an advanced Vegetative Propagation Technology.
- Micro propagation can be used commercially for asexual propagation to produce a large number of the same plant with the same genetic makeup from small pieces of plant tissues.
- The technique is useful for seed production in certain crops as genetic conservation is highly important during the seed production processes.
- A large number of plants can be produced in a short period and can also be maintained in small spaces saving some of the endangered species and germplasm.

■ Improvement in Floriculture

- Floriculture is associated with the cultivation of flowering and ornamental plants for gardens and floristry, comprising the floral industry.
- Biotechnology is playing a key role in the generation of new varieties with the change in color, scent, size, and flower through gene manipulation technique.
- Through biotechnological approaches such as tissue culture and micro propagation techniques, polyploidy induction, mutation, breeding, and genetic engineering.
- More than 50 ornamental plants are now being transformed using Agrobacterium-mediated transformation and particle bombardment techniques.

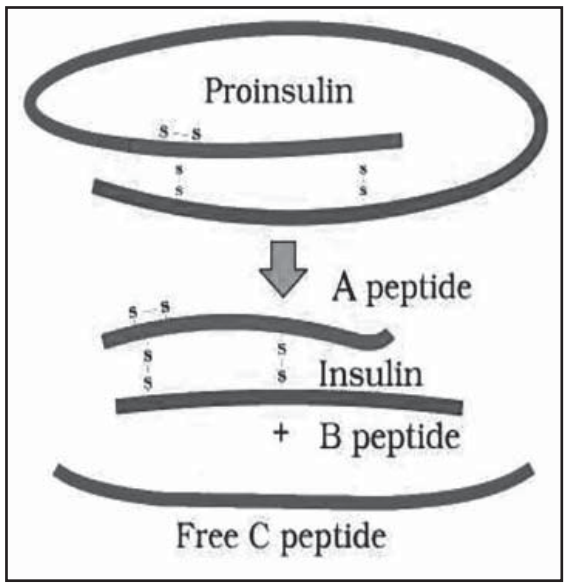
Health

Medical biotechnology is the use of living cells and other cell materials to better the health of humans. Primarily, it is used for finding cures as well as getting rid of and preventing diseases.

It heavily involves the study of DNA (Deoxyribonucleic acid) to get to know how to manipulate the genetic makeup of cells to increase the production of beneficial characteristics that humans might find useful, such as the production of insulin.

Genetically Engineered Insulin

- Insulin consists of two short polypeptide chains: chain A and chain B that are linked together by disulphide bridges.
- In mammals, including humans, insulin is synthesised as a pro-hormone (like a pro-enzyme, the pro-hormone also needs to be processed before it becomes a fully mature and functional hormone) which contains an extra stretch called the C peptide.
- This C peptide is not present in the mature insulin and is removed during maturation into insulin.
- The main challenge for production of insulin using rDNA techniques was getting insulin assembled into a mature form.
- In 1983, Eli Lilly an American company prepared two DNA sequences corresponding to A and B, chains of human insulin and introduced them in plasmids of E. coli to produce insulin chains.
- Chains A and B were produced separately, extracted and combined by creating disulfide bonds to form human insulin.



Gene Therapy

- Gene therapy is a collection of methods that allows correction of a gene defect that has been diagnosed in a child/embryo.
- Here genes are inserted into a person's cells and tissues to treat a disease.
- Correction of a genetic defect involves delivery of a normal gene into the individual or embryo to take over the function of and compensate for the non-functional gene.
- The first clinical gene therapy was given in 1990 to a 4-year old girl with adenosine deaminase (ADA) deficiency.
- In some children ADA deficiency can be cured by bone marrow transplantation; in others it can be treated by enzyme replacement therapy, in which functional ADA is given to the patient by injection.
- But the problem with both of these approaches that they are not completely curative.
- As a first step towards gene therapy, lymphocytes from the blood of the patient are grown in a culture outside the body.
- A functional ADA cDNA (using a retroviral vector) is then introduced into these lymphocytes, which are subsequently returned to the patient.
- However, as these cells are not immortal, the patient requires periodic infusion of such genetically engineered lymphocytes.
- However, if the gene isolate from marrow cells producing ADA is introduced into cells at early embryonic stages, it could be a permanent cure.

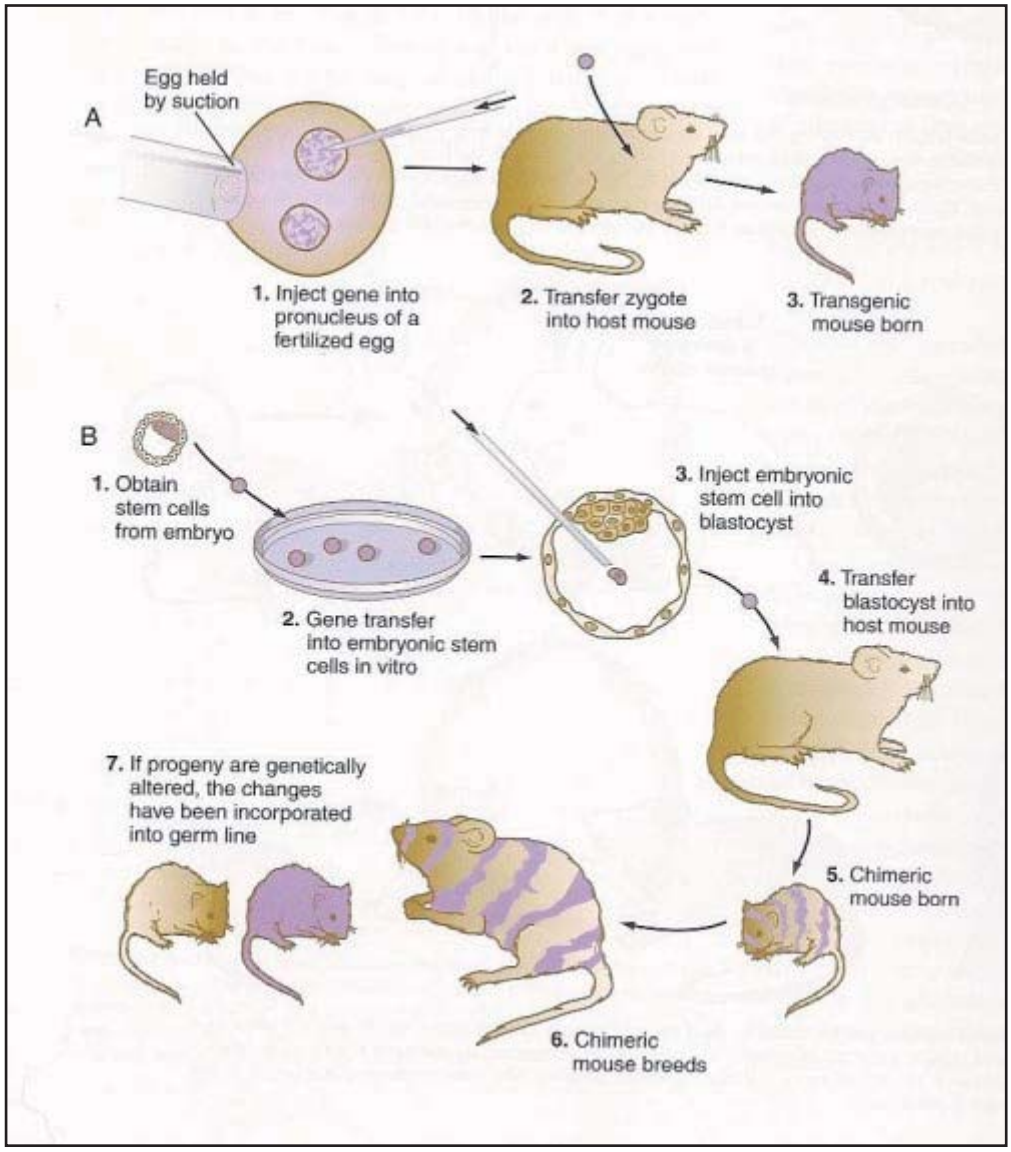
Molecular Diagnosis

- Using conventional methods of diagnosis (serum and urine analysis, etc.) early detection is not possible.
- Recombinant DNA technology, Polymerase Chain Reaction (PCR) and Enzyme Linked Immuno-sorbent Assay (ELISA) are some of the techniques that serve the purpose of early diagnosis.
- Presence of a pathogen (bacteria, viruses, etc.) is normally suspected only when the pathogen has produced a disease symptom.
- By this time the concentration of pathogen is already very high in the body.
- However, very low concentration of a bacteria or virus (at a time when the symptoms of the disease are not yet visible) can be detected by amplification of their nucleic acid by PCR.

ELISA is based on the principle of antigen-antibody interaction. Infection by pathogen can be detected by the presence of antigens (proteins, glycoproteins, etc.) or by detecting the antibodies synthesized against the pathogen.

Transgenic Animals

- Animals that have had their DNA manipulated to possess and express an extra (foreign) gene are known as transgenic animals.
- Transgenic rats, rabbits, pigs, sheep, cows and fish have been produced, although over 95 per cent of all existing transgenic animals are mice.



Reasons for the creation of transgenic animals:

- **Normal physiology and development:**
  - Transgenic animals can be specifically designed to allow the study of how genes are regulated, and how they affect the normal functions of the body and its development.
  - e.g., study of complex factors involved in growth such as insulin-like growth factor.
  - By introducing genes from other species that alter the formation of this factor and studying the biological effects that result, information is obtained about the biological role of the factor in the body.
- **Study of disease:**
  - Many transgenic animals are designed to increase our understanding of how genes contribute to the development of disease.
  - These are specially made to serve as models for human diseases so that investigation of new treatments for diseases is made possible.

- Today transgenic models exist for many human diseases such as cancer, cystic fibrosis, rheumatoid arthritis and Alzheimer's.

- **Biological products:**
  - Transgenic animals that produce useful biological products can be created by the introduction of the portion of DNA (or genes) which codes for a particular product such as human protein (α-1-antitrypsin) used to treat emphysema.
  - Similar attempts are being made for treatment of phenylketonuria (PKU) and cystic fibrosis.
  - In 1997, the first transgenic cow, Rosie, produced human protein-enriched milk (2.4 grams per litre).
  - The milk contained the human alpha-lactalbumin and was nutritionally a more balanced product for human babies than natural cow-milk.

- **Chemical safety testing:**
  - The procedure is the same as that used for testing toxicity of drugs.
  - Transgenic animals are made that carry genes which make them more sensitive to toxic substances than non-transgenic animals.
  - They are then exposed to the toxic substances and the effects studied.
  - Toxicity testing in such animals will allow us to obtain results in less time.

Ethical Issues

The manipulation of living organisms by the human race cannot go on any further, without regulation. Some ethical standards are required to evaluate the morality of all human activities that might help or harm living organisms.

Genetic modification of organisms can have unpredictable results when such organisms are introduced into the ecosystem.

- Are animals that combine species an unethical alteration of the natural order of the universe?
- Is it unethical to modify an animal's genetic make-up for a specific purpose, without knowing in advance if there will be any side-effects that will cause suffering to the animal?
- Does 'creating' animals by genetic engineering amount to treat the animals entirely as commodities?
- God laid down the structure of creation and any tampering with it is sinful.

Therefore, the Indian Government has set up organisations such as GEAC (Genetic Engineering Approval Committee), which will make decisions regarding the validity of GM research and the safety of introducing GM-organisms for public services.

Genome sequencing

- **Genome:** It is an organism's complete set of DNA, including all of its genes.
- Each genome contains all of the information needed to build and maintain that organism. In humans, a copy of the entire genome—more than 3 billion DNA base pairs—is contained in all cells that have a nucleus.
- **Genome sequencing:** It is figuring out the order of DNA nucleotides, or bases, in a genome—the order of As, Cs, Gs, and Ts that make up an organism's DNA. The human genome is made up of over 3 billion of these genetic letters.
- Genome sequencing (GS) covers the entire genome, including the noncoding regions.
  - Sequencing the genome doesn't immediately lay open the genetic information of an entire species. Even with a rough draft of the human genome sequence in hand, much work remains to be done. Scientists still have to translate those strings of letters into an understanding of how the genome works.

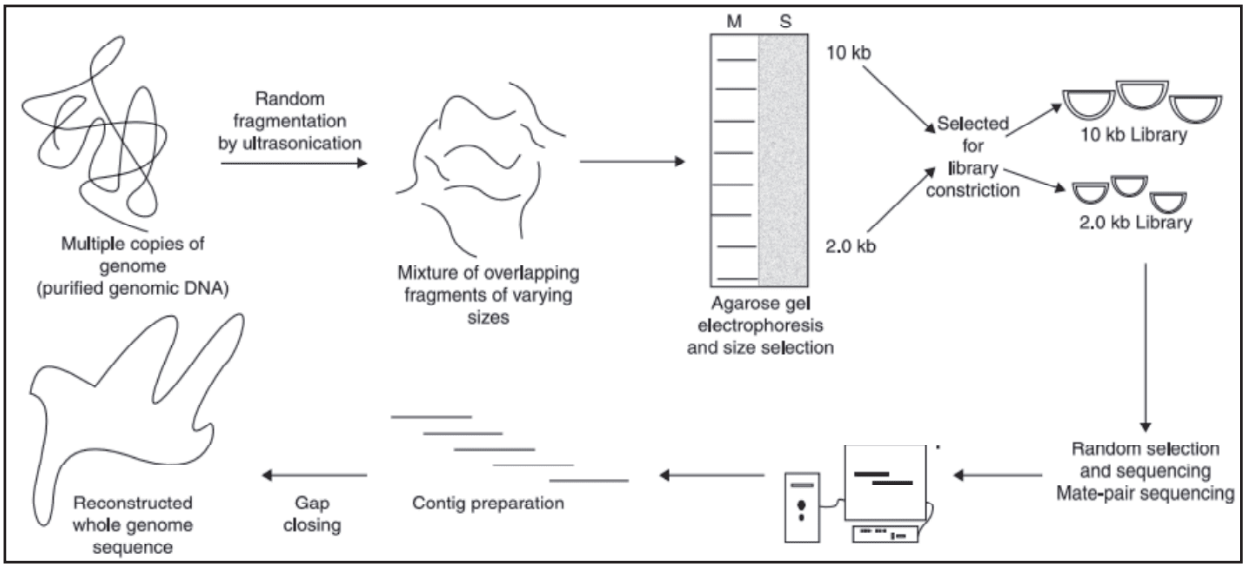
Methods for Genome Sequencing

Sanger sequencing

In Sanger sequencing, the target DNA is copied many times, making fragments of different lengths. Fluorescent "chain terminator" nucleotides mark the ends of the fragments and allow the sequence to be determined. Example- Human Genome Project.

Next-generation sequencing

- Next-generation sequencing (NGS) techniques are new, large-scale approaches that increase the speed and reduce the cost of DNA sequencing.
- Features of NGS:
  - **Highly parallel:** many sequencing reactions take place at the same time
  - **Micro scale:** reactions are tiny and many can be done at once on a chip
  - **Fast:** because reactions are done in parallel, results are ready much faster
  - **Low-cost:** sequencing a genome is cheaper than with Sanger sequencing
  - **Shorter length:** reads typically range from 505050 -700700700 nucleotides in length



Application of DNA sequencing

- DNA sequencing may be used to determine the sequence of individual genes, larger genetic regions (i.e. clusters of genes or operons), full chromosomes, or entire genomes of any organism.
- DNA sequencing is also the most efficient way to indirectly sequence RNA or proteins (via their open reading frames).
- In fact, DNA sequencing has become a key technology in many areas of biology and other sciences such as medicine, forensics, and anthropology.

Molecular biology

- Sequencing is used in molecular biology to study genomes and the proteins they encode. Information obtained using sequencing allows researchers to identify changes in genes, associations with diseases and phenotypes, and identify potential drug targets.

Evolutionary biology

- Since DNA is an informative macromolecule in terms of transmission from one generation to another, DNA sequencing is used in evolutionary biology to study how different organisms are related and how they evolved. In February 2021, scientists reported, for the first time, the sequencing of DNA from animal remains, a mammoth in this instance, over a million years old, the oldest DNA sequenced to date.

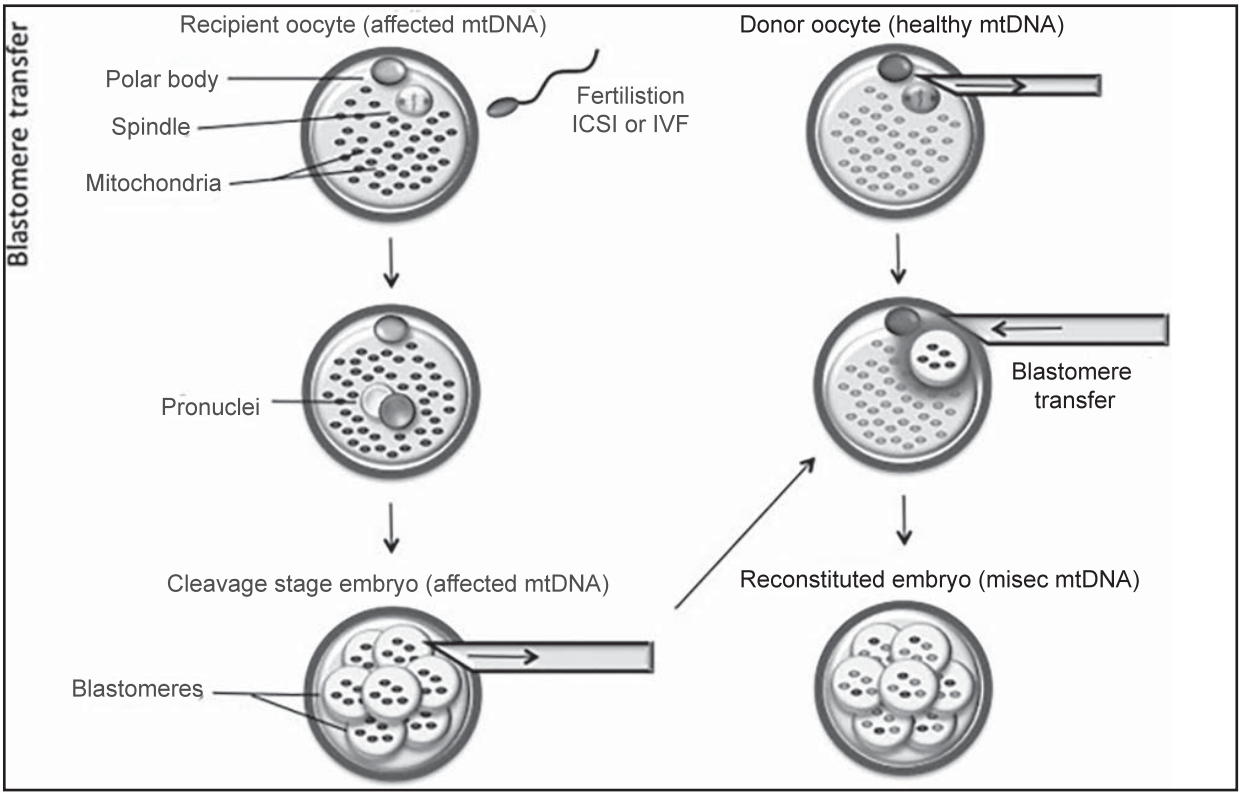
Virology

- As most viruses are too small to be seen by a light microscope, sequencing is one of the main tools in virology to identify and study the virus. Viral genomes can be based in DNA or RNA. RNA viruses are more time-sensitive for genome sequencing, as they degrade faster in clinical samples. Traditional Sanger sequencing and next-generation sequencing are used to sequence viruses in basic and clinical research, as well as for the diagnosis of emerging viral infections, molecular epidemiology of viral pathogens, and drug-resistance testing.

Forensic investigation

- DNA sequencing may be used along with DNA profiling methods for forensic identification and paternity testing. DNA testing has evolved tremendously in the last few decades to ultimately link a DNA print to what is under investigation. The DNA patterns in fingerprint, saliva, hair follicles, etc. uniquely separate each living organism from another. Testing DNA is a technique which can detect specific genomes in a DNA strand to produce a unique and individualized pattern.

Pronuclear Transfer



- In pronuclear transfer, the mother's egg is first fertilized with the father's sperm, producing a zygote. The pronuclei of the egg and sperm are then removed from the zygote and inserted into a donor egg that has been fertilized and has had its own nucleus removed (a pronucleus is the nucleus of the egg

or sperm at the stage of fertilization prior to nucleus fusion). The zygote derived from the donor egg is then implanted into the mother's uterus.

- Mitochondrial DNA (mtDNA) mutations are a common cause of genetic disease with pathogenic mtDNA mutations being detected in approximately 1 in 250 live births. MtDNA is transmitted maternally and it has been proposed that pronuclear transfer techniques may be an approach to prevent the transmission of human mtDNA disease.

UPSC CSE PRELIMS, 2020

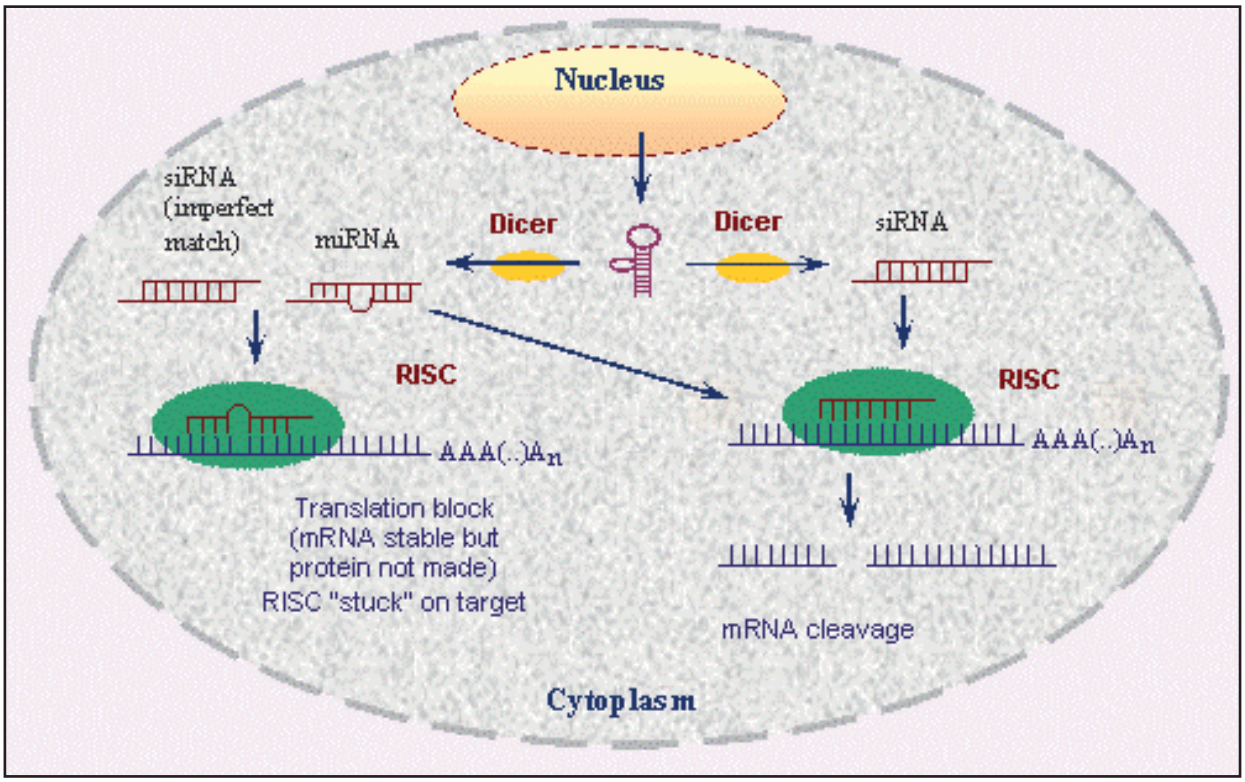
Q1: In the context of recent advances in human reproductive technology, 'Pronuclear Transfer" used for

(a) Fertilization of egg in vitro by the donor sperm
(b) Genetic modification of sperm producing cells
(c) Development of stem cells into functional embryos
(d) Prevention of mitochondrial diseases in offspring

Correct Option: (d)

RNA interference (RNAi) technology

- RNA interference (RNAi) is the biological process of mRNA degradation induced by complementary sequences double-stranded (ds) small interfering RNAs (siRNA) and suppression of target gene expression.



- Exogenous siRNAs (perfectly paired dsRNAs of ~21–25 nt in length) play an important role in host defense against RNA viruses and in transcriptional and post-transcriptional gene regulation in plants and other eukaryotes.

- Using RNAi technology by transfecting synthetic siRNAs into eukaryotic cells to silence genes has become an indispensable tool to investigate gene functions, and siRNA-based therapy is being developed to knockdown genes implicated in diseases.
- Other examples of RNAi technology include method of producing highly potent and purified siRNAs directly from Escherichiacoli cells, based on an unexpected discovery that ectopic expression of p19, a plant viral siRNA-binding protein, stabilizes a cryptic siRNA-like RNA species in bacteria.

UPSC CSE PRELIMS, 2019

Q1: RNA interference (RNAi)' technology has gained popularity in the last few years. Why?

1. It is used in developing gene silencing therapies.
2. It can be used in developing therapies for the treatment of cancer.
3. It can be used to develop hormone replacement therapies.
4. It can be used to produce crop plants that are resistant to viral pathogens.

Select the correct answer using the code given below:

(a) 1, 2 and 4 (b) 2 and 3
(c) 1 and 3 (d) 1 and 4 only

Correct Option: (a)

- **Embryo transfer technology**
  - Embryo transfer technology (ETT) is a technique by which embryos are collected from a donor female and transferred to recipient females that act as surrogate mothers for the rest of the pregnancy.
  - Embryo transfer serves at least three purposes: to support a genetic line which has difficulty reproducing, to develop disease free animals or to manipulate genetics.
  - Embryo transfer is performed by transplanting embryos from a donor animal into a pseudo pregnant recipient. It is necessary to generate a number of recipients at the same time by bringing multiple females into oestrus at the same time.

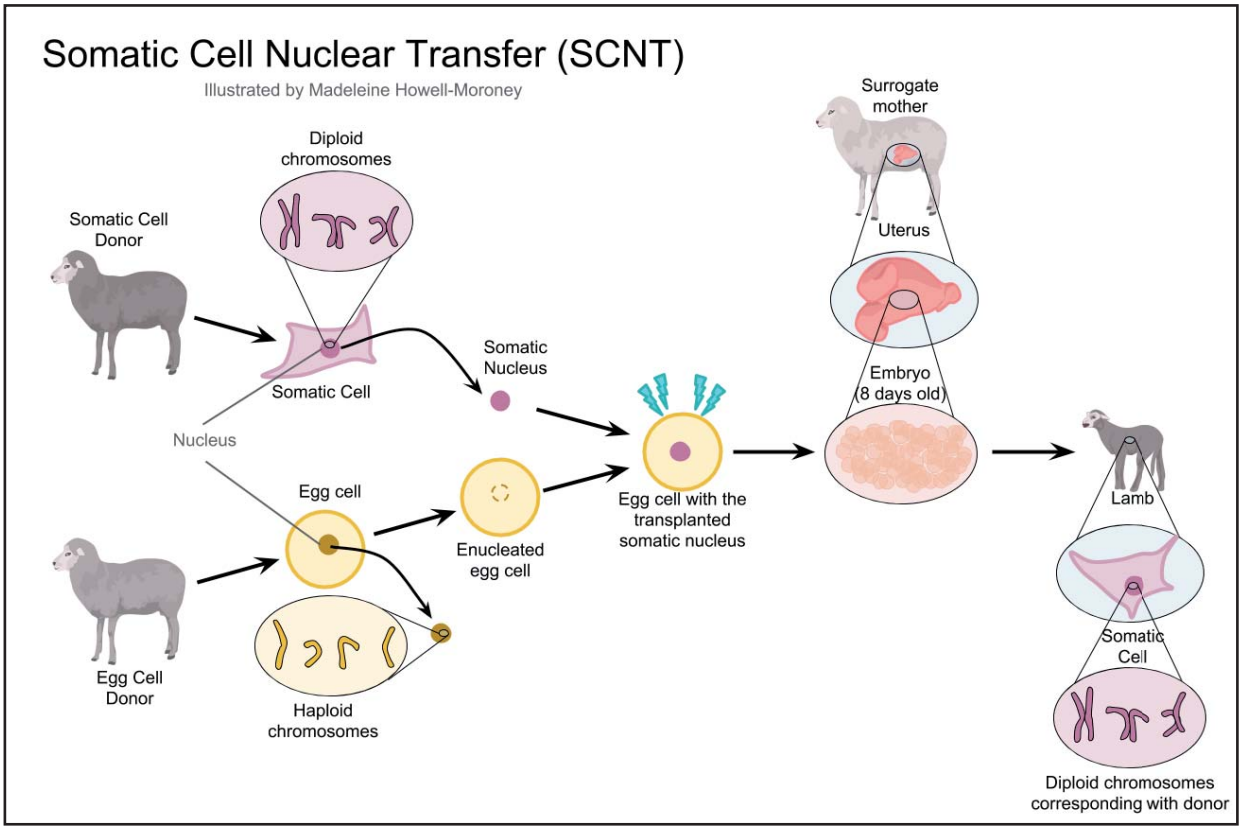
Application of Embryo Transfer Technology

- **In humans for treating infertility**
  - IVF and embryo transfer is needed in cases where natural fertilization is not an option or has difficulty occurring. There are many reasons for embryo transfer, including:
  - Ovulation disorders: If ovulation is infrequent, fewer eggs are available for successful fertilization.
  - Damage to Fallopian tubes: The Fallopian tubes are the passageway through which the embryos travel to reach the uterus. If the tubes become damaged or scarred, it is difficult for fertilized eggs to safely reach the womb.
  - Endometriosis: When tissue from the uterus implants and grows outside of the uterus. This can affect how the female reproductive system works.
  - Premature ovarian failure: If the ovaries fail, they do not produce normal amounts of oestrogen or release eggs regularly.
  - Uterine fibroids: Fibroids are small, benign tumours on the walls of the uterus. They can interfere with an egg's ability to plant itself in the uterus, preventing pregnancy.
  - Genetic disorders: Some genetic disorders are known to prevent pregnancy from occurring.

- Impaired sperm production: In men, low sperm production, poor movement of the sperm, damage to the testes, or semen abnormalities are all reasons natural fertilization may fail.

Somatic Cell Nuclear Transfer Technology

- Somatic cell nuclear transfer (SCNT), technique in which the nucleus of a somatic (body) cell is transferred to the cytoplasm of an enucleated egg (an egg that has had its own nucleus removed). Once inside the egg, the somatic nucleus is reprogrammed by egg cytoplasmic factors to become a zygote (fertilized egg) nucleus. The egg is allowed to develop to the blastocyst stage, at which point a culture of embryonic stem cells (ESCs) can be created from the inner cell mass of the blastocyst. Mouse, monkey, and human ESCs have been made using SCNT; human ESCs have potential applications in both medicine and research.



Some recent techniques in genetic engineering

Genome-scale editing tools	Short description	Applications and bacterial strains
CRISPR-Cas9	The complex of Cas9 protein and sgRNA can bind to the target DNA sequence; Cas9 protein subsequently cleaves the target DNA sequence and DNA can be repaired using the DNA mismatch repair system	Genome editing for central metabolic pathways to increase β-carotene production to 2.0 g/L in E. coli.
MAGE	Rapid, automated, and high-throughput multiplex genome engineering.	The production of isoprenoid lycopene increases by five-fold in *E. coli*.

Genome-scale engineering tools	Short description	Applications and bacterial strains
RBS (ribosome binding site) engineering	Modified 16rRNA binding sequence in mRNA to regulating gene expression at post-transcription level.	The production L-tyrosine increased to 3.0 g/L in *E. coli*. 3-methyl-3-buten-1-ol production is increased by 60% in *E. coli*.
CRISPRi	CRISPRi uses dCas9, which has no endonucleolytic function, and the binding of dCas9 to a promoter by sgRNA inhibits the binding of RNA polymerases.	7.15–11.72 g/L of p(3HB-co-4HB) is obtained by modulating the sad gene in *E. coli*.

UPSC CSE PRELIMS, 2019

Q2: What is Cas9 protein that is often mentioned in news?

(a) A molecular scissors used in targeted gene editing

(b) A biosensor used in the accurate detection of pathogens in patients

(c) A gene that makes plants pest-resistant

(d) A herbicidal substance synthesized in genetically modified crops

Correct Option: (a)

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