Biotechnology - 1

1. Recombinant DNA (rDNA) Technology

1.1. Introduction to rDNA Technology

- Recombinant DNA is an **artificially made DNA strand** that is **formed by the combination of two or more gene sequences.**
- rDNA technology involves using enzymes and various laboratory techniques to manipulate and isolate DNA segments of interest.
- The method **can be used to combine (or splice) DNA** from different species or to create genes with new functions.

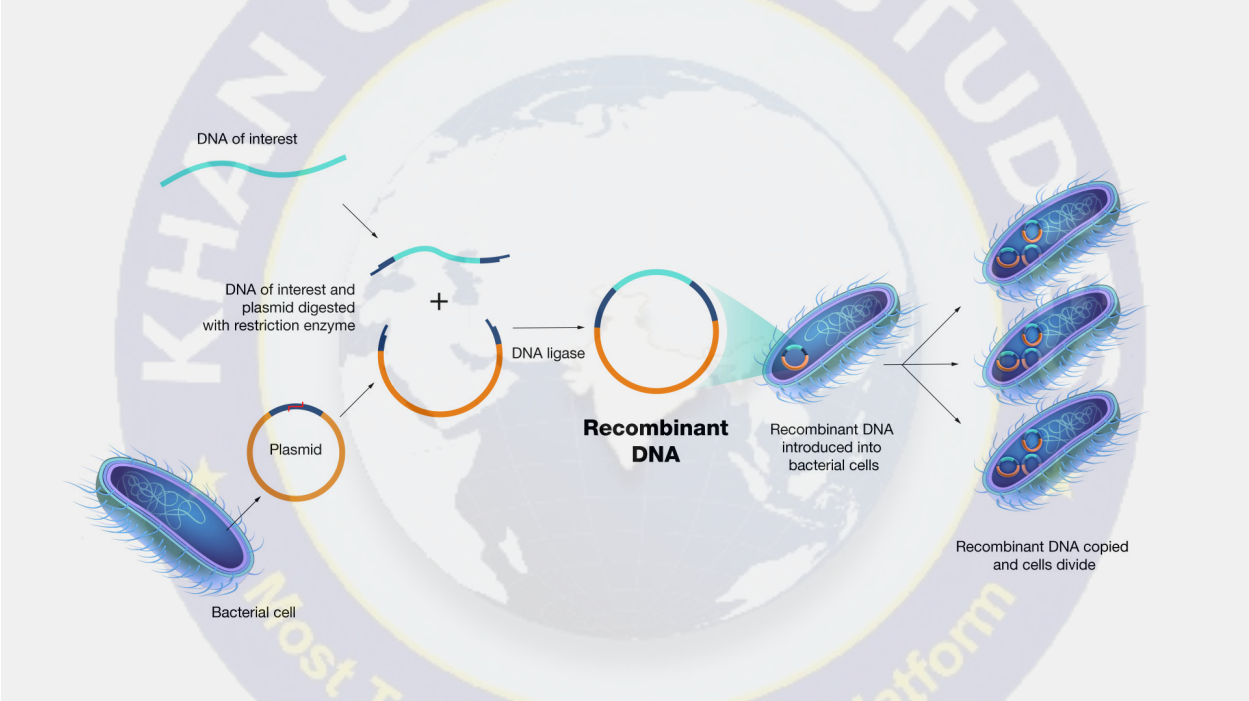

Figure.1. Recombinant DNA Technology

- rDNA is possible because the DNA of all organisms share the same chemical structure but different nucleotide sequences within the overall identical structure.
- In 1973, **Stanley N. Cohen and Herbert W. Boyer** became the first to insert recombined genes into bacterial cells.

1.2. Tools of rDNA Technology

The key tools of rDNA technology are restriction enzymes, polymerase enzymes, ligases, vectors and the host organism.

Restriction Enzymes

- A restriction enzyme is an enzyme that cleaves DNA into fragments at or near specific recognition sites within molecules known as restriction sites.
- 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.
- The restriction endonucleases are **sequence-specific** which is usually **palindrome sequences and cut the DNA at specific points.**
- 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. Restriction endonucleases are used to form **'recombinant' molecules of DNA,** which are composed of DNA from different sources/genomes.
- When cut by the same restriction enzyme, the resultant DNA fragments have the same kind of 'sticky-ends' and these can be joined together (end-to-end) using DNA ligases.

Separation and isolation of DNA fragments

- 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.**
- The most commonly used matrix is agarose which is a natural polymer extracted from sea weeds.
- The DNA fragments separate (resolve) according to their size through the sieving effect provided by the agarose gel.

Cloning Vectors

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 sequence is also responsible for controlling the copy number of the linked DNA.
- **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. Transformation is a procedure through which a piece of DNA is introduced in a host bacterium.
- **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. The ligation of alien DNA is carried out at a restriction site present in one of the two antibiotic resistance genes.
- **Vectors for cloning genes in plants and animals:** 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).

Competent Host (For Transformation with Recombinant DNA)

- In order to force bacteria to take up the plasmid, the bacterial cells must first be made 'competent' to take up DNA. This is done by treating them with a specific concentration of a divalent cation, such as calcium, which increases the efficiency with which DNA enters the bacterium through pores in its cell wall.

- Recombinant DNA can then be forced into such cells by incubating the cells with recombinant DNA on ice, followed by placing them briefly at 42 degree C (heat shock), and then putting them back on ice. This enables the bacteria to take up the recombinant DNA.
- This is not the only way to introduce alien DNA into host cells. In a method known as micro-injection, recombinant DNA is directly injected into the nucleus of an animal cell.
- In another method, suitable for plants, cells are bombarded with high velocity micro-particles of gold or tungsten coated with DNA in a method known as biolistics or gene gun.

1.3. Applications of Recombinant DNA Technology

- **Medicine:** genetic engineering has been used to mass-produce insulin, human growth hormones, follistim (for treating infertility), human albumin, monoclonal antibodies, antihemophilic factors, vaccines, and many other drugs.
- **Research:** organisms are genetically engineered to discover the functions of certain genes.
- **Industrial applications:** include transforming microorganisms such as bacteria or yeast. Mass quantities of the protein can be produced by growing the transformed organism in bioreactors using fermentation, then purifying the protein.
- **Agriculture:** Genetic engineering is also used in agriculture to create genetically-modified crops or genetically-modified organisms. Crops containing genes which will enable them to withstand biotic and abiotic stresses have been developed. Crops with a number of desirable traits have also been developed such as Golden Rice, FlavrSavr Tomato etc.
- **Environment:** Organisms have been known to help in the biodegradation of waste materials. However, there are some materials like plastics which cannot be degraded by them. To help such causes, genetic research has produced modified microorganisms which not only have the capability of doing this but are also more efficient due to the speedy process. They are used in situations which may cause severe damage to the planet earth like oil spills.

2. Transgenic Bacteria

2.1. What are Transgenic Bacteria?

- Transgenic bacteria are those bacteria that **contain genes from other species.**
- They are genetically modified microorganisms (GMOs) that have been **developed through engineering of the genome.**
- Transgenic bacteria are widely used in agriculture, medicine, and biotechnology as well as to produce food additives, such as vitamins, enzymes, antibiotics, and hormones.
- In 1973**,** scientists Herbert Boyer and Stanley Cohen created the **first genetically engineered organism — E. coli** bacteria that had the gene for resistance to the antibiotic tetracycline transferred into it.

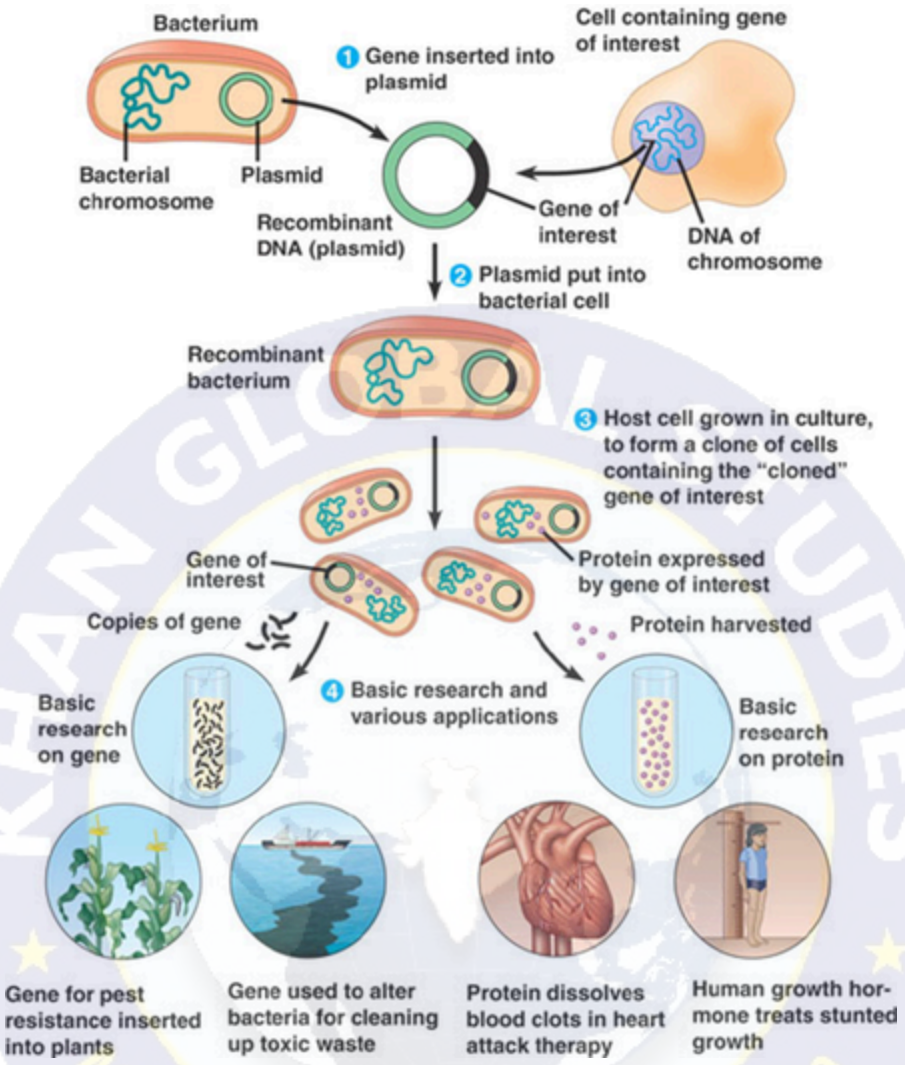

Figure.2. Procedure for Creating Transgenic Bacteria

2.2. Procedure for Creating Transgenic Bacteria

1. Take a bacterial plasmid.
2. Cut out the portion of the gene of interest using a **restriction enzyme.**
3. Same restriction enzyme is used to cut the bacterial plasmid.
4. Use DNA Ligase to **incorporate the desired portion of DNA** into the plasmid. The incorporated portion is called a **transgene.**
5. Use a vector which has a high multiplication rate to transport the transgene into the desired organism.
6. The transgene replicates within the host cell or incorporates itself into the host DNA, and commands for the production of required protein synthesis.

2.3. Application of Transgenic Bacteria

Medicine

- Production of **human insulin** to treat diabetes.

    - Before 1983, insulin was provided through animal resources that carried purified insulin similar to human insulin. The process was labour intensive, expensive and had side effects like allergic reactions.
- Other medicines produced by using transgenic bacteria include
    - **clotting factors** to treat haemophilia,
    - **human growth hormone** to treat various forms of dwarfism,
    - **interferon** to treat some cancers,
    - **erythropoietin** for anaemic patients, and
    - **tissue plasminogen activator** which dissolves blood clots.

Environment

- For **bioremediation** (where the bacteria are used to convert pollutants into a less toxic form).
- Transgenic bacteria have also been developed **to leach** copper from ore, clean up mercury pollution and detect arsenic in drinking water.
- They are used as **biofilters** in industries and can remove sulphur from coal before burning it.
- For cleaning up of oil spills by digesting hydrocarbons of crude oil.

Agriculture

- To **increase crop production** or to allow crops to be grown outside their original habitat.
- Application of *Bacillus thuringiensis* (Bt) and other bacteria can **help protect crops from insect infestation** and plant diseases.
- To increase **environmental protection** through reduced use of pesticides.
- To improve nutrition for people.
- **Increase farm profitability** through reduced cost and new product opportunities.
- Anti-freezing bacteria is sprayed onto the plants to prevent ice formation.
- Herbicides will have no effect on the transgenic crop plants and only destroy the weeds.
- Allow for crops to have a higher self life.

Mining

- To extract copper, uranium, and gold from low-grade sources.
- To **improve the bioleaching capabilities** of metals.

Industry

- Genetically modified bacteria are used to produce large amounts of proteins for industrial use.

Food

- Food products from genetically modified bacteria **include alpha-amylase,** which converts starch to simple sugars, chymosin, which clots milk protein for cheese making, and pectinesterase, which improves fruit juice clarity.

2.4. Ethical Issues and Concerns

- **Safety Concerns:** Little is known about the adaptation potential of the Transgenic Bacteria.
- **Natural VS Unnatural:** Genetic Engineering causes modifications that are not naturally possible in nature. There are unknown consequences to altering the natural state of an organism through foreign gene expression.

- **Legitimacy:** It is impossible to determine consequences linked to new scientific knowledge which could be harmful to the planet.
- **Health risks:** Potential health risks to humans include the possibility of exposure to new allergens in genetically modified foods, as well as the transfer of antibiotic-resistant genes to gut flora.
- **Patent:** The modification/usage of living organisms for public services (as food and medicine sources, for example) has also created problems with patents granted for the same.

3. Transgenic Animals

3.1. What are Transgenic Animals?

- **Animals that have had their DNA manipulated** to possess and express an extra (foreign) gene are known as transgenic animals.
- Examples include transgenic rats, rabbits, pigs, sheep, cows and fish.

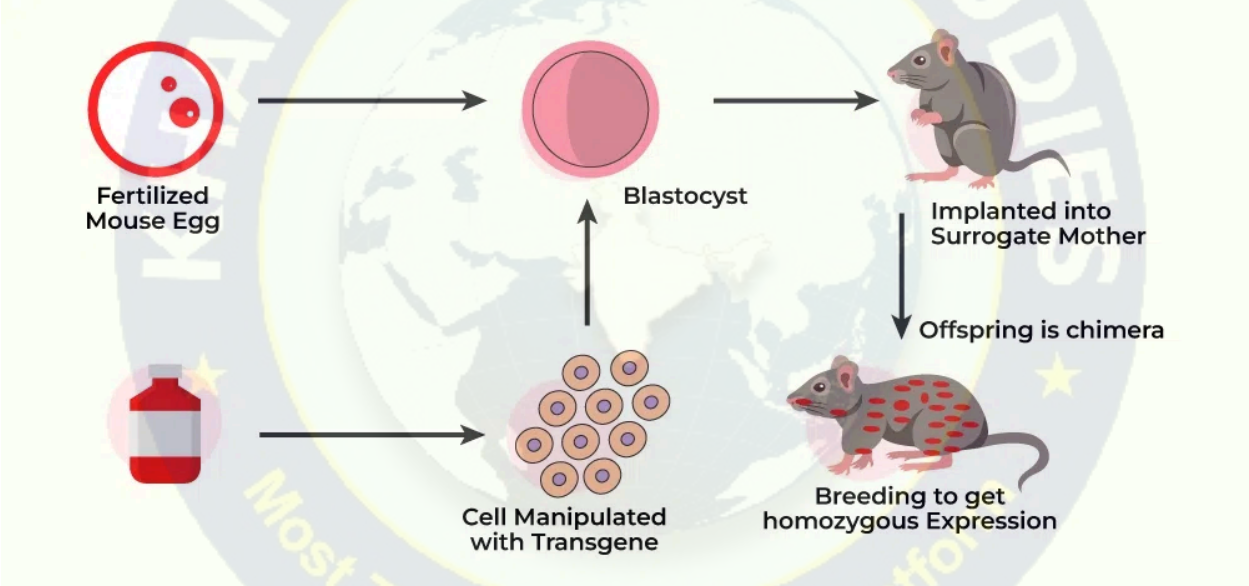

Figure.3. Procedure for Creating Transgenic Animals

3.2. Procedure for Creating Transgenic Animals

The most common method for producing transgenic animals is **gene transfer by DNA microinjection,** which involves the following steps:

- **DNA containing the desired transgene is identified** and cloned before insertion into the animal host.
- The host animals are **induced to superovulate** and their eggs are collected.
- The eggs are fertilised in a laboratory dish.
- Using a fine, hollow needle, a **solution of DNA containing the transgene is injected** into the male pronucleus of the fertilised egg before it fuses with the female pronucleus.
- The transgenic **embryos are grown in cell culture** and then **implanted into the uterus of a surrogate mother,** where they complete their development.
- Some of the baby animals will have the transgene stably integrated into their chromosomes. In others the process fails and the transgene is lost.

- Those that received the transgene and maintained it stably are called founder animals.

3.3. Applications of Transgenic Animals

Transgenic Animals in Agriculture

- Practical applications of transgenesis in livestock production include
    - enhanced prolificacy and reproductive performance,
    - increased feed utilisation and growth rate,
    - improved carcass composition,
    - improved milk production and composition, and
    - modification of hair or fibre.

Control of Vectors

- British company Oxitec has created genetically modified male mosquitoes that carry a **"self-limiting gene".** When they are released into the wild and mate with females their offspring do not reach adulthood, so they do not contribute to the spread of the Zika virus.
- Other researchers are looking at using genetic modification to curb the spread of malaria.

Advance Human Health

- Transgenic animals can improve human health by **producing novel replacement proteins,** drugs, vaccines and tissues for the treatment and prevention of human disease.

Enhanced Nutrition

- Transgenesis allows improvement of nutrients in animal products, including their quantity, the quality of the whole food, and specific nutritional composition.
- For example: Enhancing the omega-3 fatty acid in fish consumed by humans may contribute to a decreased occurrence of coronary heart disease.

Molecular Farming

- Molecular farming is also known as 'pharming', in which biopharmaceuticals are manufactured in transgenic animals.
- More than recombinant cell cultures, **animals are attractive bioreactors.** They have the correct metabolic pathways, are reproducible, easily maintained, and do not require expensive infrastructure.

Reduced Environmental Impact

- Genetically engineered animals can contribute to improving the environment and human health by consuming fewer resources and producing less waste.
- For example: Through genetic engineering, scientists have developed **Enviro-Pig.** This animal emits 30 to 60 percent **less phosphorus** than traditional pigs.
- Gene-modifying techniques also offer a **possibility to reduce the greenhouse gas emissions** from livestock.

Xenotransplantation

- A medical application of genetic modification aims to improve the suitability of animal organs for xenotransplantation.
- For example: Possibility of growing transplantable human organs in pigs.

Enhance Food Production and Quality

- Animals that are genetically engineered have improved food production capabilities, enabling them to help meet the global demand for more efficient, higher quality and lower-cost sources of food.

Improve Industrial Products

- Genetic engineering can produce high-value industrial products, such as spider silk, for both medical and defense applications.

3.4. Ethical Issues and Concerns

Ethical Issues

- Moral status of animals, animal rights and animal welfare.
- **Fear of transferring allergens** from genetically modified food to sensitive humans and animals.
- Transgenic animals may bring about **changes in natural evolutionary patterns.**
- Use of animals in biotechnology is **cruelty towards animals** which causes great suffering to them.
- Transfer of human genes into animals is a great ethnic threat for humanness.
- Where transgenic animals are concerned, it remains important to 'expect the unexpected'. Even with the best information, and the best of intentions, it is not possible to predict with certainty how they will impact upon the experimental animal.
- Many of the embryos that undergo genetic engineering procedures do not survive, and of those that do survive only a small proportion (between 1% to 30%) carry the genetic alteration of interest.

Environmental Impact

- Use of genetically engineered animals could harm the environment indirectly by changing demand for feed, number of animals used, or amount of resulting waste, and by the **effects of wastes containing novel gene products** on microbial and insect ecologies.
- Transgenic farm animals may also have harmful environmental effects if they escape or are released from captivity and mate with wild individuals of the same species.
- The risk of an escaped transgenic animal becoming established in the natural environment depends on its ability to survive and reproduce in the wild.

Patenting

- Genetic engineering also brings with it concerns over intellectual property, and patenting of created animals and/or the techniques used to create them.
- Preserving intellectual property can breed a culture of confidentiality within the scientific community, which in turn limits data and animal sharing.
- Such limits to data and animal sharing may create situations in which there is unnecessary duplication of genetically engineered animal lines, thereby challenging the principle of Reduction.

Other Concerns

- Proteins designed to produce a pharmaceutical product in the animal's milk might find their way to other parts of the animal's body, possibly causing adverse effects.
- Interfering with the genome by inserting or removing fragments of DNA **may result in alteration of the animal's normal genetic homeostasis.**

- Studies have revealed that cloned mammals may suffer from developmental abnormalities, including extended gestation, large birth weight and effects in organs and tissues.
- If the use of cloning became more widespread in the animal breeding industry, there is a danger that the level of genetic diversity could fall to an unacceptable degree.

5. Human Genome Project (HGP)

5.1. What was the Human Genome Project?

- The Human Genome Project was a large, well-organized, and highly collaborative international effort that generated the first sequence of the human genome and that of several additional well-studied organisms.
- It was **carried out from 1990–2003**.
- The idea of the HGP was **first publicly advocated by Renato Dulbecco** in an article published in 1984, in which he argued that knowing the human genome sequence would facilitate an understanding of cancer.

5.2. Whose DNA was Sequenced?

- The sequence of the human genome generated by the HGP reflects a patchwork from multiple people whose identities were intentionally made anonymous to protect their privacy.
- The project researchers used a process to recruit volunteers, acquire their informed consent, and collect their blood samples.

5.3. Who carried out the Human Genome Project?

- HGP has been completed with the participation of an international consortium of thousands of researchers.
- In the United States, the researchers were funded by the Department of Energy and the National Institutes of Health, which created the Office for Human Genome Research in 1988 (Renamed the National Human Genome Research Institute in 1997).
- The sequencing of the human genome involved researchers from 20 separate universities and research centers across the United States, United Kingdom, France, Germany, Japan and China.
- The groups in these countries became known as the **International Human Genome Sequencing Consortium.**

5.4. Significance of Human Genome Project

- HGP has transformed biology through its integrated big science approach to deciphering a reference human genome sequence.
- It established an open approach to data sharing and open-source software, thereby making the data resulting from the project accessible to all.
- The genome sequences of microbes, plants and animals have revolutionized many fields of science, including microbiology, virology, infectious disease and plant biology.
- Moreover, deeper knowledge of human sequence variation has begun to alter the practice of medicine.

- HGP has inspired subsequent large-scale data acquisition initiatives such as the International HapMap Project, 1000 Genomes, and The Cancer Genome Atlas, as well as the Human Brain Project and the emerging Human Proteome Project.

5.5. Genome India Project

Taking inspiration from the Human Genome Project, the **Department of Biotechnology** (DBT) initiated the "Genome India Project" (GIP) **on 3rd January 2020.**

Aim

- The GIP aims **to collect 10,000 genetic samples** from citizens across India, to build a reference genome.
- This project is **led by the Centre for Brain Research** at Bengaluru-based Indian Institute of Science, which acts as the central coordinator between a collaboration of 20 leading institutions.

Method

- For conducting the project, investigators in hospitals will lead the data collection through a simple blood test from participants and the information will be added to biobanks.
- Some of the priority areas are Precision health, Rare genetic disorders, Mutation spectrum of genetic and complex diseases in the Indian population, Genetic Epidemiology of Multifactorial Lifestyle Diseases, and Translational Research.

Significance

- This initiative reflects India's progress in gene therapies and precision medicine, and its movement towards emerging next-generation medicine which yields the possibilities for greater customization, safety, and earlier detection.
- The initiative would help lay the foundation of personalized healthcare for a very large group of persons on the planet.