

# TARGET PRELIMS 2024

## BOOKLET-9; S&T-9

### BIOTECHNOLOGY

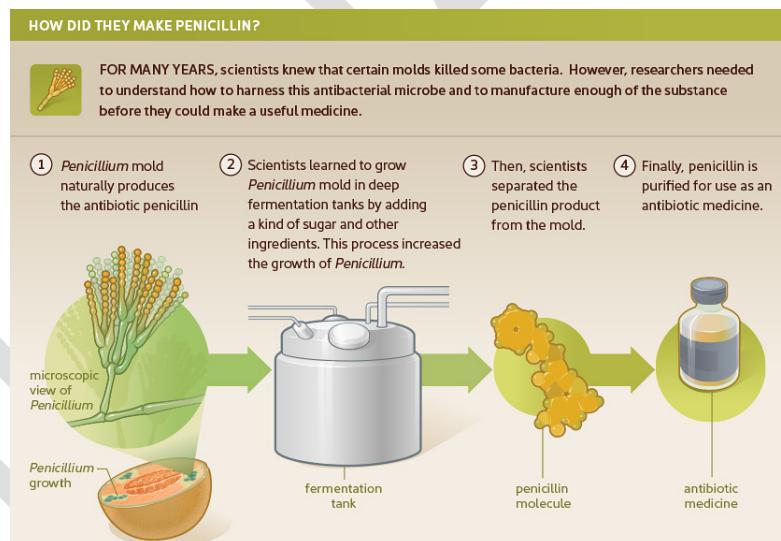
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## 1. INTRODUCTION

- **Definitions**
  - Biotechnology is the use of biological processes, organisms, or systems to manufacture products intended to improve the quality of human life.
    - E.g., Curd, Alcohol, GM crops, test-tube baby, developing a DNA vaccine or correcting a defective gene, are all part of Biotechnology.
  - Depending on the tools and applications, it often overlaps with the (related) fields of bioengineering, biomedical engineering, bio manufacturing, molecular engineering etc.
- **Two Sections of Biotechnology:** The entire field of Biotechnology can be divided into two sections
  - **Classical/traditional/Old Biotechnology**
    - E.g.
      - Curd being prepared with the help of microbes
      - Brewing alcohol
      - Cheese, bread and vinegar
      - Penicillin
    - In all the above product only natural capabilities of the microorganisms and cells were exploited.



- **Modern Biotechnology**
  - Modern biotechnology refers to manipulation of genome or innate capabilities of organisms for making it more desirable or to synthesis a valuable product.
  - E.g.
    - Genetic Engineering
  - Tissue/Cell Culture (it refers to growth of tissue or cells in an artificial medium separate from the organisms)

## 2. BASIS OF BIOTECHNOLOGY

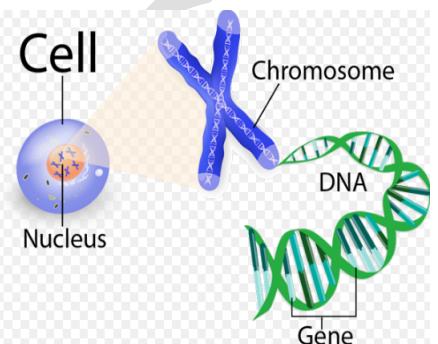
- Most living organisms have DNA as genetic material, DNA (Deoxyribonucleic Acid).
  - Some viruses have RNA as genetic material (e.g. Tobacco Mosaic viruses, QB bacteriophage, etc.)

- Now since all living organisms have DNA, it is possible to make changes, mix and match and this gives rise to possibility of the use of biotechnology.

## 1) BASICS UNDERSTANDING OF GENETIC MATERIAL

### A) GENE

- It is basic physical and functional unit of heredity. It contains the code for a molecule that has a function. They act as instructions to make molecules called proteins
- Genes are located on DNA. It is a short section of DNA. DNA can be cut and separated, forming a sort of 'bar code' that is different from one person to the next.
- In humans, genes vary in size from a few hundred DNA bases to more than 2 million bases.
- The Human Genome Project has estimated that humans have between 20,000 and 25,000 genes.

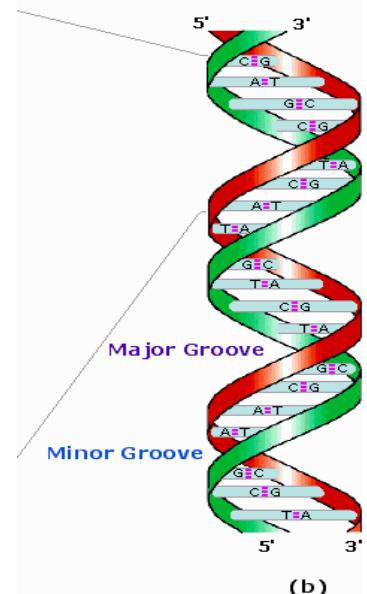


#### Gene Mapping

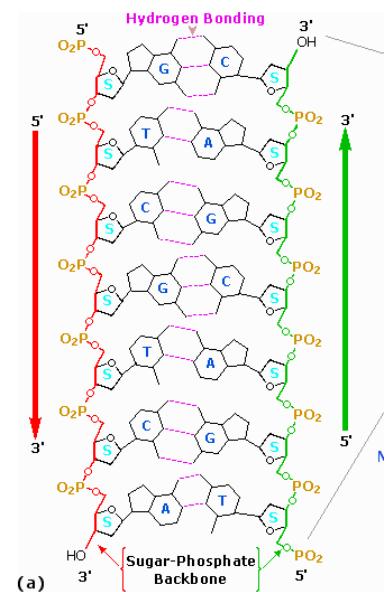
- Determining the gene's functionality and position of the gene in the chromosome is called gene mapping.

### B) DNA (DEOXYRIBONUCLEIC ACID)

- DNA is the hereditary material in humans and almost all other organisms. Nearly every cell in a person's body has the same DNA. Most DNA is located in the cell nucleus (where it is called nuclear DNA), but a small amount of DNA can also be found in the mitochondria (where it is called **mitochondrial DNA or mtDNA**)
- DNA is long polymer of deoxyribonucleotides. I.e. a deoxyribonucleotide is the monomer, or single unit, of DNA, or deoxyribonucleic acid.
- The length of the DNA is usually defined as number of nucleotides (or a pair of nucleotides referred to as base pairs) present in it.
- Human DNA is  **$3.3 \times 10^9$  base pairs**.
- Structure of Polynucleotide Chain**



- A nucleotide has three components - a nitrogenous base, a pentose sugar, (deoxyribose in case of DNA), and a phosphate group.
- There are two types of nitrogenous base.
  - Purines** (Adenine and Guanine)
  - Pyrimidines** (Cytosine, Uracil and Thymine)
- Note: Thymine is only found in DNA and Uracil only in RNA
- DNA bases pair up with each other, A with T and C with G, to form units called base pairs.
- The bases in two strands are paired through hydrogen bond (H-bonds) forming base pairs (bp). Adenine forms two hydrogen bonds with Thymine from opposite strand and vice-versa. Similarly, Guanine is bonded with Cytosine with three H-bonds.
- The structure of double helix is somewhat like a ladder, with the base pairs forming the ladder's rungs and sugar and phosphate molecules forming the vertical sidepieces of the ladder.
- The two chains are coiled in right-handed fashion.



### a) WHAT IS DNA FINGERPRINTING?

- DNA fingerprinting, also called DNA typing, DNA profiling, genetic fingerprinting, genotyping, or identity testing is a method of isolating and identifying variable elements in the base pair sequence of DNA.
- This technique was developed in 1984 by British geneticist **Alec Jeffreys**, after he noticed that certain sequences of highly variable DNA (known as **minisatellites**), which don't contribute to the function of genes, are repeated within genes.
- It was also noticed that each individual has a unique pattern of minisatellites (the only exceptions being multiple individuals from a single zygote, such as identical twins).
- DNA fingerprinting is a technique** that simultaneously detects lots of mini satellites in the genome to produce a pattern unique to an individual. This is a **DNA Fingerprint**.
- How is DNA fingerprint created?**
  - Obtaining a sample of cells**: such as skin, hair, or blood cells which contain DNA.
  - Extract** and purify DNA from these cells.
  - PCR** is used to amplify the desired fragments of DNA many times over creating thousands of copies of the fragments.
  - Once an adequate amount of DNA has been produced using PCR, the exact sequence of nucleotide pairs in a segment of DNA can be determined by using one of several **biomolecular sequencing methods**.

- **Application of DNA Fingerprinting:**
  - **Identification:** It is a forensic technique used to identify individuals/ dead bodies by characteristics of their DNA.
  - **Solving legal disputes:**
    - **Physically connect a piece of evidence to a person** or rule out someone as a suspect.
    - To determine **paternity and other relationships**
  - **Medical applications:**
    - Match tissue of organ donors with those of people who need transplant
    - Identify diseases that are passed down through your family
    - Help find cure for those diseases, called hereditary diseases.
- **Problems:**
  - **Sources of errors:** Sample contamination, faulty preparation procedures, and mistakes in interpretation of results are major sources of error.

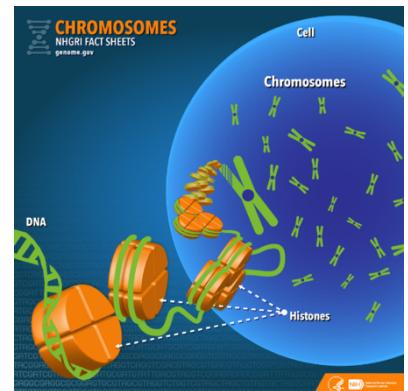
## b) DNA BARCODING

- **DNA Barcoding** is a tool for **rapid species identification** based on DNA sequence. It uses as short section of DNA from a specific gene or genes.
  - The way barcodes on a product, uniquely identifies a commercial product, in the same way, short gene segments – known as **DNA barcodes** – are unique for each species.
  - DNA barcoding has emerged as a global standard for fast and reliable genetic species identification of animals, plants and fungi.
- **Different gene regions are used to identify the different organismal groups using barcoding:**
  - For e.g., for animals (birds, butterflies, fish) and some protists – a short DNA sequence of COI gene found in mitochondrial DNA is used.
  - Similarly, Species identification of land plants is enabled by the combination of two different chloroplast gene regions – matK and rbcL.
  - Fungi species can be determined by the ITS region.
- **The ultimate goal of DNA barcoding is to build a publicly accessible reference database with species-specific DNA barcode sequences.**
- **Various methods of DNA Barcoding:** Barcoding can be done from tissue from a target specimen, from a mixture of organisms (bulk samples), or DNA present in environmental samples (e.g. water or soil). The methods barcoding will differ in each of these cases:

- **Tissue Samples**
- **Bulk Samples:** This sample contains several organisms from the taxonomic group under study.
  - E.g. – Aquatic macroinvertebrate samples collected by kick-net, or insect samples collected with a Malaise trap.
- **eDNA samples:** The environmental DNA (eDNA) method is a non-invasive approach to detect and identify species from cellular debris or extracellular DNA present in environmental samples (e.g., water or soil).
  - The main difference between bulk samples and environmental samples is that the bulk sample usually provides a large quantity of good-quality DNA.
- **Applications of DNA Barcoding:**
  - Identifying plant leaves (even when flowers and fruits are not available)
  - Identifying pollen collected on the bodies of pollinating animals
  - Identifying insect larvae which may have fewer diagnostic characteristics than adults
  - Investigating the diet of an animal based on its stomach content

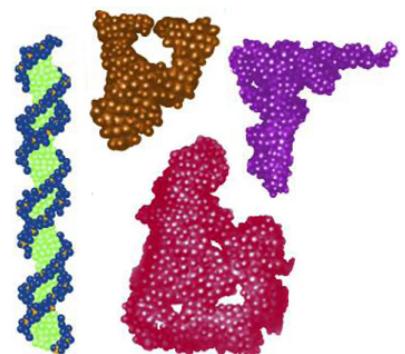
### C) CHROMOSOMES

- In the nucleus of each cell, the DNA molecule is packaged into thread-like structure called chromosomes.
- Each chromosome is made up of DNA tightly coiled many times around protein called histones that support the structure.
- The adjacent figure shows the relation between chromosome and DNA molecule



### D) RNA

- RNA stands for ribonucleic acid. It is a molecule with long chain of nucleotides. A nucleotide contains a nitrogenous base, a ribose sugar, and a phosphate.
- Like DNA, RNA is also vital for living cells.
- **Shape and structure**
  - It comes in a variety of different shapes.
  - Unlike double-stranded DNA, RNA is a single-stranded molecule in many of its biological roles and has a much shorter chain of nucleotides.

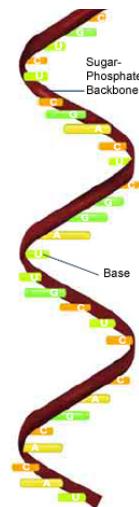


*RNA comes in a variety of different shapes.  
Double-stranded DNA is a staircase-like molecule.  
Image Credit: National Institute of General Medical Sciences*

- However, RNA can, by complementary base pairing, form intra-strand (i.e., single-strand) double helixes, as in tRNA

- **Functions of RNA**

- Carrying **genetic material** in some viruses
- The main job of RNA is to **transfer the genetic code needed for the creation of proteins from the nucleus to the ribosome**. The process prevents DNA from having to leave the nucleus. This keeps the DNA and genetic code protected from damage. Without RNA, proteins could never be made.
- Some RNAs act as enzymes. Such RNA enzymes are called ribozymes and they exhibit many of the features of a classical enzyme.



*Ribonucleic acid (RNA) has the bases adenine (A), cytosine (C), guanine (G), and uracil (U). Image Credit: National*

- **mRNA, rRNA, and tRNA**

- RNA is central to protein synthesis.
  - First a type of RNA called messenger RNA (mRNA) carries information from DNA to structure called ribosomes.
  - These ribosomes are made from proteins and ribosomal RNA (rRNAs).
  - These all come together and form a complex that can read messenger RNAs and translate the information they carry into proteins. This requires the help of transfer RNA or tRNA.
- RNA is formed from DNA by a process called transcription. This uses enzymes like RNA polymerase.
- **Transcriptome** is the set of all messenger RNA molecules in one cell or a population of cells.
  - Because transcriptome includes all mRNA transcripts in the cell, the transcriptome reflects the genes that are being actively expressed at any given time.

**Biotechnology makes it possible to move gene which is responsible for some particular feature from one organism to another.**

### a) RNA INTERFERENCE TECHNOLOGY

- » RNA Interference Technology (RNAi) is a biological process in which RNA molecules inhibit gene expression or translation, by neutralizing targeted mRNA molecules.
- » It is also known as **co-suppression, post-transcriptional gene silencing (PTGS), and quelling**.
- » Here mechanisms are developed to degrade mRNA molecules. This decreases their activity by preventing translation, via gene silencing.
- » **Functions/Applications**
  - » RNA interference is a vital part of the immune response to viruses and other foreign genetic material, especially in plants where it may prevent the self-propagation of transposons.

- » RNA interference has an **important role** in defending cells against parasitic nucleotide sequences – virus etc.
- » It can be useful to **study the function of a gene** in experimental biology in cell culture.

### 3. TWO CORE TECHNIQUES THAT ENABLED BIRTH OF MODERN BIOTECHNOLOGY ARE:

#### 1) GENETIC ENGINEERING

- » Technique to alter the chemistry of genetic material (DNA and RNA), to bring about desired modifications into host organisms and thus change the phenotype of the host organisms.  
Jelly fish glow at night. If we want other living organism to glow at night, we can extract the gene which is responsible for this glow and put it in the new host organism.
- » **Advantage of genetic engineering over traditional hybridization process**
  - » Traditional hybridization processes -> can lead to inclusion and multiplication of undesirable genes along with desired genes.
  - » Genetic engineering solves the above problem by isolating and introducing only one or a set of desirable genes without introducing undesirable genes.

#### 2) MAINTENANCE OF STERILE (MICROBIAL CONTAMINATION-FREE) AMBIENCE IN CHEMICAL ENGINEERING PROCESS

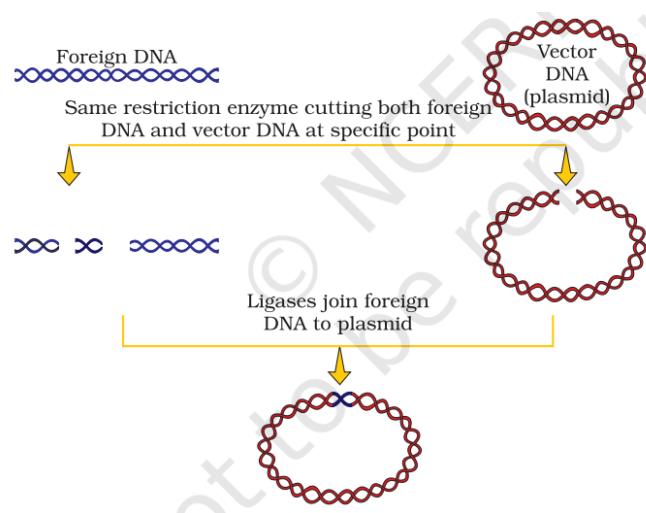
- » To enable growth of only the desired microbe / eukaryotic cell in large quantities for the manufacture of biotechnological products like antibiotics, vaccines, enzymes etc

### 4. TOOLS OF RECOMBINANT DNA TECHNOLOGY

Genetic engineering or recombinant DNA technology can be accomplished only if we have key tools, i.e., **restriction enzymes, polymerase enzymes, ligases, vector and the host organisms.**

#### 1) RESTRICTION ENZYMES

- A restriction enzyme or restriction endonuclease is an enzyme that cuts DNA at a near specific recognition nucleotide sequence known as restriction sites.
  - To cut DNA, all restriction enzymes make two incisions, once through each sugar-phosphate backbone (i.e. each strand) of the DNA double helix.



- **Restriction endonuclease** are used in genetic engineering to form 'recombinant' molecule of DNA, which are composed of DNA from different sources/genomes.
- When cut by 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**.

## 2) CLONING VECTOR

- They are used to transfer the foreign DNA to host DNA.
- Vectors used at present are engineered in such a way that they help easy linking of foreign DNA.

## 3) DNA LIGASE

- » It is a specific type of enzyme, a ligase that facilitates the joining of DNA together by catalyzing the formation of a phosphodiester bond.

## 4) HOST ORGANISMS

- The organism where the gene would be inserted.
- Techniques such as micro-injection are used. Here recombinant DNA is directly injected into nucleus of an animal cell.
- In other methods suitable for plants, the cells are bombarded with high velocity microparticles of gold or tungsten coated with DNA in a method known as **biolistic or gene gun**.
- Another method is using 'disarmed pathogen' vectors, which when allowed to infect the cell, transfer the recombinant DNA into the host.

## 5. CRISPR-CAS9

- What is **(CRISPR/CAS9)**?
  - CRISPR-CAS9 is a new genome editing tool, which is simpler, faster, cheaper, more versatile and more accurate than the previous techniques of editing DNA and has wide range of potential applications.
  - **Background: The inspiration for CRISPR:**
    - The inspiration of developing CRISPR CAS9 came from the **CRISPR system used by several bacteria** to fight against bacteriophages.
    - CRISPR (Clustered Regularly Interspaced Short Palindromic Sequence) are short DNA sequences found in the genome of Prokaryotic organisms such as bacteria, which are reminders of various bacteriophage (virus) attacks that the bacteria successfully defended against. Cas9 enzyme (part of the bacteria's defence mechanism) uses these

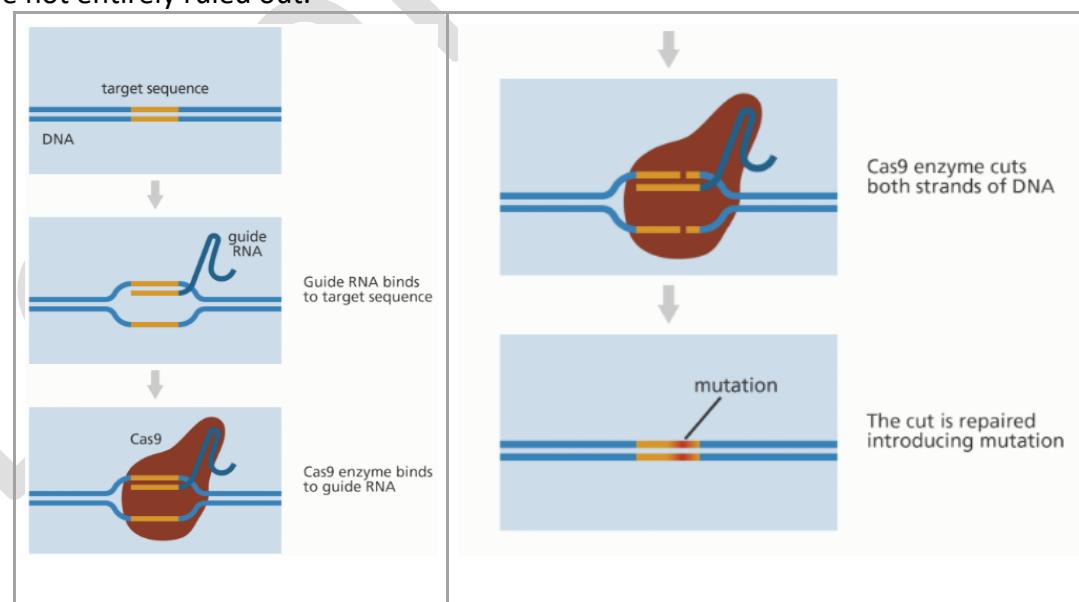
flags to precisely target and cut any foreign DNA, thus protecting the bacteria from future attacks by similar bacteriophages.

- Emmanuelle Charpentier of France and Jennifer Doudna of the US won the Nobel Chemistry Prize in 2020 for developing CRISPR-Cas9. This was the first time a Nobel Science prize has gone to a women-only team.

**NOTE:** Prof. Charpentier, 51, and Prof. Doudna, 56, were just **the sixth and seventh women to receive the Nobel Prize in Chemistry.**

- **How does CRISPR-CAS9 work? (Clustered Regularly interspaced short palindromic repeats)**

- [https://www.youtube.com/watch?v=UKbrwPL3wXE&ab\\_channel=MayoClinic](https://www.youtube.com/watch?v=UKbrwPL3wXE&ab_channel=MayoClinic)
- The first task is to identify the particular sequence of genes that is cause of problem and thus have to be deleted.
- Once this is done, an RNA molecule (called guideRNA) is programmed to locate this sequence of DNA stand, just like the 'find' or 'search' function of a computer.
- After this, a special protein called Cas9 (CRISPR associated Protein 9), which is often described as 'genetic scissors / molecular scissors', is used to break the DNA strand at specific points so that bits of DNA can then be added or removed.
  - A DNA strand, when broken, has a natural tendency to re-attach and heal itself. But if the auto-repair mechanism is allowed to continue, the bad sequence can regrow. So, scientists intervene during the auto-repair process by supplying the correct sequence of genetic codes, which attaches to the broken DNA strand.
- The entire process is programmable, and has remarkable efficiency, though chances of error are not entirely ruled out.



- **Applications of CRISPR-CAS9**

- The technology has had a **revolutionary impact** on life science.

- Its applications include:
  - **Curing diseases genetic in nature** – i.e., the diseases are caused by unwanted changes or mutations in genes. These include common blood disorders like sickle cell Anaemia, eye diseases including color blindness etc.
  - **Deformities arising out of abnormalities in gene sequences** – like stunted or slow growth, speech disorders, or inability to stand or walk can also be treated by CRISPR.
  - **Developing GM crops and animals.**
    - For e.g., Japan has already approved the commercial cultivation of a tomato variety that has been improved using CRISPR-based intervention.
    - In India, several research groups are working on CRISPR-based enhancements for various crops including rice and banana.

- **Limitation**

- **Potential of misuse:** (bioterrorism; designer babies)
- **Collateral Damage (Knock-on Effect):**
- **Ethics of CRISPR** – Should humans be allowed to modify how the nature works?

#### 4) HOW GENE THERAPY USING CRISPR CAN CURE CANCER (DEC 2022: SOURCE THE HINDU)

- **What is T-cell acute lymphoblastic leukaemia (T-ALL)**
  - It is a type of cancer where the T-cells, which are a class of white blood cells, equipped to hunt and neutralize threats to the body, turn against the body and end up destroying healthy cells that normally help with immunity. The disease is rapid and progressive and is usually treated by chemotherapy and radiation therapy.
- **How gene therapy treated this?**
  - Alyssia, a teenage girl, had tried several of the standard treatments including chemotherapy and radiation. But the treatment wasn't successful.
  - Then she enrolled in an experimental trial conducted by doctors and scientists at the University College, London and Great Ormond Street Hospital. She was the **first patient to receive experimental gene therapy that relied on a new technique called 'base-editing'**.
  - **What is base editing?**
    - When a misarrangement in the sequence of nitrogen bases (ATCG) is edited to arrange it properly, it is called base editing. David Liu, of the Broad Institute, Massachusetts has improvised on the CRISPR-cas9 to be able to directly change certain bases: thus, a C can be changed into G and T into an A. While still a nascent technology, **base editing is reportedly more effective at treating blood disorders**

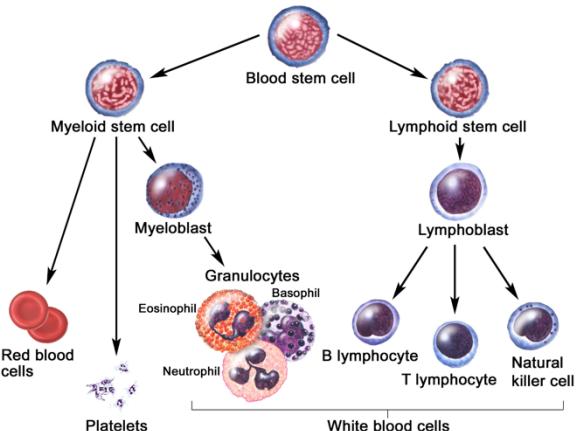
which were caused by so-called single point mutations, or when a change in a single base pair can cause terminal disease.

- Alyssia's case:

- In Alyssia's case, her T-cells – perhaps because of a misarrangement in the sequence of bases – had become cancerous. The objective of the gene therapy in the case of T-cell leukemia was to fix her immune system in a way that it stops making cancerous T-cells.
- First, healthy T-cells were extracted from a donor and put through a series of edits.
  - The first base edit blocked the T-cells targeting mechanism so it would cease attacking Alyssia's body.
  - The second removed a chemical marking, called CD7, which is on all T-cells.
  - Third prevented the T-cells from being killed by a chemotherapy drug.
  - Finally, the T-cells were programmed to destroy all cells – cancerous or protective – with CD7 marked on it.
  - After spending a month in remission, she was given a second donor transplant to regrow her immune system that would contain healthy T-cells.
- **How effective was the treatment?**
  - Her cancer doesn't seem to have re-surfaced.
- **More verification needed:**
  - It has been 1.5 years since she was first diagnosed with the disease and whether the treatment has reliably and entirely fixed her immune system, remains to be established.

#### A) UNDERSTANDING T-CELLS IN MORE DETAILS

- T cells are a type of white blood cells. They are part of immune system and develop from **hematopoietic stem cells** (blood stem cells) present in bone marrow. They help protect body from infection and may help fight cancer. They are also called T Lymphocyte and thymocyte.
- After getting born from blood stem cells, they migrate to thymus gland to develop. T-cells derive their name from the thymus. In thymus, the precursor cells mature into several distinct type of T cells. This differentiation continues after they have left the thymus.



- One of the important functions of T-cells is immune mediated cell death – it is carried out by two major subtypes – CD8+ “Killer” and CD4+ “helper” T cells. These are named for the presence of the cell surface proteins CD8 and CD4.
- T cells, also known as “Killer T-cells”, are cytotoxic – this means that they are able to directly kill virus-infected cells, as well as cancer cells.
- T-cells can be distinguished from other lymphocytes by the presence of a T-cell receptor (TCR) on their cell surface.

## 6. DARK DNA – CLASS DISCUSSION

## 7. SOMATIC CELL NUCLEAR TRANSFER

- In genetics and developmental biology, somatic cell nuclear transfer (SCNT) is a **laboratory technique for creating an ovum with a donor nucleus**.
  - In SCNT the nucleus, which contains the organism's DNA, of a somatic cell (a body cell other than a sperm or egg cell) is removed and the rest of the cell discarded.
  - At the same time, the nucleus of an egg cell is removed.
  - The nucleus of the somatic cell is then inserted into the unnucleated egg cell.
  - After being inserted into the egg, the somatic cell nucleus is reprogrammed by the host cell.
  - The egg, now containing the nucleus of a somatic cell, is stimulated with a shock and will begin to divide.
  - **After many mitotic divisions in culture**, this single cell forms a blastocyst (an early stage embryo with about 100 cells) with almost identical DNA to the original organism

It can be used in embryonic stem cell research, or in regenerative medicine where it is sometimes referred to as "therapeutic cloning." It can also be used as the first step in the process of reproductive cloning.

## 8. APPLICATIONS OF BIOTECHNOLOGY

### 2) GM CROPS

- **GM Crops, Advantages and Controversies**

- Crops whose DNA has been altered are known as GM crops. This genetic modification of crops can add or remove certain characteristics from the plant and thus can bring many advantages.
  - Make crops **more tolerant to anti-biotic stresses** (cold, drought, salt, heat) etc.
    - E.g., GM Rubber developed by Rubber Research Institute of India
  - Make plants **Pest Tolerant**.
    - Reduces reliance on chemical pesticides.
    - E.g. BT cotton, BT Brinjal (in Bangladesh)
  - Help to **reduce post-harvest losses**
  - Enhance the **nutritional value** of food, e.g., Golden Rice (Vitamin A enriched rice)
  - Tailor-made plants to supply **alternative resources** to industries, in the form of starches, fuels, and pharmaceuticals.

#### A) BT COTTON

- Specific BT Toxic gene (*cry1Ac*) were isolated from *Bacillus thuringiensis* and incorporated into several crop plants such as cotton. This produces proteins that kill certain insects such as lepidopterans (tobacco budworm, armyworm), beetles, etc.
- It has been grown in India since 2002 and over the years have given increase productivity and area under crop cultivation. It has also led to decrease in insecticide which fought bollworms by 97%.
- **Note:** **Bollgard® Bt Cotton** (single gene technology) is India's first biotech crop technology approved for commercialization in 2002, followed by Bollgard® II – double gene technology in mid-2006, by the GEAC.
  - **Bollgard® cotton** provides in-built protection for cotton against destructive American Bollworm *Heliothis Armigera* infestations, and contains an **insecticidal protein from a naturally occurring soil microorganism, Bacillus thuringiensis (Bt)**.
  - Bollgard® II technology contains a superior double-gene technology - Cry1Ac and Cry 2Ab which provides protection against bollworms and *Spodoptera caterpillar*, leading to better boll retention, maximum yield, lower pesticides costs, and protection against insect resistance.
  - Both, Bollgard® II and Bollgard® insect-protected cotton are widely planted around the world as an environmentally friendly way of controlling bollworms.
- But it has also raised concerns like increased water consumption, and emergence of pesticide resistant pests (e.g., pink bullworm), and increased use of insecticide for controlling pests like sucking pests.

#### B) BT BRINJAL

- Transgenic Brinjal created by inserting a **crystal protein gene (Cry1Ac)** from the soil bacterium Bacillus thuringiensis into the genome of various brinjal cultivar. It gives resistance against lepidopteron insects in particular the Brinjal fruit and shoot border (BFSB), the most common pest which affects 30-50% of the Brinjal crops.
- The crop also cleared the GEAC's biosafety test in 2009. But, government yielded to anti-GM activists and declared a moratorium in 2010 on the crop.
- But some cases of illegal BT Brinjal cultivation was observed in Haryana in 2019
  
- **Why are some groups are calling for allowing of BT Brinjal in India?**
  - It had cleared the GEAC's biosafety test in 2009.
  - **Increased benefit for farmer**
  - When GM Crops are not officially available, farmers turn to **unapproved knock offs** that may not conform to accepted biosafety standards.
  
- **Why is BT Brinjal not allowed in India? Why is it opposed by various activists?**
  - There are fears that it may **impact India's plant biodiversity**.
  - Further, **cross pollination** may lead to **herbicide resistant super weeds** that can further threaten environment and biodiversity.
  - **Health Impact** is something that needs to be studied more.
  - **Not so obvious benefits:** A recent study from surveys of farmers indicate that 2/3<sup>rd</sup> of the farmers who moved to BT Brinjal have had a 'bad' or 'very bad' experience.

### C) GM MUSTARD

- **What is GM Mustard?**
  - DMH-11 (Dhara Mustard Hybrid) is a genetically modified (GM) mustard Hybrid.
  - GM mustard is the country's first genetically modified food crop.
  - It was developed by a team of scientists led by former Vice Chancellor Deepak Pental, of DU at Center for Genetic Manipulation of Crop Plants (CGMCP), Delhi University by crossing Indian mustard cultivars with juncea lines of East European origin like 'Early Heera' and Donskaja.
  
- **Claim of higher yield:**
  - Claims around 30% more yield than the traditional varieties
  
- **What genetic modification was achieved and what are its benefit?**
  - **Barnase gene and Barster gene** from *Bacillus amyloliquefaciens*
    - Barnase impairs pollen production

- Barster blocks the function of Barnase
  - Hybridization becomes possible:
    - This method was used to developed DMH-11 by crossing a popular Indian mustard variety 'Varuna' (the barnese line) with an East European 'Early Heera-2' mutant (barstar).
- **Arguments for and against approval of GM mustard**
- » **For**
    - **Higher Production**
    - **Reducing Import Dependency**
    - **Saving Forex**
    - **Keeping India Scientifically relevant**
  - » **Against**
    - The main contention is that the GM mustard incorporates three alien genes - barnase, barstar, and bar - rendering it inherently unsafe for human and animal health.
      - But these genes have already been deployed in Canola, and we import it freely.
    - Mustard is a food crop unlike cotton, so both should not be compared
    - All health effects not properly known yet
    - Environmental damages should be studied properly first.
    - Yield claims have been challenged by many organizations
  - » **GEAC Approval (Oct 2022)**
    - In Oct 2022, GEAC approved commercial cultivation of genetically modified mustard yet again. The approval allowed environmental release of two varieties of genetically engineered mustard, so that it can be used for developing new parental lines and hybrids under the supervision of ICAR. The environmental release of DMH-11 will allow for its seed production and testing as per existing ICAR guidelines and other extant rules/ regulations prior to commercial release. The field demonstration studies on the effect of GE mustard on honeybees and other pollinators was also allowed to be conducted.

#### **D) GM RUBBER – DEVELOPED BY KERALA BASED - RUBBER RESEARCH INSTITUTE OF INDIA**

- Rubber Research Institute of India have developed a plant tailored for the climatic conditions in the Northeast.
- Rubber board research farm at Sarutari on the outskirts of Guwhati now sports world's first GM rubber plant, tailored for climatic condition in the north-east.

- **Genetic Modification:** The GM rubber has additional copies of the gene **MnSOD**, or manganese-containing superoxide dismutase, inserted in the plant, which is **expected to tide over the severe cold conditions during winter** – a major factor affecting the growth of young rubber plants in the region

## E) INCREASING THE NUTRIENT CONTENT – GOLDEN RICE

### ▫ Golden Rice

#### - What is Golden Rice?

- The IRRI and its national research partners have developed golden rice to complement existing interventions to address vitamin A deficiency (VAD). It is a serious public health problem affecting millions of children and pregnant women globally.
- Golden rice is variety of rice produced through genetic engineering to biosynthesize beta-carotene. Beta-carotene is a nutrient similar to what is found in orange colored fruits and vegetables and is converted into Vitamin-A as needed by the body.
- Thus, golden rice can help south and south-east Asian countries, where two-thirds or more of daily calorific intake is obtained from rice. Research has indicated that the golden rice can provide upto 50% of the daily requirement of an adult for vitamin A.



- Golden rice was one of the 7 winners of the 2015 Patents for Humanity Awards by the United States Patent and Trademark Office
- **Safety Evaluation by International Rice Research Institute**
  - The safety evaluation of Golden rice has shown that it is as safe and nutritious as conventional rice but comes with added benefit of beta-carotene.
- **About International Rice Research Institute:**
  - IRRI is the world's premiere research organization dedicated to reducing poverty and hunger through rice science; improving the health and welfare of rice farmers and consumers; and protecting the rice growing environment for future generation.
  - It is an independent, non-profit, research and educational institute, founded in 1960 by the Ford and Rockefeller foundations with support from the Phillipines government.
  - The institute is headquartered in Los Banos, Philippines and has offices in 17 rice-growing countries in Asia and Africa.
  - It works with in-country partners to develop advanced rice varieties that yield more grain and better withstand pests and disease as well as flooding, drought, and other harmful effects of climate change.

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#### F) ISSUE OF ILLEGAL CULTIVATION OF GM CROPS:

- **BT Brinjal** Illegal cultivation in Haryana Rajasthan etc.
- **Sale of Illegal HTBt (Herbicide tolerant Bt) cotton seeds** has doubled this year(June 2021)
  - The HTBt cotton variant adds another layer of modification to BT cotton, making the plant resistant to the herbicide glyphosate, but has not been approved by regulators.
  - **Support for HTBt:** Groups like Shetkari Sangathan are demanding the legalization of HTBt cotton.
    - **Saves cost:** Weeding labour cost reduces, only one round of glyphosate spraying is needed to deal with the weed.
    - **Illegal sales** reduce accountability, hampers government revenue and farmers are at risk of getting wrong information.
  - **Concerns/Fears:**
    - Glyphosate have carcinogenic effect
    - Unchecked spread of herbicide resistance to nearby plants through pollination, creating a variety of superweeds etc.

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#### G) SCIENTISTS ARE ENGINEERING PLANTS TO PRODUCE INSECT 'SEX PERFUME' TO REPLACE PESTICIDES (APRIL 2023)

- Researchers are engineering tobacco plants to produce moth pheromones that could potentially be used to create traps that can lure insects as a replacement for harmful pesticides.
- **Note:** Pheromones are chemicals that are produced and released by animals. When they are released by an individual of a species, they effect the behaviour of other individuals. Animals secrete these pheromones to trigger different kinds of behaviour. The pheromones that trigger sexual arousal can be thought of as a kind of 'sex perfume', attracting other individuals of the same species.
- The researchers engineered plants to produce chemicals that mimic these pheromones.
- **Note:**
  - Chemically produced insect pheromones are already used for pest control and have been for some decades. Some insect traps contain pheromones to attract the insect to them, for use in the house garden, and in food production systems.
  - **Disadvantages of these chemically produced pheromones:** It is not possible to make complex pheromones by this mechanism. Moreover, chemical manufacturing process produces a number of other pollutants.
- **GM Crop Route:**
  - Researchers used Nicotiana benthamiana, a species of tobacco.
  - Note: The same plant has been engineered to produce ebola antibodies and even coronavirus like particles for use in COVID vaccine.

- Here, scientists built a sequence of DNA in the lab that mimic moth's genes and also put in place a few molecular switches that can precisely regulate how the molecules are formed. The switches can turn the manufacturing process on and off.
- **Advantages of using pheromones:** They are highly species specific and unlike broad spectrum pesticides don't kill other species of pollinators.

### 3) REDUCED HEIGHT GENES (RHT): ADVANTAGES AND LIMITATIONS

#### Introduction

- Since the 1960s and the Green Revolution, **reduced height (Rht) genes have increased global yields** because the short-stemmed wheat they produce puts more investment into the grains rather than into the stems and has improved standing ability. It leads to reduced risk of lodging, increase in partitioning and assimilation of grains, more fertile florets per spriglet and higher harvest index (the proportion of plant weight in grains).
- The high yielding wheat variety developed by **Borlaug**, which required higher use of fertilizers and pesticides, produced bigger grains. However, the heavier grains caused the plants to become unstable and prone to lodging. Therefore, **Borlaug introduced dwarfing genes** into wheat giving plants a stronger, shorter stem that resisted lodging.
  - i) **21 reduced height genes** in wheat Rht1 – Rht21, have been described so far.
  - ii) In India, the presently available semi-dwarf varieties, which were explored during the Green Revolution, carry conventional Rht1 dwarfing alleles (variant form of a given gene) and produce optimum yields under high-fertility irrigated conditions.
- **Limitations of Dwarf wheats:**
  - a) Dwarf wheats are not well adapted to deeper sowing conditions. This is due to shorter coleoptiles, and low early vigor often results into reduced seedling emergence. Further shorter coleoptiles lead to crop residue posing a problem for seedling emergence.
  - b) These wheats also don't work in drought conditions they can't be planted deep inside the soil to access moisture. They will fail to reach the surface of the soil.
- **Key Research to solve the issue:**
  - Scientists at Agharkar Research Institute (ARI), an autonomous institute of DST, have mapped to alternative dwarfing genes of Rht14 and Rht18. These genes are associated with better seedling vigor and longer coleoptiles (sheath protecting the young shoot tip).
  - **Advantages:**

- a) The new wheat variety will be suitable for sowing under rice stubble retained condition and in **dry environments**. It would thus reduce the need of water and also contribute to reduction in crop stubble burning.
  - b) It also diversifies the genetic base of dwarfing genes considering diverse wheat growing zones in India.
2. Recent research published in the *Proceedings of the National Academy of Sciences (PNAS)* journal on 23<sup>rd</sup> Nov 2022 says that Scientists at the John Innes Centre, in collaboration with an international team of researchers, have discovered **the new “reduced height” or semi dwarf gene called Rht13**. The varieties of wheat with Rht13 gene could be rapidly bred into wheat varieties to enable farmers to grow reduced-height wheat in **drier soil conditions**.
- Rht13** overcome this problem of seedling emergence because the **gene acts in tissues higher-up in the wheat stem**. So, the dwarfing mechanism only takes effects once the seedling has fully emerged. This gives farmers a significant advantage when planting deeper in dry conditions.

### 3) BIOTECHNOLOGICAL APPLICATION IN MEDICINES

The recombinant DNA technological processes have had a great impact in the area of health care by enabling mass production of safe and more effective therapeutic drugs.

- Further, the recombinant therapeutics do not induce unwanted immunological responses as is common in case of similar products isolated from non-human sources.
- At present, more than 30 recombinant therapeutics have been approved for human-use the world over.
  - In India, around 12 of these are presently being marketed.

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#### A) VACCINES (COVERED SEPARATELY WITH HEALTH SECTION)

- For e.g., various vaccines for COVID-19 were developed with the help of biotechnology – mRNA vaccines, vaccines with attenuated virus

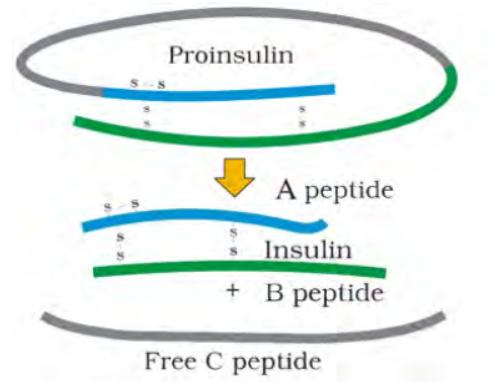
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#### B) MASS PRODUCTION OF EFFECTIVE THERAPEUTICS

- The recombinant DNA technological processes have had a great impact in the area of health care by enabling mass production of safe and more effective therapeutic drugs.
- **Advantages of recombinant therapeutics:** Further, the recombinant therapeutics do not induce unwanted immunological responses as is common in case of similar products isolated from non-human sources.
- At present, more than 30 recombinant therapeutics have been approved for human-use the world over.
- In India, around 12 of these are presently being marketed

### C) GENETICALLY ENGINEERED INSULIN

- Earlier, Insulin used for diabetes was extracted from pancreas of slaughtered cattle and pigs.
  - » Caused patients to develop some kind of allergies or other kinds of reactions to the foreign protein.
- Structure of 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 synthesized as a pro-hormone (like a pro-enzyme, pro hormone also needs to be processed before it becomes a fully mature and functional hormone) which contains an extra stretch called 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 technique was getting insulin assembled into a mature form.
- How this was achieved through Biotechnology
  - 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 disulphide bonds to form human insulin.

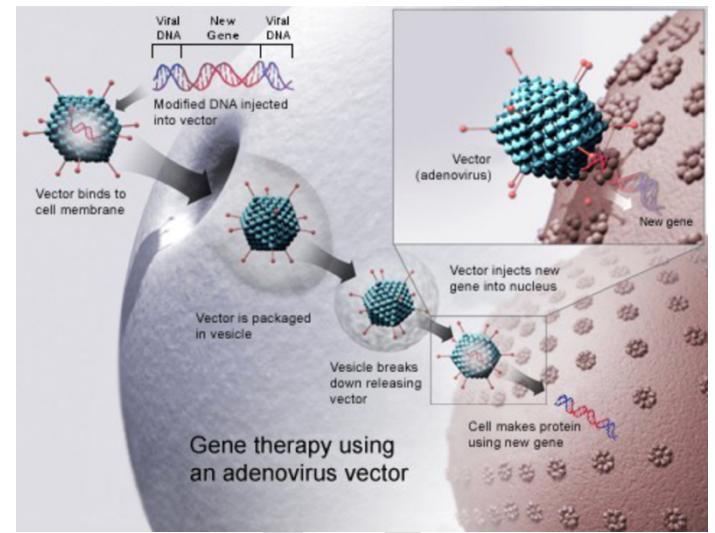
### D) GENE THERAPY

#### - Introduction

- » If a person is born with a hereditary disease, can a corrective therapy be taken for such disease? Gene therapy is an attempt to do this.
- » Gene therapy refers to the process of introduction, removal or change in the content of an individual's genetic material with the goal of treating the disease and a possibility of achieving long term cure.

» **Gene Therapy Products (GTPs)** include the mechanisms to deliver nucleic acid components by various means for therapeutic benefit to patients. They include entities that are used for things like gene augmentation, gene editing, gene silencing, synthetic or chimeric gene augmentation etc.

- **Note:** Not all medical procedures that introduce alterations to a patient's genetic makeup can be considered a gene therapy. For e.g.: Bone Marrow transplantation and organ transplants in general have been found to introduce foreign DNA into patients.



#### - Advantages of promoting gene therapy

- **Permanent result may be a possibility:**
- **High burden of rare genetic diseases in India:** Around 7 crore of India's population suffers from rare genetic diseases. Gene therapy can prove to be a turning point in treatment of such genetic diseases.
- **Worldwide market for the gene therapy products** is expected to go to \$250 billion by 2025.

#### - Concerns/Limitations

- Promotion of development of gene therapy also brings along with it unique technical risks and ethical challenges.
- **Technical Challenges**
  - The gene therapy may be associated with **unwanted immune system reactions**. For e.g., when vectors (viruses) are attacked by the immune system of the body.
  - Current gene therapy mechanisms can sometimes **target the wrong cells**.
  - **The delivery viruses may mutate** and become harmful.
- **Ethical Challenges**
  - For e.g. creation of GM babies using germline gene editing by a Chinese scientist attracted global criticism and fueled debate on ethical concerns regarding applications of gene therapy technologies.
  - **Playing god** debate.

#### - National Guidelines for Gene Therapy Product Development and Clinical Trials – Released by ICMR in Dec 2019: Key Highlights

- » The guidelines are **aimed** at ensuring that **gene therapies are introduced in India** and **clinical trials for gene therapy can be performed in an ethical, scientific and safe manner.**
- » They provide the **general principles for developing gene therapy products (GTPs)** for any **human ailment and provide a framework** for all areas of GTP production including **pre-clinical testing, clinical administration, human clinical trials, as well as long term follow up.** These must follow the established general principles of biomedical research.
- » They **apply to all stakeholders** involved in the field of gene therapy including **researchers, clinicians, oversight/regulatory committees, industry, patient support groups and any other involved in GTP development** or their application in humans and their derivatives.
- » The guidelines will serve as a **roadmap** for those in the field trying to develop gene and cell **therapies** and will thus **contribute to accelerating the development** of advanced therapeutic options
- ICMR has also proposed setting up of **task force to promote gene technology research in the country.**

#### a) CAR-T CELL THERAPY

- **Why in news?**
  - The CDSCO has granted **market authorization for NexCAR19**, India's first indigenously developed CAR-T cell therapy, to ImmunoACT (Nov 2023)
- **Background: How Cancer has been treated before CAR T-Cell Therapy:**
  - **Surgery** (removing the cancer)
  - **Radiotherapy** (delivering ionizing radiation to the tumour)
  - **Systematic Therapy** (administering medicines that act on tumour)
    - The **earliest form** of systematic therapy was **chemotherapy**. It **preferentially acts on cancer cells** because of the latter's rapid, unregulated growth and poor healing mechanisms. These drugs have **modest response rate** and **significant side effects** as they effect numerous cell types in the body.
    - 
    - The next stage in its evolution was **targeted agents** a.k.a. **immunotherapy**: The drugs bind to **specific target on the cancer or in the immune cells** that help the tumour grow or spread. This method often has **less side effects** as the impact on non-tumour cells is limited. However, it is **effective only against tumours that express these targets.**
- **CAR-T Cell Therapy** has emerged as a **new development in this front.**
  - It is a revolutionary therapy that **modifies immune cells**, specifically T-Cells, by **turning them into potent cancer fighters known as CAR-T Cells.**
  - **How it works?**
    - In CAR T-cell therapy, **the patient's blood is drawn to harvest T-cells** – immune cells that play a major role in **destroying tumour cells.**

- Researchers modify these cells in the laboratory so that they express specific proteins on their surface, known as **chimeric antigen receptors** (CAR): they have an affinity for proteins on the surface of tumour cells. This modification in the cellular structure allows CAR T-cells to effectively bind to the tumour and destroy it.
- These modified cells are then infused back into the patient's blood stream after conditioning them to multiply more effectively.
- The cells are even more specific than targeted agents and directly activate the patient's immune system against cancer, making the treatment more clinically effective. This is why they are called '**living drugs**'.
- **Advantages of CAR-T Cell therapy over other Cancer fighting methods:**
  - It is very accurate and only targets cancer cells.
  - It makes the treatment easier with onetime therapy (unlike several sessions of chemotherapy)
  - It can also fight non-responsive cancer patients.
  - It is designed to cure and provide lifelong benefits.

- **Where is it being used today?**

- CAR T-cell therapies are approved for **Leukaemias** (cancers arising from the cells that produce white blood cells) and **Lymphomas** (arising from the lymphatic system)
- It is also being used among patients with cancers that have returned after an initial successfully treatment or which haven't responded to previous combinations of chemotherapy or immunotherapy.

- **CAR T-Cell Therapy in India:**

- The first major clinical trial showing they were effective was published almost a decade ago. The first indigenously developed therapy in India was successfully performed only in 2021.
- **In Oct 2023, the Central Drugs Standard Control Organization (CDSCO) granted market authorization for **NexCAR19**, India's first indigenously developed CAR-T cell therapy, to **ImmunoAct**, a company incubated by IIT Bombay. This paves the way for commercial launch of this therapy in India.**
  - It is designed to target cancer cells that carry the CD19 protein. This protein acts like a flag on cancer cells, which allows CAR-T cells to recognize and attach themselves to the cancer cells and start process of elimination.
  - **Who can get the NexCAR19 therapy?**
    - The therapy is for people with B-Cell lymphomas who didn't respond to standard treatments like chemotherapy, leading to relapse or reoccurrence of the cancer.
    - **B-Cell leukaemia is most common among children. Are they also eligible?**
      - » For now, therapy's approval is only for patients aged 15 years and above.
      - » The pediatric trial phase is currently underway at the **Tata Memorial Hospital**, in collaboration with IIT-Bombay.

- **Significance:**
  - India is one of the first developing country to have its own Car-T therapy. Even some developed nations don't have their own CAR-T therapies and they import from USA or Europe.
  - This reduces the cost of treatment to about 1/10<sup>th</sup> of the cost abroad and has the potential of boosting medical tourism in India. It costs around Rs 3.3 crores abroad while in India it will cost somewhere between 30-40 lakh rupee.
  - Lab and animal studies have shown that **NexCAR19** lead to significantly lower drug-related toxicities. For e.g., it causes minimal damage to neurons and the central nervous system, a condition known as neurotoxicity. The therapy also leads to minimal Cytokine Storm Syndrome (CRS), which is characterized by inflammation and hyperinflammation in the body due to the death of a significant number of tumour cells, as CAR-T cells are designed to target and eliminate cancer cells.

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#### b) WHAT IS B-CELL LYMPHOMA

- B-Cell Lymphoma is a form of cancer that starts in a white B-cell called a **Lymphocyte**. B-Cell Lymphocytes make antibodies, the proteins in the immune system that help fight infections. They are often found in lymph nodes or other lymphoid tissues such as the spleen.
- **In B-Cell Lymphoma**, some lymphocytes are no longer healthy and don't fight infections. Instead, they grow out of control, crowding out the normal cells and causing the Lymph nodes to get bigger.

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#### c) GENE THERAPY TO TREAT SICKLE CELL ANAEMIA AND THALASSEMIA (NOV 2023) (WILL BE COVERED WITH HEALTH BOOKLET)

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#### d) PFIZER'S HEMOPHILIA B GENE THERAPY SUCCEEDS IN LATE-STAGE STUDY (DEC 2022: SOURCE – THE HINDU)

- **About Haemophilia B:**
  - It is a hereditary bleeding disorder. It hampers body's ability to make a blood-clotting protein called factor IX.
  - **What happens when you bleed?**
    - At the time of bleeding, a series of reactions take place in the body that helps blood clots to form. This process is called coagulation. It needs various proteins called coagulation, or clotting factors. A person has higher chances of bleeding if one or more of these factors are missing and are not functioning like they should.
    - **Factor IX (nine)** is one such coagulation factor. **Haemophilia B** is the result of the body not making enough factor IX. It is caused by an inherited X-linked recessive trait, with the defective gene located on the X chromosome.
  - **Most people with haemophilia B are male.** (Reason – Class discussion)

- **Pfizer's haemophilia B gene therapy succeeds in late-stage study:**
  - The study showed that a single dose of the therapy was superior to the current standard of care in helping reduce the bleeding rate in patients with moderately severe to severe forms of haemophilia B.
  - Pfizer's therapy, fidanacogene elaparovec, is designed to help patients produce factor IX themselves after a one-time treatment, as opposed to current treatments, which focus on regular infusions of the protein.
- **Pfizer is also testing other experimental gene therapies in late-stage trials as potential treatments for the bleeding disorder haemophilia A and muscular disorder Duchenne muscular dystrophy.**

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#### e) NOTE: HAEMOPHILIA A

- It is also called factor VIII(8) deficiency or classic haemophilia. It is a genetic disorder caused by missing or defective factor VIII (FVIII), a clotting protein.

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#### f) DUCHENNE MUSCULAR DYSTROPHY

- **About muscular dystrophy:**
  - It is a group of diseases that cause progressive weakness and loss of muscle mass. In muscular dystrophy, abnormal genes (mutations) interfere with the production of proteins needed to form healthy muscle.
  - There are many kinds of muscular dystrophy. The Symptoms of most common variety begin in Childhood, mostly in boys. Other types don't surface until adulthood.
  - **Sign:** The main sign of muscular dystrophy is progressive muscle weakness. Specific signs and symptoms begin at different ages and in different muscle groups, depending on the type of muscular dystrophy.
- **About Duchenne muscular dystrophy:**
  - Most common type of muscular dystrophy.
  - Although girls can be carriers and mildly affected, it's much more common in boys.
  - **Signs and symptoms** which typically appear in Childhood are:
    - Frequent falls
    - Difficulty rising from a lying or sitting position
    - Trouble running and jumping
    - Walking on the toes
    - Large calf muscle
    - Delayed growth

- Learning disabilities.
- Other types of muscular dystrophy include: Becker Muscular Dystrophy

## E) MOLECULAR DIAGNOSIS

- For treatment of any disease, early diagnosis and understanding its pathophysiology is very important. 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 detection.
  - PCR is a technique used in molecular biology to amplify a single copy or a few copies of a piece of DNA across orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.
    - It is now routinely used to detect HIV in suspected AIDS patients. It is being used to detect mutations of genes in suspected cancer patients too.
  - 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 pathogens
- E.g. Tests During COVID-19
  - RT-PCR Test
    - The test detects the presence of viral RNA in human samples.
    - In this test first the viral RNA is converted into DNA (reverse transcription)
    - PCR is a process where a few copies of DNA are amplified to produce millions of copies.
    - This is done with the help of enzymes, primers, and probes.
  - Rapid Anti-Body Test
    - A rapid test is conducted to determine if there has been any kind of recent viral infection in a person's body. When a pathogen enters a human body, specific anti-bodies are released as a response to the virus. A rapid test can detect the presence of such anti-bodies in blood, serum or plasma samples question.
    - This is a simple test and can give results in 10-30 minutes.
    - It should be noted that it is not a confirmatory test for COVID-19. It is only a preliminary screening for diagnosis of coronavirus infection.
    - Further, a negative test doesn't rule out COVID-19 infection. A rapid test comes positive after 7-10 days of viral infection and remains positive for several weeks after that.

## F) DISEASE CONTROL THROUGH GENETICALLY MODIFIED ORGANISMS

- By introducing sterile mosquitoes (genetically formed). (concept - not done yet)
- Synthetic vector genome which is incapable of hosting the parasite and/or virus.

## G) PERSONAL GENOMICS

- It is the branch of genomics concerned with sequencing and analysis of the genome of an individual. The genotyping stage employs different techniques, including single-nucleotide polymorphism (SNP) analysis chips (typically 0.02% of the genome), or partial or full genome sequencing.
- **Uses**
- Once the genotypes are known, the individual's genotype can be compared with the published literature to determine likelihood of trait expression and disease risk.
- Personalized medicines
  - It is a medical method that targets treatment structures and medicinal decisions based on patient's predicted response or risk of disease.
  - Various subcategories of personalized medicines
    - Predictive Medicines
    - Precision Medicines
    - Stratified Medicines
- It predicts the right kind of treatment
  - Efficacy of toxicity of chemotherapy, or radiotherapy etc.

## 4) TRANSGENIC ANIMALS

- Animals that have 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% of all existing transgenic animals are mice.
  - **Why so much medical research on mice, rat?**
    - **Genetic, biological and behaviour characteristics** closely resemble that of humans and many symptoms of human conditions can be replicated in mice and rats.
      - We share between 95% of the same genes, and our immune system are even more compatible.
      - Therefore, the result of mouse experiment often correlates to human biology
      - Further, mice can be genetically manipulated to mimic virtually any human disease or condition.
  - **Convenience**
    - Rodents are small, easily housed and maintained, and adapt well to the new surroundings.
  - **Reproduce quickly and short lifespan:** Reproduce quickly and have short life span of 2-3 years - so several generations of mice can be observed in sort span of time.

- **Relatively Inexpensive**
  - Can be brought in large quantities from commercial producers
- **Mild tempered and docile**
  - Rodents are also generally mild tempered and docile, making them easy for researchers to handle.
- **How transgenic animals are helpful?**
  - **Normal physiology and development**
    - Experimenting on how alteration of genes would affect humans.
  - **Study of disease**
    - Many transgenic animals are designed to increase our understanding of how genes contribute to the development of disease.
  - **Biological Products**
    - Some medicines might require some biological products which are often expensive to produce.
    - Transgenic animals that produce useful biological products can be created by the introduction of portion of DNA (or genes) which code for a particular product.
      - E.g. : Human protein ( $\alpha$ -1-antitrypsin) used to treat emphysema.
      - In 1997, the first transgenic cow - Rosie, produced human protein-enriched milk (2.4 grams per liter).
        - The milk contained the human alpha-lactalbumin and was nutritionally a more balanced product for human babies than natural cow milk.
    - **Vaccine Safety**
      - Transgenic mice are being developed for use in testing of safety of vaccines before they are used on humans.
      - Transgenic mice are being used to test the safety of the polio vaccine.
    - **Chemical safety testing**
      - This is known as toxicity safety testing.
      - The procedure is same as used for testing toxicity of drugs.

## 5) BIOTECHNOLOGY AND ENVIRONMENT

### H) GM ALGAE, CROPS ETC. CAN PROVIDE MORE BIOMASS FOR BIOFUEL.

### B) BIODIVERSITY CONSERVATION

- a. E.g. -> De-extinction of species; **Colossal** is a new bioscience and genetics company, with the idea of bringing many extinct species back to life. Scientists at Harvard University in the USA would insert

the Giant Woolly mammoth's (extinct 4,000 years ago) genes responsible for tiny ears, subcutaneous fat and hair length and color into living elephant skin cells. Once they are successful in bringing these hybrids back to life, Colossal will proceed with the ultimate goal of reviving the ancient extinct animals by producing more such hybrids.

**Criticism:** Immoral; revival of these species may threaten the existing ecosystem and disturb the food chain which has evolved over the years; Rather than focusing on revival of long extinct species, biotechnology should focus on protecting the existing ones.

### C) TO DETECT INVASIVE SPECIES:

- Environmental DNA based assay to detect invasive catfish in waterbodies (Nov 2022 – Source: DTE)
  - Conventional methods to detect invasive species like using nets, traps, and visual observations, are cumbersome, the researchers from CCMB now have developed Environmental DNA (e-DNA) based molecular methods to provide a time and cost-effective alternative.
  - eDNA is defined as “genetic material obtained directly from environmental samples (soil, sediments, water etc.) without any obvious signs of the biological source material. It is an efficient, non-invasive and easy-to-standardize sampling approach. It can be obtained from ancient as well as modern environment. With scientific advancements in DNA sequencing technologies, the technique is increasingly being used for biodiversity monitoring.
  - CSIR-CCMB has designed a molecular assay utilizing eDNA to specifically detect this invasive catfish in Indian ecosystem, which is affordable and quick, and will be very useful tool in conservation management. They use a reliable eDNA-based quantitative PCR assay to detect the African Sharptooth Catfish from water samples in the aquatic system.

### 5) GM INSECTS

- A genetically modified (GM) insect refers to insects whose DNA has been engineered through various genetic engineering tools like CRISPR CAS9.
- Various GE insects are available globally today. The development and application of GE insects offers applications in various fields:
  - **Improving Human Health:**
    - **Vector Management** in human and livestock health: GE mosquitoes for e.g. can be designed to carry genes that limit their ability to transmit diseases such as dengue, malaria etc.
    - **Reduction in use of chemicals** -> Maintenance and improvement of both human health and environmental health.
  - **Food Security:**
    - **Management of crop insect pests:** Insects can be genetically engineered to carry traits that reduce the population of agricultural pests.

- » For e.g. introducing sterile males can help control pest population.
  - **Increased food production:** Protein production for healthcare purposes; honey production etc.
    - » Engineering honeybees to make better-quality and/or quantities of honey can contribute to reduced imports and may facilitate exports.
  - **Improvement in beneficial insects** like pollinators, predators, parasitoids etc.
  
  - **Economic Application:**
    - Other than improved agri production, improvements in productive insects (e.g. silkworm, lac insect) etc can promote economic growth.
      - » E.g. GE silkworms can produce finer and/or cheaper silk, affecting prices and boosting sales.
  
  - **Fighting pollution and ensuring environmental sustainability:**
    - Reduction in use of chemical will contribute to reduced pollution and environmental sustainability. Similarly, improved pollinators can contribute to biodiversity production.
    - Some GE insects can be used as bio-indicators to monitor pollution or detect some specific substance in environment.
- **Some Concerns:**
- **Ecological Risk:** Once introduced in the environment, it's very difficult to contain these insects. And if some future problem emerges, it would be difficult to control.
  - **Unforeseen health implications** when these GM insects interact with humans.
  - **Bioweapons:** GE insects may be used to produce bioweapons.
  - **Regulatory challenges:** Government guidelines like Guidelines for Genetically Engineered insects; National Guidelines for Gene Therapy Product Development and Clinical Trials' have similar ambiguity.
  - **Ethical concerns:** GE insects raise a question – “If human being should act as God” and make changes in the living organisms around it.

#### **A) GUIDELINES FOR GENETICALLY ENGINEERED (GE) INSECTS: RELEASED BY DBT IN APRIL 2023**

- The guidelines provide procedural roadmaps for those interested in creating GE insects.
  - It intends to help Indian researchers navigate regulatory requirements.
  - The guidelines are harmonized to guidance from WHO on GE mosquitoes.
  
- But **experts have identified some issues with the guidelines:**
  - b) **Uncertainty of Purpose:** The guidelines don't specify the purpose for which GE insects may be approved in India. It only provides regulatory procedures for R&D on insects with some beneficial applications.

- c) **Uncertainty for Researchers:** The guidelines are applicable only to research and not to confined trials or deployment.
  - » Government authorities will also have to closely follow the deployment of these insects. Once deployed, the GE insects can't be recalled, and unlike GM foods, they are not amenable to individual consumer choice.
- d) **Uncertainty of Ambit:** The guidelines offer SOPs for GE mosquitoes, crop pests, and beneficial insects – but what 'beneficial' means, in the context is GE insect is not clear.

## 9. OTHER TOPICS (ONLY CLASS DISCUSSION)

### 1) GENE MAPPING / GENE SEQUENCING

### 2) EARTH BIO GENOME PROJECT

### 3) DARK DNA

### 4) STEM CELL RESEARCH

- Adult Stem Cells
  - Induced pluripotent stem cells
- Embryonic Stem Cells
  - 1) **Totipotent Stem Cells:** These can differentiate into all possible types of stem cells.
  - 2) **Pluripotent Stem Cells:** These are the cells from an early embryo and can differentiate into any cell type.
  - 3) **Multipotent Stem Cells:** These differentiate into a closely related cell type. E.g., the hematopoietic stem cells differentiate into red blood cells and white blood cells.
  - 4) **Oligopotent Stem Cells:** Adult lymphoid or myeloid cells are oligopotent. They can differentiate into a few different types of cells.
  - 5) **Unipotent Stem Cells:** They can produce cells only of their own type. Since they have the ability to renew themselves, they are known as unipotent stem cells. E.g., Muscle stem cells.

### 5) CHIM STUDIES IN INDIA

### 6) SYNTHETIC BIOLOGY

## 10. RELEVANT PYQS

1	<p>Which of the following professional(s) are more likely to run the risk of permanent change in their cell's DNA? [Prelims 1996]</p> <ol style="list-style-type: none"> <li>1. Researchers using Carbon 14 isotope</li> <li>2. X-Ray Technician</li> <li>3. Coal Miner</li> <li>4. Dyer and Painter</li> </ol> <p>Select the correct answer using the codes given below:</p> <ol style="list-style-type: none"> <li>A. 2 alone</li> <li>B. 1, 2 and 3</li> <li>C. 1, 2 and 4</li> <li>D. 1, 3 and 4</li> </ol>
2	<p>Which of the following techniques can be used to establish the paternity of a child? [Prelims 1997]</p> <ol style="list-style-type: none"> <li>(a) Protein analysis</li> <li>(b) Chromosome counting</li> <li>(c) Quantitative analysis of DNA</li> <li>(d) DNA fingerprinting</li> </ol>
3	<p>[Prelims 1999]</p> <p>Assertion(A): Insect resistant transgenic cotton has been produced by inserting BT gene  Reason(R): The Bt gene is derived from a bacterium</p> <ol style="list-style-type: none"> <li>(a) Both A and R are true and R is the correct explanation of A</li> <li>(b) Both A and R are true and R is not a correct explanation of A</li> <li>(c) A is true and R is false</li> <li>(d) A is false and R is true</li> </ol>
4	<p>[Prelims 1999]</p> <p>Assertion(A): Dolly was the first cloned Mammal  Reason(R): Dolly was produced by in vitro fertilization</p> <ol style="list-style-type: none"> <li>(a) Both A and R are true and R is the correct explanation of A</li> <li>(b) Both A and R are true and R is not a correct explanation of A</li> <li>(c) A is true and R is false</li> <li>(d) A is false and R is true</li> </ol>
5	<p>[2000]</p> <p>Assertion(A): DNA fingerprinting has become a powerful tool to establish paternity and identity of criminals in rape and assault cases  Reason(R): Trace evidences such as hairs, saliva and dried semen are adequate for DNA analysis</p>

	<p>(a) Both A and R are true and R is the correct explanation of A          (b) Both A and R are true and R is not a correct explanation of A          (c) A is true and R is false          (d) A is false and R is true</p>
6	<p>Insect Resistant Cotton plants have been genetically engineered by inserting a gene from a/an [2000]          (a) virus          (b) bacterium          (c) Antibiotics          (d) Alcohol</p>
7	<p>The American multinational company, Monsanto, has produced an insect resistant cotton variety that is undergoing field trials in India. A toxic gene from which one of the following bacteria has been transferred to this transgenic cotton? [2001]</p> <p>A. <i>Bacillus Subtilis</i>          B. <i>Bacillus thuringiensis</i>          C. <i>Bacillus amyloliquefaciens</i>          D. <i>Bacillus globlii</i></p>
8	<p>With reference to latest developments in stem cell research, consider the following statements:</p> <ol style="list-style-type: none"> <li>1. The only source of human stem cells are the embryos at blastocyst stage</li> <li>2. The stem cells can be derived without causing destruction to blastocyst</li> <li>3. The stem cells can regenerate themselves in vitro virtually forever</li> <li>4. Indian research centres also created a few cell lines which can be developed into many types of tissues</li> </ol> <p>Which of the statements are correct?</p> <p>A. 1, 2 and 4          B. 1, 2 and 3          C. 3 and 4 only          D. 1 and 3</p>
9	<p>Genetically modified 'golden rice' has been engineered to meet human nutritional requirements. Which of the following statements best qualifies golden rice? [2010]</p> <p>(a) the grain has been fortified with genes to provide three times higher grain yield per acre than other high yielding varieties          (b) Its grains contain pro-vitamin A which upon ingestion is converted to vitamin A in the human body          (c) Its modified genes cause the synthesis of all the nine essential amino acids          (d) Its modified genes cause the fortification of its grains with vitamic D</p>

10	<p><i>At present, scientists can determine the arrangement or relative positions of genes or DNA sequences on a chromosome. How does this knowledge benefit us? (2011 Pre)</i></p> <ol style="list-style-type: none"> <li>1. It is possible to know pedigree of livestock.</li> <li>2. It is possible to understand the causes of all human diseases.</li> <li>3. It is possible to develop disease-resistant animal breeds.</li> </ol> <p>Which of the statements given above are correct?</p> <ol style="list-style-type: none"> <li>a. 1 and 2 only</li> <li>b. 2 only</li> <li>c. 1 and 3 only</li> <li>d. 1, 2 and 3 only</li> </ol>
11	<p><i>A genetically engineered</i> from of Brinjal, known as the Bt-brinjal, has been developed. The objective of this is [prelims 2011]:</p> <ol style="list-style-type: none"> <li>(a) to make it pest-resistant</li> <li>(b) to improve its taste and nutritive qualities</li> <li>(c) to make it drought resistant</li> <li>(d) to make its shelf-life longer</li> </ol>
12	<p>With reference to 'stem cells', frequently in the news, which of the following statements is/are correct? [2012]</p> <ol style="list-style-type: none"> <li>1. Stem cells can be derived from mammals only</li> <li>2. Stem cells can be used for screening new drugs</li> <li>3. Stem cells can be used for medical therapies</li> </ol> <p>Select the correct answer using the codes given below:</p> <ol style="list-style-type: none"> <li>(a) 1 and 2 only</li> <li>(b) 2 and 3 only</li> <li>(c) 3 only</li> <li>(d) 1, 2 and 3</li> </ol>
13	<p><b>What are the reasons for the people's resistance to the introduction of Bt brinjal in India (2012)</b></p> <ol style="list-style-type: none"> <li>1. Bt Brinjal has been created by inserting a gene from a soil fungus into its genome</li> <li>2. The seeds of Bt brinjal are terminator seeds and therefore, the farmers have to buy the seeds before every season from the seed companies</li> <li>3. There is an apprehension that the consumption of Bt Brinjal may have adverse impact on health</li> <li>4. There is some concern that the introduction of Bt brinjal may have adverse effect on the biodiversity</li> </ol> <p>Select the correct answer using the codes given below:</p>

	<ul style="list-style-type: none"> <li>a. 1, 2 and 3 only</li> <li>b. 2 and 3 only</li> <li>c. 3 and 4 only</li> <li>d. 1, 2, 3 and 4</li> </ul>
14	<p><i>Other than resistance to pests, what are the prospects for which genetically engineered plants have been created? (Prelims 2012)</i></p> <ul style="list-style-type: none"> <li>1. To enable them to withstand drought</li> <li>2. To increase the nutritive value of the produce</li> <li>3. To enable them to grow and do photosynthesis in spaceships and space and space stations</li> <li>4. To increase their shelf life</li> </ul> <p>Choose the correct answer from the codes provided below:</p> <ul style="list-style-type: none"> <li>A. 1 and 2 only</li> <li>B. 3 and 4 only</li> <li>C. 1, 2 and 4 only</li> <li>D. 1, 2, 3 and 4</li> </ul>
15	<p>Recombinant DNA technology (Genetic Engineering) allows genes to be transferred (Pre 2013)</p> <ul style="list-style-type: none"> <li>1. Across different species</li> <li>2. From Animals to plants</li> <li>3. From microorganisms to higher organisms</li> </ul> <p>Select the correct answer using the codes given below:</p> <ul style="list-style-type: none"> <li>a. 1 only</li> <li>b. 2 and 3 only</li> <li>c. 1 and 3 only</li> <li>d. 1, 2 and 3</li> </ul>
16	<p>The Genetic Engineering Appraisal Committee is constituted under the: [Prelims 2015]</p> <ul style="list-style-type: none"> <li>(a) Food Safety and Standards Act, 2006</li> <li>(b) Geographical Indications of Goods (Registration and Protection) Act, 1999</li> <li>(c) Environment (Protection) Act, 1972</li> <li>(d) Wildlife (Protection) Act, 1972</li> </ul>
17	<p>In the context of the development in Bio-informatics, the term 'Transcriptome', sometimes seen in the news, refer to: (Pre 2016)</p> <ul style="list-style-type: none"> <li>a. A range of enzymes used in genome editing</li> <li>b. The full range of mRNA molecules expressed by an organism</li> </ul>

	<p>c. The description of the mechanism of gene expression d. A mechanism of genetic mutations taking place in cells</p>								
18	<p>What is the application of Somatic Cell CJ Nuclear Transfer Technology? (Pre 2017)</p> <p>a. Production of bio larvicides b. Manufacture of biodegradable plastics c. Reproductive cloning of animals d. Production of organisms free of diseases</p>								
19	<p><b>Consider the following pairs:</b> [Prelims 2018]</p> <table border="1"> <thead> <tr> <th>Terms sometimes seen in news</th><th>Context/Topic</th></tr> </thead> <tbody> <tr> <td>i. Belle II Experiment</td><td>Artificial intelligence</td></tr> <tr> <td>ii. Blockchain Technology</td><td>Digital/ Cryptocurrency</td></tr> <tr> <td>iii. CRISPR – Cas9</td><td>Particle Physics</td></tr> </tbody> </table> <p>Which of the pairs given above are correctly matched?</p> <p>A. 1 and 3 only B. 2 only C. 2 and 3 only D. 1, 2 and 3 only</p>	Terms sometimes seen in news	Context/Topic	i. Belle II Experiment	Artificial intelligence	ii. Blockchain Technology	Digital/ Cryptocurrency	iii. CRISPR – Cas9	Particle Physics
Terms sometimes seen in news	Context/Topic								
i. Belle II Experiment	Artificial intelligence								
ii. Blockchain Technology	Digital/ Cryptocurrency								
iii. CRISPR – Cas9	Particle Physics								
20	<p>With reference to the Genetically modified mustard (GM mustard) developed in India, consider the following statements (Prelims 2018)</p> <ol style="list-style-type: none"> <li>1. GM Mustard has the genes of a soil bacterium that give the plant the property of pest resistance to a wide variety of pests</li> <li>2. GM Mustard has the genes that allow the plant cross-pollination and hybridization</li> <li>3. GM Mustard has been developed jointly by IARI and Punjab Agricultural University</li> </ol> <p>Which of the statements given above is/are correct?</p> <p>a. 1 and 3 only b. 2 only c. 2 and 3 only d. 1, 2 and 3 only</p>								
21	<p><b>What is cas9 protein that is often mentioned in news ?</b> (Pre 2019)</p> <p>(a) A molecular scissors used in targeted gene editing (b) A biosensor used in the accurate detection of pathogens in patients.</p>								

	<p>(c) A gene that makes plants pest-resistant          (d) A herbicidal substance synthesized in generally modified crops</p>
22	<p>With reference to the recent developments in science which one of the following statements is not correct? (Pre 2019)</p> <p>(a) Functional chromosomes can be created by joining segments of DNA taken from cells of different species          (b) Pieces of artificial functional DNA can be created in laboratories.          (c) A piece of DNA taken out from an animal cell can be made to replicate outside a living cell in a laboratory.          (d) Cells taken out from plants and animals can be made to undergo cell division in laboratory petri dishes.</p>
23	<p>'RNA interference (RNAi)' technology has gained popularity in the last few years. why? (Pre 2019)</p> <p>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 virtual pathogens.</p> <p>Select the correct answer using the code given below.</p> <p>(a) 1, 2 and 4          (b) 2 and 3          (c) 1 and 3          (d) 1 and 4 only</p>
24	<p>Bollgard I and Bollgard II technologies are mentioned in the context of: [Prelims 2021]</p> <p>(a) Clonal Propagation of crop plants          (b) Developing GM crop plants          (c) Production of plant growth substance          (d) Production of biofertilizers</p>
25	<p>Consider the following statements: [Prelims 2022]</p> <p>DNA Barcoding can be a tool to:</p> <ol style="list-style-type: none"> <li>1. Assess the age of a plant or animal.</li> <li>2. Distinguish among species that look alike.</li> <li>3. Identify undesirable animal or plant materials in processed foods.</li> </ol> <p>Which of the statements given above is/are correct?</p>

	<p>A. 1 only</p> <p>B. 3 only</p> <p>C. 1 and 2 only</p> <p>D. 2 and 3 only</p>
26	<p>Microsatellite DNA is used in the case of which one of the following? [Prelims 2023]</p> <p>A. Studying the evolutionary relationship among various species of fauna</p> <p>B. Stimulating 'stem cells' to transform into diverse functional tissues</p> <p>C. Promoting Clonal Propagation of horticulture plants</p> <p>D. Assessing the efficacy of drugs by conducting a series of drug trials in a population</p>