IMPORTANT TOPICS FROM BIOTECHNOLOGY

DNA

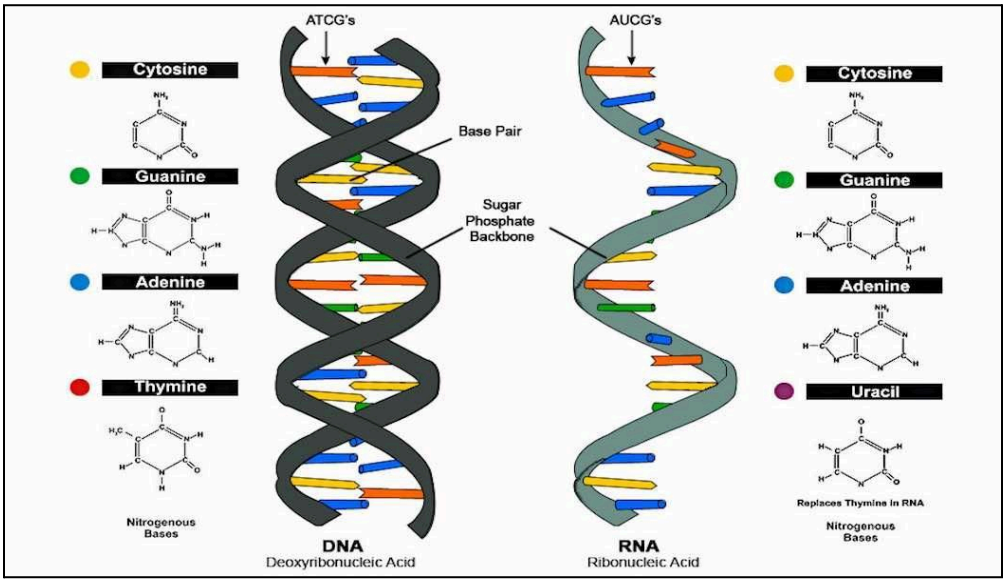
- Nucleic acid present in all organism: in the form of DNA and RNA.
- The structure of DNA defines the basic genetic makeup of the body.
- It carries and transmits the hereditary material/genetic instruction from parents their generation.
- Works for production of proteins.

Structure of DNA

- The DNA molecule consists of two strands that wind around one another to form a shape known as a double helix.
- Each strand has a backbone made of alternating sugar (deoxyribose) and phosphate groups.
- Attached to each sugar is one of four bases--adenine (A), cytosine (C), guanine (G), and thymine (T).
- The two strands are held together by bonds between the bases; adenine bonds with thymine, and cytosine bonds with guanine.

Application of DNA

- Solving crimes
- Identifying human remains
- Testing for paternity
- Genetic testing.
- Develops genetically transformed plants.



Junk DNA

Junk DNA, also known as non-functional DNA, refers to regions of DNA that do not code for proteins. Despite its name, it doesn't mean that these regions are useless or irrelevant. In fact, they perform important functions in genome regulation and other activities.

- **Definition**: Junk DNA is a DNA sequence that does not have a known biological function. Most organisms have some junk DNA in their genomes, which mostly consists of pseudogenes and fragments of transposons and viruses.
- **Function**: Although junk DNA does not directly form proteins, it plays a crucial role in regulating gene expression and other genomic activities.

- **Composition**: Junk DNA includes various types of non-coding DNA, such as introns, transposons, pseudogenes, regulatory elements, and repetitive sequences.
- **Controversy**: There is ongoing debate among scientists about the functionality of junk DNA. Some believe that these regions are preserved by natural selection, while others argue that they are merely genetic leftovers.

EXTRACHROMOSOMAL DNA (ecDNA)

Fundamentals of Cells and DNA

- Human cells contain 23 pairs of chromosomes (total 46 chromosomes).
- Chromosomes are thread-like structures located in the cell nucleus, made of DNA and proteins.
- DNA (deoxyribonucleic acid) is a molecule in the nucleus that carries genetic information in the form of genes, which provide instructions for building and maintaining an organism.
- Most DNA is found in chromosomes (nuclear DNA), but it can also exist as:
  - Mitochondrial DNA (mtDNA)
  - Extrachromosomal DNA (ecDNA)
  - Cell-free DNA (cfDNA)

About extrachromosomal DNA (ecDNA)

ecDNA are small circular DNA fragments that float freely in the nucleus, separate from chromosomes.

Formation of ecDNA:

It forms when fragments of DNA break away from the main chromosomes due to processes such as chromosomal damage or replication errors. This can occur through mechanisms like:

- Chromothripsis: A catastrophic event where chromosomes shatter and are reassembled incorrectly, leaving some fragments detached.
- Replication Errors: Mistakes during DNA replication can result in fragments being excluded from chromosomes.

Role of ecDNA in Cancer:

- ecDNA is present in up to 90% of certain tumor types, such as brain tumors, liposarcomas, and breast cancers.
- It often carries multiple oncogenes, which drive tumor growth and contribute to drug resistance.
- Oncogenes are mutated genes that can trigger cancer and are essential for activating tumor development.

Violates Mendel's Third Law:

- Mendel's Law states that genes located on different chromosomes are inherited independently, meaning they are passed on randomly to the next generation.

- However, ecDNA breaks this rule by keeping genes grouped together and passing them as a cluster during cell division. This helps cancer cells inherit beneficial genes more easily, speeding up tumor growth.
- Unlike regular chromosomes, which are distributed randomly, ecDNA is passed along as a package.

RNA

- **Structure of RNA**
  - RNA consists of ribose nucleotides (nitrogenous bases appended to a ribose sugar) attached by phosphodiester bonds, forming strands of various lengths.
  - The nitrogenous bases in RNA are adenine, guanine, cytosine, and uracil, which replaces thymine in DNA.
- **Utility of RNA**
  - Helps in synthesis of protein in our body.
  - Production of new cells in human body.
  - Translation of DNA into proteins.
  - Messenger between ribosome and the DNA.

- **Types of RNA**
  - tRNA (transfer RNA)- choose the right protein required by the body , which then helps the ribosomes.
  - rRNA (ribosomal RNA)- synthesis and translation of m RNA into proteins.
  - mRNA (messenger RNA)-transfers the genetic material to ribosomes and pass the instructions about the type proteins, necessary for blood cells.
- **Transcriptome:** The term "transcriptome" can also be used to describe the array of mRNA transcripts produced in a particular cell or tissue type. So a transcriptome is the full range of messenger RNA, or mRNA, molecules expressed by an organism.

RNA Editing

Context: US Based Biotech firm became first company to treat genetic condition through RNA editing at clinical trials.

- **RNA Editing:** It is a process where the RNA made from DNA is changed before it is used to make proteins. This means the final RNA can be slightly different from the original DNA instructions.
- **Key Points**
  - RNA editing happens after transcription (after RNA is copied from DNA).
  - It changes some letters (nucleotides) in the RNA sequence.
  - This can lead to different proteins being made from the same DNA.
  - It increases the variety of proteins in the body.

GENOME SEQUENCING

- **Genome:** A genome is the **complete set of genetic information** in an organism. It provides all of the information the organism requires to function.
  - All living things (bacteria, plants, and mammals) have a distinct genetic code, or genome, **made up of nucleotide bases (A, T, C, and G).**
  - An organism's own DNA fingerprint, or pattern, can be detected if one knows **the bases' sequence.**

- **Sequencing:** It is the process of **establishing the order of bases**.
    - An organism's **genome's base order can be determined** in one step via a laboratory approach called whole genome sequencing.

Transcription and Translation

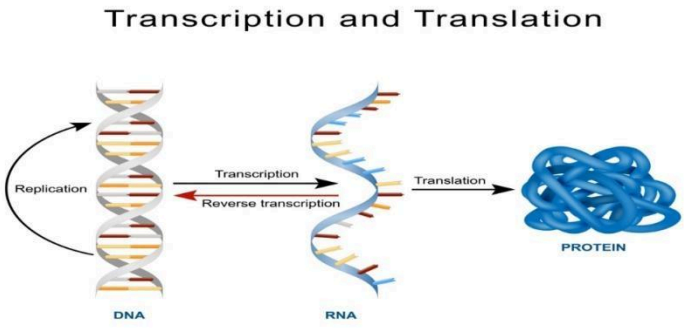
- **Protein synthesis:** It is the process in which cells make proteins. It occurs in two stages: transcription and translation.

Transcription

- It is the **transfer of genetic instructions in DNA to mRNA** in the **nucleus**.
- After the mRNA is processed, it carries the instructions to a ribosome in the cytoplasm.
- Thus, transcription is regarded as the first step of gene expression.

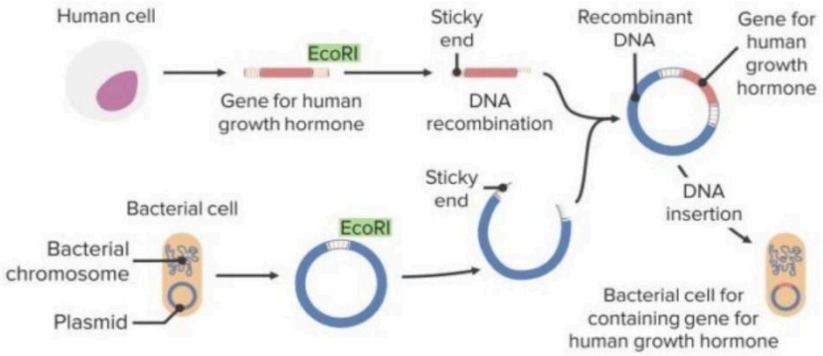
Translation

- Translation occurs at the **ribosome**, which consists of **rRNA and proteins.**
- The instructions in mRNA are read, and tRNA brings the correct sequence of amino acids to the ribosome.
- Then, rRNA helps bonds form between the amino acids, producing a **polypeptide chain**.
- After a polypeptide chain is synthesized, it may undergo additional processing to form the finished protein.



Recombinant DNA technology

- Recombinant DNA (rDNA) is a technology that **uses enzymes to cut paste together DNA sequence of interest**.
- The **recombinant DNA sequence** can be placed into **vehicle** called **vectors** that ferry the DNA into a suitable host cell where it can be copied or expressed.



Tools involved in Recombinant DNA technology

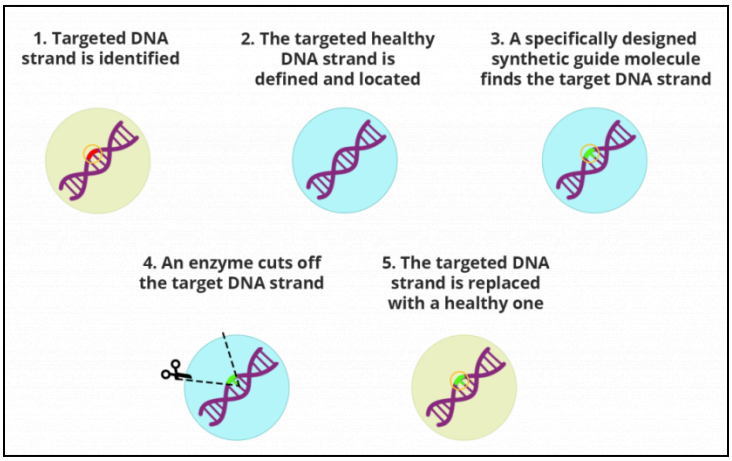
- **Restriction Enzymes:**
  - Restriction enzymes belong to a larger class of enzymes called nucleases.
  - These are of two kinds; exo-nucleases and endo-nucleases.
  - Exo-nucleases remove nucleotides from the ends of the DNA whereas endonucleases make cuts at specific positions within the DNA.
  - Restriction endonucleases are used in genetic engineering to form 'recombinant' molecules of DNA, which are composed of DNA from different so urces/genomes.
- **Separating DNA molecules:**
  - The most common separation technique used is gel electrophoresis.
  - This technique takes advantage of the negative charge on DNA molecules by using an electrical field to provide the force necessary to separate DNA molecules based on size.
  - The separated DNA fragments can be visualised only after staining the DNA with a compound known as ethidium bromide followed by exposure to UV radiation.
- **Methods of INSERTION OF rDNA in Host Cells**
  - **Micro-injection:** Recombinant DNA is directly injected into the nucleus of an animal cell.
  - **Biolistics or gene gun:** Cells are bombarded with high velocity micro-particles of gold or tungsten coated with DNA in a method known as biolistic or gene gun.
  - This method is suitable for plants
- **Steps involved in process are:**
  1) **Isolation of genetic material:** it is to isolate the desired DNA in its pure form I.e. free from other macromolecules.
  2) **Restriction enzyme digestion:** restriction enzymes act molecular scissors that cut DNA at specific locations. These reactions are called restriction enzyme digestion.
  3) **Amplification using PCR:** polymerase chain reaction or PCR is a method of making multiple copies of a DNA sequence using the enzyme z-DNA polymerase in vitro.
  4) **Ligation of DNA molecules:** the process of joining two pieces together using the enzyme DNA ligase is a ligation.
     - The resulting DNA molecules is a hybrid of two DNA molecules
     - Hence this new hybrid DNA is also called a rDNA molecule and technology is referred as recombinant DNA technology.
  5) Insertion of recombinant DNA into host
  6) Isolation of recombinant cells.
- **Applications**
  - Disease diagnosis- used to detect the presence of HIV in a person
  - Food and agriculture- manufacture of GM crops and climate resilient crop
  - Medicines- production of insulin
  - **Gene therapy-** it is used an attempt to correct the gene defects which give rise to hereditary diseases
  - Development of vaccines and recombinant hormones

Gene Editing

- **Biotechnology** deals with techniques of using live organisms or enzymes from organisms to produce products and processes useful to humans.
- The **two core techniques** that enabled birth of modern biotechnology are:

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

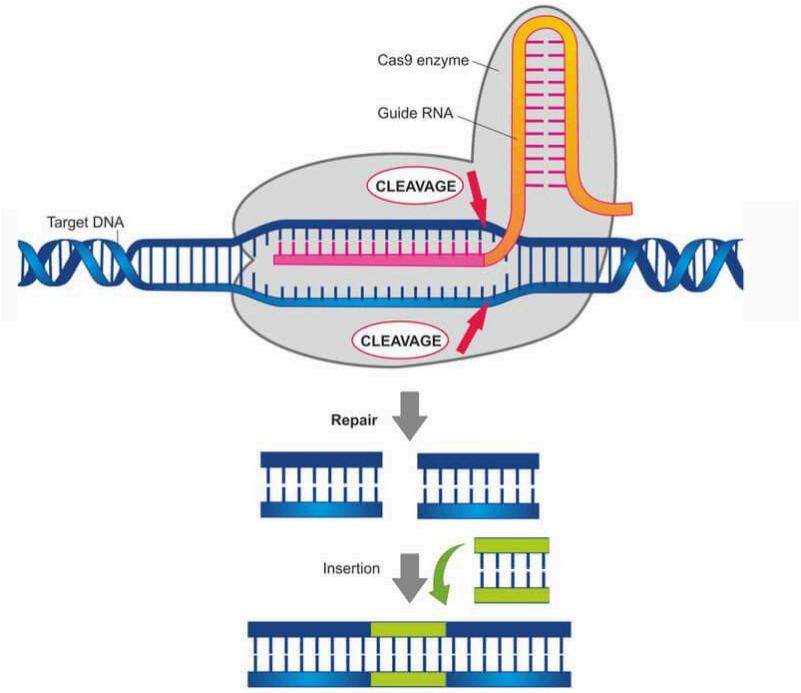
- **Types of gene editing**
  - **Germline editing:** The genome of an individual is edited in a way that the change is heritable. This is achieved through genetic alterations within the germ cells, or the reproductive cells, such as the egg and sperm.
  - **Somatic cell gene editing:** Introduction of genes into bone marrow cells, blood cells, skin cells that will not be inherited later generations.
- **How gene editing is done**
  - Enzymes which cut DNA are known as engineered nucleases.
  - It is performed using enzymes, particularly nucleases whose purpose is to target a specific DNA sequence, where they introduce cuts into the DNA strands which enables the removal of existing DNA and the insertion of replacement DNA.



- **Applications-**
  - Treating inherited diseases ☾ sickle cell anemia
  - Helps to understand what specific genes do
  - Prevents inheritance of genetic diseases
  - Generates more resilient crops
  - Detecting species in environment
- **Genome Editing Techniques:** The core technologies now most used to facilitate genome editing, are :
  - **Clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9)**
  - Transcription activator-like effector nucleases (TALENs)
  - Zinc-finger nucleases (ZFNs)
  - Homing endonucleases or mega nucleases

Clustered regularly interspaced short palindromic repeats (CRISPR) and Cas9

- **CRISPR:** It is the **DNA-targeting component** of the system, and it is made **up of an RNA molecule**, or **'guide**,' that is engineered to attach to certain DNA bases via complementary base-pairing.
- **CRISPR-associated protein 9 (Cas9**): It is the **nuclease component** that cuts the DNA.
    - It is an **enzyme**.
    - It relies on guide RNA to find the DNA sequence of interest. It will then introduce a double strand break at a specific location within a strand of DNA.
- The CRISPR-Cas9 genetic scissors were discovered by E**mmanuelle Charpentier and Jennifer A. Do udna**, who won the **Nobel Prize in Chemistry in 2020**



Gene Therapy

- It is a therapy that **uses genes to prevent diseases**.
- It might allow doctors to **treat a disorder by inserting a gene into a patient's cells** instead of using drugs or surgery.
- **Types of gene therapy: Somatic** cell gene therapy and **Germline** gene therapy.
- **Advantages**
    - Gene therapy has the potential to eliminate and prevent hereditary disease.
    - It is possible to cure heart diseases, cancer and AIDS.
    - It can be used to discard diseases from the future generation.

Types of cells (from perspective of Gene editing)

Somatic cells	Stem cell	Reproductive cell

• Somatic cells are diploid cells, which contain two pairs of chromosomes, one received from each parent. • Any cell other than germ cells (sperm and egg), gametocytes (cells that divide to form germ cells), and undifferentiated stem cells are known as somatic cells. • Somatic cells are not capable of producing offspring. But, they form all the internal organs and tissues and contribute to their functionalities. • They are responsible for growth, repair and regeneration. • Some of the specialized somatic cells are: - Skin cells - Muscle cells - Nerve cells - Blood cells.	• Stem cells are body's raw material, i.e. cells from which all other cells with specialized functions are generated. • They can divide through mitosis□ single cell divided into two identical daughter cells. • Limitlessly to replenish other cell types of multi-cellular organisms throughout their life. • After stem cell division, each newly produced cell can either remain as a stem cell or differentiate to form any other cell type with more defined functions, such as muscle cell, blood cell, or neural cell. • Types of stem cells: - Embryonic Stem Cells. - Embryonic germ cells. - Adult Stem Cells. • Application – - Treatment of brain diseases. - Treatment of blood diseases. - Treatment of cardiovascular. - Tissue regeneration.	• A cell whose nucleus unites with that of a cell of the opposite sex to form a new organism□ reproductive cell. • Gametes are an organism's reproductive cells□ sex cells. • Female gametes are called ova or egg cells, and male gametes are called sperm. • Gametes □these are haploid cells, and each cell carries only one copy of each chromosome. • Gamete is capable of fusing with another haploid reproductive cell to form a **diploid zygote**. The zygote is formed by the fusion (or combining) of two gametes, i.e. male gamete and female gamete. This union of gametes resulting in a zygote is called **fertilization.** • These reproductive cells are produced through **meiosis.**

Embryonic stem cells

- Found in the three germ layer of the embryo.
- Inner cell mass of the embryo contains embryonic stem cells
- Embryonic stem cells are the most potent, as their job is to become every type of cell in the body. The full classification includes:
    - **Totipotent**: These stem cells can differentiate into all possible cell types. The first few cells that appear as the zygote starts to divide are totipotent.
    - **Pluripotent**: These cells can turn into almost any cell. Cells from the early embryo are pluripotent.

- **Multipotent**: These cells can differentiate into a closely related family of cells. Adult hematopoietic stem cells, for example, can become red and white blood cells or platelets.
- **Oligopotent**: These can differentiate into a few different cell types. Adult lymphoid or myeloid stem cells can do this.
- Embryonic stem cells are considered pluripotent instead of totipotent because they cannot become part of the extra-embryonic membranes or the placenta.

Stem cell therapy

- It is the introduction of new adult stem cells into damaged tissue in order to treat an ailment or injury.
- The ability of stem cells to self-renew and give rise to different cells that can potentially replace diseased and damaged areas in the body with minimal side effects.
- A number of stem cell therapies exist, but most are at experimental stages, costly or controversial.
- Any disease which involves tissue degeneration can be a potential disease for stem cell therapies. Some of them are:
  - Tissue repair
  - Alzheimer's disease
  - Heart disease
  - Severe burns
  - Diabetes
  - Spinal cord injury
  - Leukemia and cancer
  - Rheumatoid arthritis

Animal Cloning

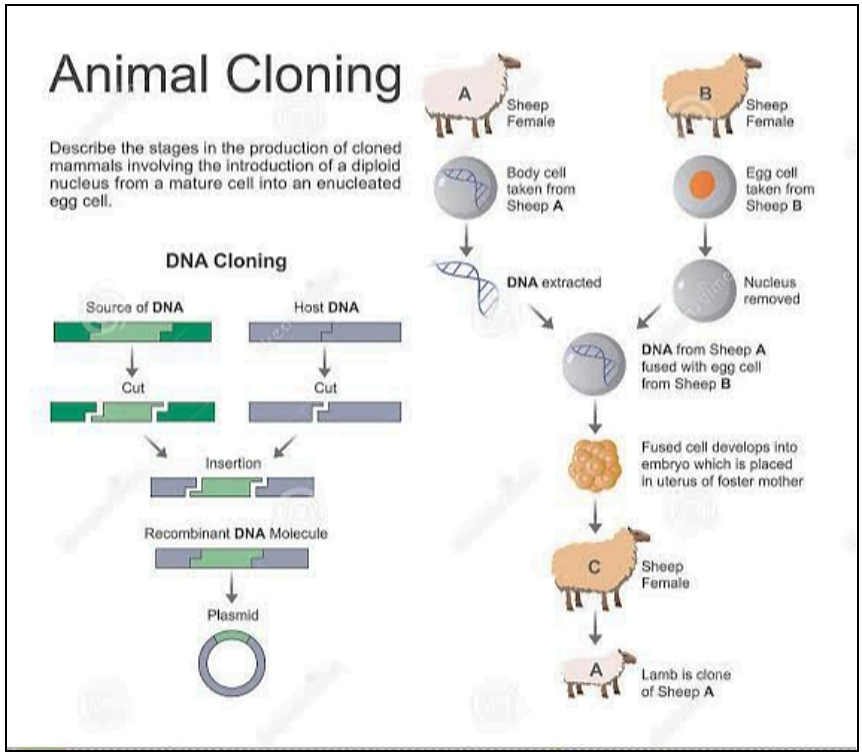
Cloning: it is the process of generating identical copy of a cell or an organism.

Animal cloning

- Procedure of creating a whole new multi-cellular organism which is genetically identical to original organism.
- Non-sexual process
- No fertilization or inter-gamete contact takes place.

Different levels of cloning

- Molecular cloning
  - Isolation of DNA sequence from any species (often a gene) in its insertion into a vector (host bacterium) for propagation without alteration.
- Cellular cloning
  - Copies of cells are made resulting in the formation of identical cells from a single cell. This type of cloning is used in stem cell research.
- Organism cloning
  - A multi-cellular organism is created, genetically identical to other organism.



Types of cloning

- Gene cloning
    - It creates copies of genes or segments of DNA.
- Reproductive cloning
    - It involves the implantation of a cloned embryo into a real or an artificial uterus. The embryo develops into a fetus that is genetically identical to the donor of the original nucleus.
- Therapeutic/ biomedical cloning
    - It is used to clone embryos for the purpose of extracting stem cells for use in replacing or repairing damaged tissues or organs, achieved by transferring a diploid nucleus from a body cell into an egg whose nucleus has been removed.

Applications of cloning-

- Biomedical: disease models, bioreactors.
- Agricultural:
    - can be used to create copies of animals with highly valued traits, like dairy cow with high milk production
    - Livestock improvement
    - Genetic improvement
    - Conservation of endangered species.
- Research
    - Stem cell research
    - Understanding embryology.

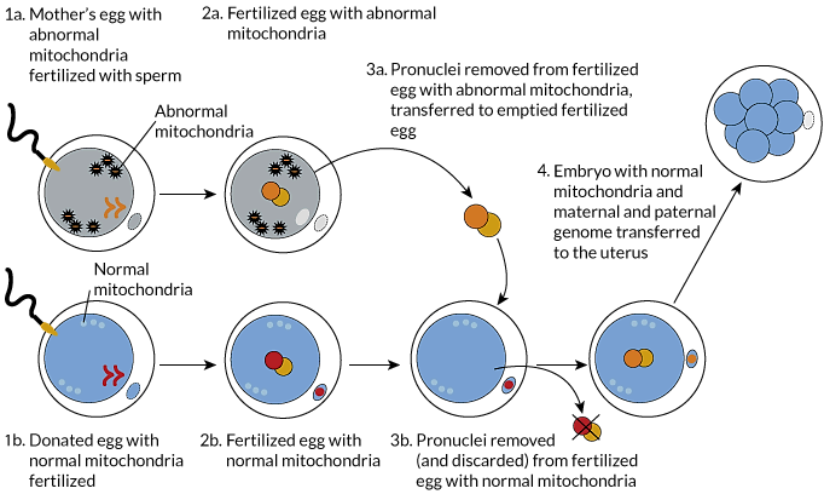
Criticism

- Cloning contradicts natural reproductive process and hence has its negative effects-
- Sometimes a clone is not able to perform metabolic process like eating, drinking, etc.
- Clone may have short life span.

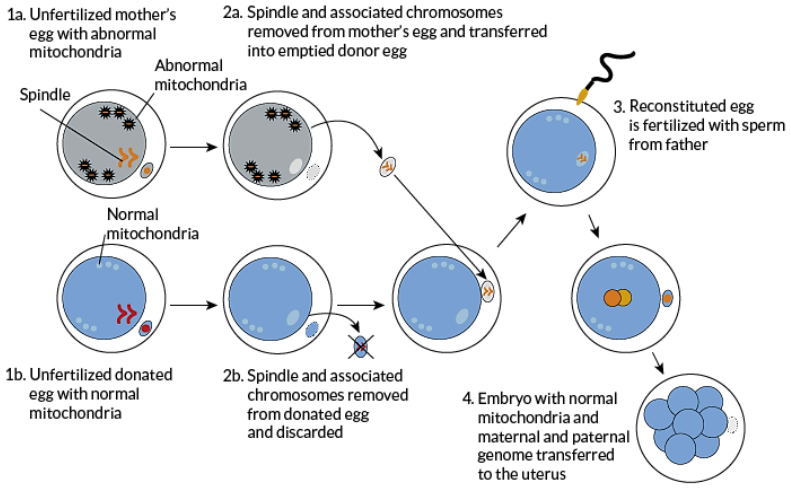
- They can experience developmental anomalies.
- Reproductive cloning is considered unnatural by some and hence it can violate religious belief.

3- Parent baby

- A baby in which the vast majority of nuclear DNA comes from the mother and the father and a small amount of mitochondrial DNA comes from a female donor.
- This reproductive technology focuses on reducing the effects of mutations that occur in the DNA of mitochondria, which reside in cytoplasm. This could prevent the transmission to their offspring of mitochondria diseases.
- There are **two main techniques** involved:
- **Mitochondrial spindle transfer:**
    - Inside laboratory, scientists remove the nucleus from a healthy donor egg and replace it with a nucleus taken from the egg cell of a woman who carries a rare neurological disease called Leigh syndrome.
    - This leaves the donor's healthy mitochondria intact. The scientists then fertilize the modified egg with the father's sperm.
    - After this the 'reconstructed embryo' is implanted into the mother's uterus.



- **Pronuclear transfer:**
    - At the time of fertilization, genetic material is fused. One set comes from the egg and another comes from dad's sperm.
    - At early stage in development, the two (egg and sperm) have not yet fused into a single nucleus. This is called Pronuclei.
    - Pronuclei is the central, DNA-containing parts of fertilized eggs.
    - In this technique researchers fertilize the mother mouse's egg and a donor egg at the same time. The pronuclei are removed from the donor's fertilized egg and discarded. These are then transferred to an egg cell containing a healthy mitochondria.



Biofilms

Context: Researchers at IISc have developed a technique using enzymes from the cow's gut to break down biofilm barriers. This innovation may significantly enhance treatment of drug-resistant infections, especially in hospital settings.

About:

- Biofilms are protective layers formed by bacteria to shield themselves from external threats.
- These structures consist of sugars, proteins, lipids, and DNA.
- Found on various surfaces, including medical devices, tissues, and water pipelines.

Bridge Recombinase Mechanism (BRM)

Context: Scientists have discovered a naturally existing DNA editing tool called the Bridge Recombinase Mechanism (BRM). This breakthrough could revolutionize gene editing by allowing complex modifications of long DNA sequences.

- **About:** The Bridge Recombinase Mechanism is a naturally occurring genetic editing system found across all forms of life. It operates using mobile genetic elements, commonly known as jumping genes.
- Jumping genes are small DNA segments that can move from one location in the genome to another.
- They consist of:
    - A recombinase enzyme that cuts and pastes DNA.
    - Special DNA sequences at the ends that help bind and manipulate DNA.
- These genes can insert, delete, duplicate, invert, or rearrange segments of DNA, enabling real-time, flexible genome editing
- **BRM Different from other tools**: Unlike artificial tools like CRISPR-Cas9, BRM:
    - Is naturally existing
    - Can manipulate very long DNA sequences
    - Is capable of more complex and dynamic editing operations

CAR T-Cell Therapy

CAR T-cell therapy is an advanced form of **immunotherapy** where a patient's T cells are genetically modified to attack cancer. These modified cells produce **chimeric antigen receptors (CARs)** that recognize and destroy cancer cells.

Key Steps:

1. **T Cell Collection** – T cells are separated from the patient's blood.
2. **Genetic Engineering** – T cells are modified in a lab to express CARs.
3. **Expansion** – Modified cells are multiplied.
4. **Preparation** – Patient may receive chemo to help CAR T cells work better.
5. **Infusion** – CAR T cells are infused back into the body.
6. **Monitoring** – Patients are observed for response and side effects.

Advantages:

- Targets cancer cells specifically
- May offer long-term remission
- Helps patients unresponsive to other treatments
- Expanding use beyond blood cancers

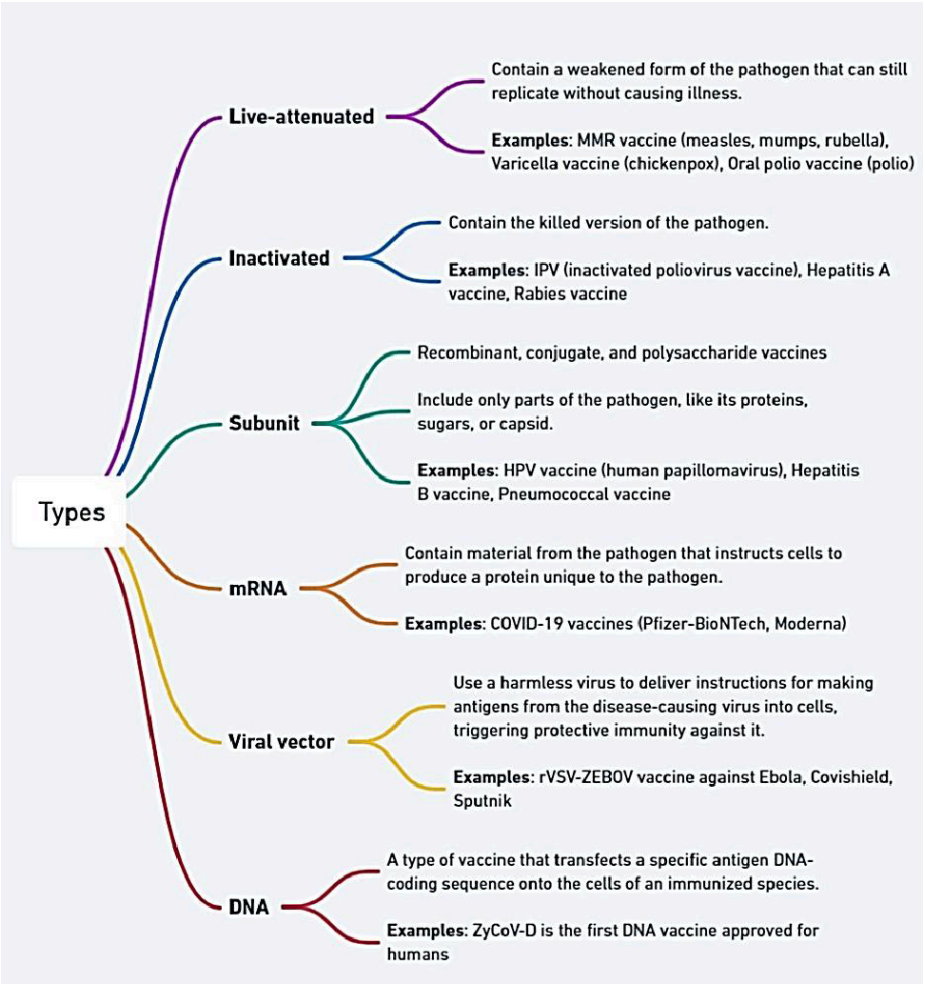
Genome India Project

Context: India has successfully completed the sequencing of 10,000 human genomes from 83 population groups across the country.

- **About:** The Genome India Project is a national initiative to sequence and catalogue the genetic makeup of Indian populations.
- It is funded and coordinated by the Department of Biotechnology (DBT), Ministry of Science & Technology.
- The data is now available to researchers worldwide through the Indian Biological Data Centre (IBDC), Faridabad.
- Inspired by Human Genome Project.

Vaccination

- Vaccination is the process of administering a vaccine to stimulate an individual's immune system to develop adaptive immunity to a pathogen.
- **Working:**
    - **Exposure to Antigens**: The vaccine introduces antigens into the body, which the immune system detects as foreign.
    - **Immune Response Activation**: This detection triggers an immune response, leading to the production of antibodies.
    - **Memory Cell Formation**: Some of the immune cells become memory cells, which will remember the antigen.
    - **Long-term Immunity**: If the actual pathogen later infects the body, these memory cells recognize and respond more rapidly and effectively.


Types
Live-attenuated
Contain a weakened form of the pathogen that can still replicate without causing illness.
Examples: MMR vaccine (measles, mumps, rubella), Varicella vaccine (chickenpox), Oral polio vaccine (polio)
Inactivated
Contain the killed version of the pathogen.
Examples: IPV (inactivated poliovirus vaccine), Hepatitis A vaccine, Rabies vaccine
Subunit
Recombinant, conjugate, and polysaccharide vaccines
Include only parts of the pathogen, like its proteins, sugars, or capsid.
Examples: HPV vaccine (human papillomavirus), Hepatitis B vaccine, Pneumococcal vaccine
mRNA
Contain material from the pathogen that instructs cells to produce a protein unique to the pathogen.
Examples: COVID-19 vaccines (Pfizer-BioNTech, Moderna)
Viral vector
Use a harmless virus to deliver instructions for making antigens from the disease-causing virus into cells, triggering protective immunity against it.
Examples: rVSV-ZEBOV vaccine against Ebola, Covishield, Sputnik
DNA
A type of vaccine that transfects a specific antigen DNA-coding sequence onto the cells of an immunized species.
Examples: ZyCoV-D is the first DNA vaccine approved for humans