Session 5-6: Stem Cells, Gene Therapy, Gene Editing & CRISPR, Cloning, Mitochondrial Replacement Therapy, DNA Barcoding

Table of Contents

Stem Cells

Introduction to Cells and Stem Cells

Cells are the fundamental and structural units of life, forming the basis of all organisms. They range from being unicellular to multicellular and are integral in providing structure and converting nutrients into energy. Cells are the most basic level of organization in all life forms.

Stem cells differ significantly from regular cells. They possess the unique ability to develop into various specialized cell types in the body. Stem cells are essential for growth, as they generate new cells, and replace specialized cells that are damaged or lost.

Properties of Stem Cells

Stem cells are characterized by three distinct properties:

1. **Capability of dividing and renewing themselves** for prolonged periods.
2. **Unspecialized nature**, meaning they do not have tissue-specific structures to perform specialized functions.
3. **Potential to differentiate** into specialized cell types.

Types of Stem Cells based on Source

Type of Stem Cell	Source	Characteristics/Functions
Adult Stem Cells	Brain, bone marrow, blood vessels, skeletal muscles, skin, liver	Can self-renew indefinitely; produce various cell types from the originating organ
Embryonic Stem Cells	Blastocysts (4-5 days old); sourced from extra IVF embryos	Can develop into any cell type in the body
Induced Pluripotent Stem Cells	Lab-created from tissue-specific cells	Similar to embryonic stem cells; can give rise to all different cell types in the body
Mesenchymal Stem Cells	Connective tissues (stroma) around tissues and organs	Initially found in bone marrow; can develop into bones, fat cells, cartilage

Types of Stem Cells based on Potency

Type of Stem Cell	Source	Characteristics/Functions
Totipotent Stem Cells	Fertilized eggs	Can differentiate into any cell type, including extra-embryonic cells

Pluripotent Stem Cells	Embryos, induced pluripotent stem cells	Can differentiate into all cell types except extra-embryonic cells
Multipotent Stem Cells	Various tissues (e.g., bone marrow, neural tissue)	Can develop into a limited number of cell types within a specific lineage
Oligopotent Stem Cells	Certain tissues (e.g., skin, intestine)	Can differentiate into a few closely related cell types
Unipotent Stem Cells	Certain tissues (e.g., hair follicles, blood)	Can produce only one cell type, but have self-renewal capability

iPS Cells

iPS Cells (Induced Pluripotent Stem Cells)

iPS cells, also known as **induced pluripotent stem cells**, are **a type of stem cell that can be generated from adult somatic cells through genetic reprogramming**.

iPS cells have various applications in the field of regenerative medicine and biotechnology.

Discovery of iPS Cells

- **Discovery**: iPS cells were first described in **2006** by **Shinya Yamanaka**, a Japanese physician and researcher, along with his colleagues, using mouse cells.
- **Human Cell Derivation**: In **2007**, Yamanaka successfully derived iPS cells from human adult fibroblast cells.
- **Nobel Prize Recognition**: For this groundbreaking discovery, Shinya Yamanaka was awarded the **2012 Nobel Prize** in Physiology or Medicine, shared with Sir John Gurdon.

Development of iPS Cells

- **Generation Process**: iPS cells are generated from adult somatic cells, like skin fibroblasts or peripheral blood mononuclear cells (PBMCs), through **genetic reprogramming**.
- **Key Factors**: The process involves introducing reprogramming genes, namely **Oct4, Sox2, Klf4, and c-Myc** (collectively known as **Yamanaka factors**), which transform somatic cells into pluripotent stem cells.

Applications of iPS Cells

1. **Drug Screening**: iPS cells are utilised for testing the **efficacy and safety** of new drugs.
2. **Disease Modeling**: They aid in understanding diseases on a **patient-specific basis**.
3. **Cell Therapy**: iPS cells can differentiate into any desired cell type for transplantation to replace damaged or diseased cells.
4. **Personalized Medicine**: The possibility of generating patient-matched iPS cells allows for the creation of individual-specific pluripotent stem cell lines.
5. **Industrial-Scale Manufacturing**: iPS cells have the potential for large-scale cell production for various applications.
6. **Future Prospects**: Other potential applications include **clean meat production**.

Current Status

While iPS cell technology holds significant promise, it is currently at a stage where therapeutic transplants are not yet deemed safe. The primary use of iPS cells remains in research and drug discovery.

Stem Cell Therapy

Stem cell therapy involves using stem cells to treat or prevent a disease or condition. This field has gained significant attention due to its potential in treating various diseases.

Therapeutic Use	Principle of Treatment	Application
Blood Stem Cell Transplantation	Use of blood stem cells to replace diseased or damaged bone marrow	Treating leukemia, lymphoma, and certain anemias; involves transplanting healthy stem cells to restart blood cell production
Skin Grafts for Burn Victims	Growth of new skin cells from stem cells	Generating new skin tissue in the lab for severe burn patients, followed by grafting onto burn sites
Corneal Regeneration	Regeneration of corneal tissue using limbal stem cells	Treatment for corneal blindness and other corneal injuries by regenerating damaged corneal tissue
Regenerative Heart Therapy	Repair of damaged heart tissue with stem cells	Experimental treatments for heart attack damage; injecting stem cells to

		improve heart function and reduce scar tissue
Neurodegenerative Diseases	Replacement of damaged neurons in the brain	Clinical trials for Parkinson's and Alzheimer's, focusing on restoring cognitive and motor functions
Type 1 Diabetes Treatment	Differentiation into insulin-producing cells	Clinical trials using transplanted insulin-producing cells to regulate blood sugar levels in type 1 diabetes patients
Musculoskeletal Disorders	Differentiation into bone, cartilage, or muscle cells	Treatment for osteoarthritis and other joint issues through MSC injections to reduce inflammation and regenerate cartilage
Spinal Cord Injuries	Regeneration of nerve cells or repair of myelin sheath	Clinical trials testing stem cells for nerve tissue regeneration to restore function and mobility in spinal cord injuries

Integration of Stem Cells in Gene Therapy

- **Procedure**: In gene therapy, stem cells are often utilized due to their adaptability. The process typically involves **removing blood-forming stem cells** from the patient. These cells are responsible for creating all types of blood and immune cells.
- **Gene Modification**: Once extracted, a **viral vector** is used to introduce a new or corrected copy of a gene into the DNA of the patient's cells. This is done in a laboratory setting.
- **Application**: The modified cells are then reintroduced into the patient’s body, where they can produce cells with the corrected gene, thereby treating or mitigating the disease.

Cord Blood Banks

Overview

Cord blood banks are specialized facilities that store **umbilical cord blood** for future medical use. The cord blood is a rich source of **stem cells**, which have the potential to treat a variety of life-threatening diseases.

Creation and Purpose

- **Establishment**: Cord blood banks were established to harness the potential of cord blood in treating blood and immune system diseases.
- **Collection Process**: After a baby's birth, cord blood is collected from the umbilical cord and placenta. This process is simple, painless, and safe for both the newborn and the birthing individual.
- **Processing and Storage**: The collected blood is then screened, tested, processed, frozen, and stored for future use.

Benefits

1. **Life-Saving Potential**: Cord blood contains stem cells that can be vital in treating diseases like leukemia, genetic disorders, and immune system diseases.
2. **Ease of Collection**: Compared to bone marrow collection, cord blood collection is less complicated and poses minimal risk to the donor.
3. **Resource for Medical Advancements**: Stored cord blood is a valuable asset for future medical research and treatments.

Limitations

1. **Limited Stem Cell Quantity**: The amount of stem cells in cord blood is relatively small, often requiring multiple donors for adult transplants.
2. **Storage Costs**: Private cord blood banking can be expensive, with ongoing fees for storage.
3. **Collection Fees**: Some hospitals might charge for public cord blood collection.
4. **Availability of Service**: Not all hospitals offer cord blood collection for public storage.

Regulations of Stem Cells in India

In India, stem cell therapy, along with **stem cell banking** and **preservation**, is a growing field. Regulations are in place to ensure the ethical and safe use of stem cells in medical practices.

ICMR Guidelines: National Guidelines for Stem Cell Research (NGSCR) 2017

- The **Indian Council of Medical Research (ICMR)** has issued comprehensive guidelines — **National Guidelines for Stem Cell Research (NGSCR) 2017** — for stem cell research.
- These guidelines cover:
  - Ethical and scientific considerations
  - Categorization of research
  - Delineation of responsibilities of various stakeholders
- Stem cells are categorised into different groups, with guidelines specifying:
  - Permissible research areas
  - Restrictive research areas
  - Prohibited research areas

New Drugs and Clinical Trial Rules, 2019

- The **'New Drugs and Clinical Trial Rules, 2019'**, introduced by the Union Health Ministry, classify stem-cell-derived products as "**new drugs**".
- This classification **requires doctors to seek government permission for using new stem-cell therapy**.
- The rules prioritize transparency and accountability in the approval process.
- The rules also promote research and development.

Government Support and Funding

- The Government, via the **ICMR**, **Department of Biotechnology (DBT)**, and **Department of Science and Technology (DST)**, supports stem cell research.
- More than **40 health research and educational institutes** have developed advanced infrastructure for this field, complementing industry initiatives.
- The DBT has invested **Rs. 73.46 Crore** in stem cell-related projects, encompassing basic biology, translational research, gene editing technologies, and creation of animal models for human diseases.

Gene Therapy

What is Gene Therapy

Gene Therapy is a medical intervention that involves the modification or manipulation of genetic material within an individual's cells. This process is used to treat or prevent diseases.

The methods include replacing a malfunctioning gene with a healthy copy, deactivating a harmful gene, or introducing a new or modified gene to combat disease.

Thus, it generally entails the following:

1. introduction of DNA within the cells,
2. alteration of DNA within the cells, or
3. removal of DNA within the cells.

Need for gene therapy

1. **Treatment of Genetic Disorders:** Gene therapy is crucial for treating genetic disorders where conventional treatments are ineffective or unavailable. It offers a potential cure by correcting the underlying genetic defect, rather than just addressing the symptoms.
2. **Potential to Treat a Wide Range of Diseases:** Its applicability extends beyond genetic disorders to a wide range of diseases, including cancer, viral infections, and degenerative diseases.
3. **Personalised Medicine:** Gene therapy allows for personalised treatment strategies tailored to an individual’s genetic makeup. This personalised approach can improve treatment efficacy and reduce the likelihood of adverse reactions.
4. **Long-lasting Effects:** Unlike traditional treatments, many gene therapies have the potential to provide long-lasting or even permanent therapeutic effects with a single treatment.
5. **Advancements in Medical Research and Biotechnology:** Gene therapy drives innovation in medical research and biotechnology, leading to new discoveries and technologies.

Beginning of Gene Therapy

The first approved gene therapy procedure was performed on September 14, 1990, by **Dr. French Anderson** and his colleagues at the NIH of the USA. They treated a four-year-old girl, **Ashanthi DeSilva,** born with **adenosine deaminase deficiency, a genetic disorder that severely weakens the immune system**. The disease is called **Severe combined immunodeficiency (SCID)**. The term "**bubble baby**" is

often used to describe infants with SCID because they are extremely vulnerable to infections and must be protected from exposure to infectious agents.

This treatment involved the insertion of a correct gene **adenosine deaminase** into her white blood cells, partially restoring her immune function.

Human Genetic Diseases Targeted by Gene Therapy

Gene therapy shows promise in treating various **genetic diseases**, including:

- **Cystic Fibrosis**: Impacts the lungs and digestive system.
- **Hemophilia**: A disorder leading to impaired blood clotting.
- **Sickle Cell Anemia**: Causes red blood cells to become sickle-shaped.
- **Muscular Dystrophy**: Leads to muscle degeneration and weakness.
- **Severe Combined Immunodeficiency (SCID)**: A group of disorders severely weakening the immune system.

Types of Gene Therapy

1. **Somatic Cell Gene Therapy**
    - Involves the transfer of therapeutic genes to a patient's **somatic cells**, which are any cells other than reproductive cells.
    - The modifications and effects are restricted to the patient and are not inherited by future generations.
    - Somatic cell gene therapy has been used in clinical trials to treat various diseases, including **cystic fibrosis**, certain **infectious diseases**, and single-gene disorders like **haemophilia** and **thalassaemia**.
2. **Germ Line Gene Therapy**
    - Involves the modification of **reproductive cells**, such as sperm and egg cells, by the introduction of functional genes, which are integrated into the genome.
    - The modifications are hereditary and pass on to subsequent generations.
    - This approach is potentially highly effective against genetic and hereditary diseases but is controversial due to ethical and safety concerns.
    - Currently, germ line gene therapy is not approved for clinical use anywhere in the world.

In-vivo and Ex-vivo Gene Therapy

In vivo gene therapy

- **In vivo gene therapy** refers to the direct delivery of the therapeutic gene into the patient's body. This is done without extracting the patient’s cells for laboratory modification.
    1. A vector, typically a genetically engineered virus, is used to carry the therapeutic gene directly to the patient's cells.
    2. This vector is introduced into the body through methods like intravenous injection.
    3. The vector is designed to target specific cells in the body that are affected by the disease.
    4. Once the vector reaches the target cells, it delivers the therapeutic gene into the cell's genome.

Ex Vivo Gene Therapy

- **Ex vivo gene therapy** involves extracting cells from the patient, genetically modifying them in a laboratory, and then reintroducing them into the patient's body.
- **Steps in Ex Vivo Gene Therapy**
    1. **Cell Extraction:** Cells are extracted from the patient, often stem cells or cells from affected tissues.
    2. **Laboratory Modification:** These cells are then genetically modified in a laboratory using a vector to introduce the therapeutic gene.
    3. **Cell Cultivation:** Modified cells are cultivated in the lab to increase their numbers.
    4. **Reintroduction into the Patient:** The genetically modified cells are infused back into the patient's body.
- **Stem Cells as Targets: Stem cells are often targeted in ex-vivo gene therapy due to their ability to differentiate into various cell types**. This makes them ideal for treating a wide range of genetic disorders. Stem cells have the capacity to regenerate and repair damaged tissues, making them particularly useful in regenerative medicine.

Gene Therapy Treatment Procedure

1. **Identification of the Target Gene and Disease:**
    - The first step involves identifying the specific gene associated with the disease. This requires an understanding of the disease's genetics and pathophysiology.
2. **Vector Selection and Design:**
    - **Vectors** are carriers used to deliver therapeutic genes into a patient's cells, including:
        - **Viral Vectors**: Modified viruses like retroviruses, adenoviruses, and adeno-associated viruses.
        - **Non-Viral Vectors**: Direct DNA injection, liposomes, and nanoparticles.
    - A suitable vector is chosen to deliver the therapeutic gene. The vector's design must ensure it can effectively deliver the gene to the targeted cells without causing adverse effects.
3. **Gene Modification and Integration:**
    - The therapeutic gene is inserted into the vector. This gene must be designed to:
        1. replace a missing or faulty gene,
        2. silence a malfunctioning gene, or
        3. introduce a new gene to help fight a disease.
4. **Cell Targeting and Vector Administration:**
    - The vector carrying the therapeutic gene is administered to the patient. This can be done either directly to the patient (**in vivo therapy**) or to cells (stem cells) taken from the patient and then returned (**ex vivo therapy**).
    - In vivo therapy involves direct delivery to the body, often via intravenous injection.
    - Ex vivo therapy involves removing cells from the patient, modifying them in the laboratory, and then reinfusing the modified cells back into the patient.
5. **Integration into the Patient’s DNA:**
    - Once the vector reaches the targeted cells, the therapeutic gene is delivered into the patient's DNA. This step is crucial for the gene to be expressed and produce the needed protein or therapeutic effect.

6. **Monitoring and Follow-Up:**
    - After treatment, patients are closely monitored for any adverse reactions and the effectiveness of the therapy.
    - The monitoring process includes regular medical check-ups and specific tests to assess the expression of the new gene and its impact on the disease.
7. **Ethical and Regulatory Compliance:**
    - Throughout the gene therapy process, compliance with ethical guidelines and regulatory requirements is essential.

Benefits of Gene Therapy

- **Potential Cure for Genetic Disorders**: Offers a possible cure rather than symptomatic treatment.
- **Targeted Treatment**: Specific targeting of affected cells and tissues.
- **Reduced Side Effects**: Potentially fewer side effects compared to conventional treatments.

Success of Gene Therapy

Disease	Description
Cystic Fibrosis	Affects the lungs and digestive system, causing thick mucus.
Hemophilia	Leads to impaired blood clotting due to lack of clotting factors.
Sickle Cell Anemia	Causes red blood cells to become sickle-shaped, leading to various complications.
Muscular Dystrophy	Characterized by muscle degeneration and weakness.
Severe Combined Immunodeficiency (SCID)	A group of disorders that severely weaken the immune system.
Leber's Congenital Amaurosis	A genetic disorder causing severe vision loss at birth.
ADA-SCID	A form of SCID caused by Adenosine Deaminase deficiency.
Beta-Thalassemia	A blood disorder reducing the production of

	hemoglobin.
Spinal Muscular Atrophy	Causes muscle wasting and weakness, usually apparent from birth.
Metachromatic Leukodystrophy (MLD)	Affects the nervous system, leading to motor and cognitive decline.
Lymphangioleiomyomatosis (LAM)	A rare lung disease affecting mostly women in their childbearing years.

Limitations of Gene Therapy

- **Immune Response**: Potential for immune system rejection.
- **Short-lived Nature**: May require repeated treatments.
- **Insertional Mutagenesis**: Risk of gene insertion causing other genetic issues, like cancer.
- **Ethical and Regulatory Concerns**: Debates over germ line therapy and genetic manipulation ethics.
- **Cost and Accessibility**: High costs and limited availability, particularly in countries like India.

Gene Therapy Regulation in India

- **Regulated by the Indian Council of Medical Research (ICMR) and the Central Drugs Standard Control Organisation (CDSCO):** These bodies oversee the development and implementation of gene therapy in India.
- **National Guidelines for Gene Therapy Product Development & Clinical Trials (2019):**
    - Established by the ICMR to promote and regulate gene therapies.
    - Aimed at ensuring ethical, scientific, and safe conduct of clinical trials for gene therapies in India.
    - **Objectives and Main Features**
        1. The guidelines cover the development of gene therapy products for various human ailments.
        2. These guidelines require approval from the CDSCO for gene therapy, and the establishment of the Gene Therapy Advisory and Evaluation Committee (GTAEC) to supervise proposed therapies.

3. The guidelines also mandate long-term follow-up for all clinical trials, with up to 10 years of follow-up recommended after commercialization.
4. The guidelines outline procedures and standards for conducting clinical trials involving gene therapy.
5. The guidelines particularly address treatments for rare diseases and inherited genetic diseases like Gaucher’s disease, haemophilia, thalassemia, sickle-cell anaemia, and certain forms of muscular dystrophies.
6. **Prohibited Research Areas:** Human germline gene therapy, reproductive cloning, and clinical trials involving xenogeneic cells (cells from different species) are not allowed.
7. **Permitted In Vitro Studies:** Genome modifications to an embryo that will not be carried to term are allowed, focusing on in vitro research.

Gene Editing & CRISPR

Gene Editing

Gene editing, also known as genome editing, refers to a group of technologies that enable scientists to **make precise changes to an organism's DNA**.

These technologies **allow genetic material to be added, removed, or altered at specific locations** in the genome.

One well-known method is **CRISPR-Cas9**, which acts like molecular scissors,

1. cutting the DNA at a specific spot, and then
2. allowing scientists to remove, add, or replace the DNA at that location.

Genome editing is used in research to understand diseases and is being explored in clinical trials for a wide variety of conditions, including single-gene disorders and more complex diseases such as cancer and HIV infection.

The technology has the potential to modify genes in various organisms, including plants, bacteria, and animals, and has become faster, cheaper, and more efficient with the development of new methods.

CRISPR

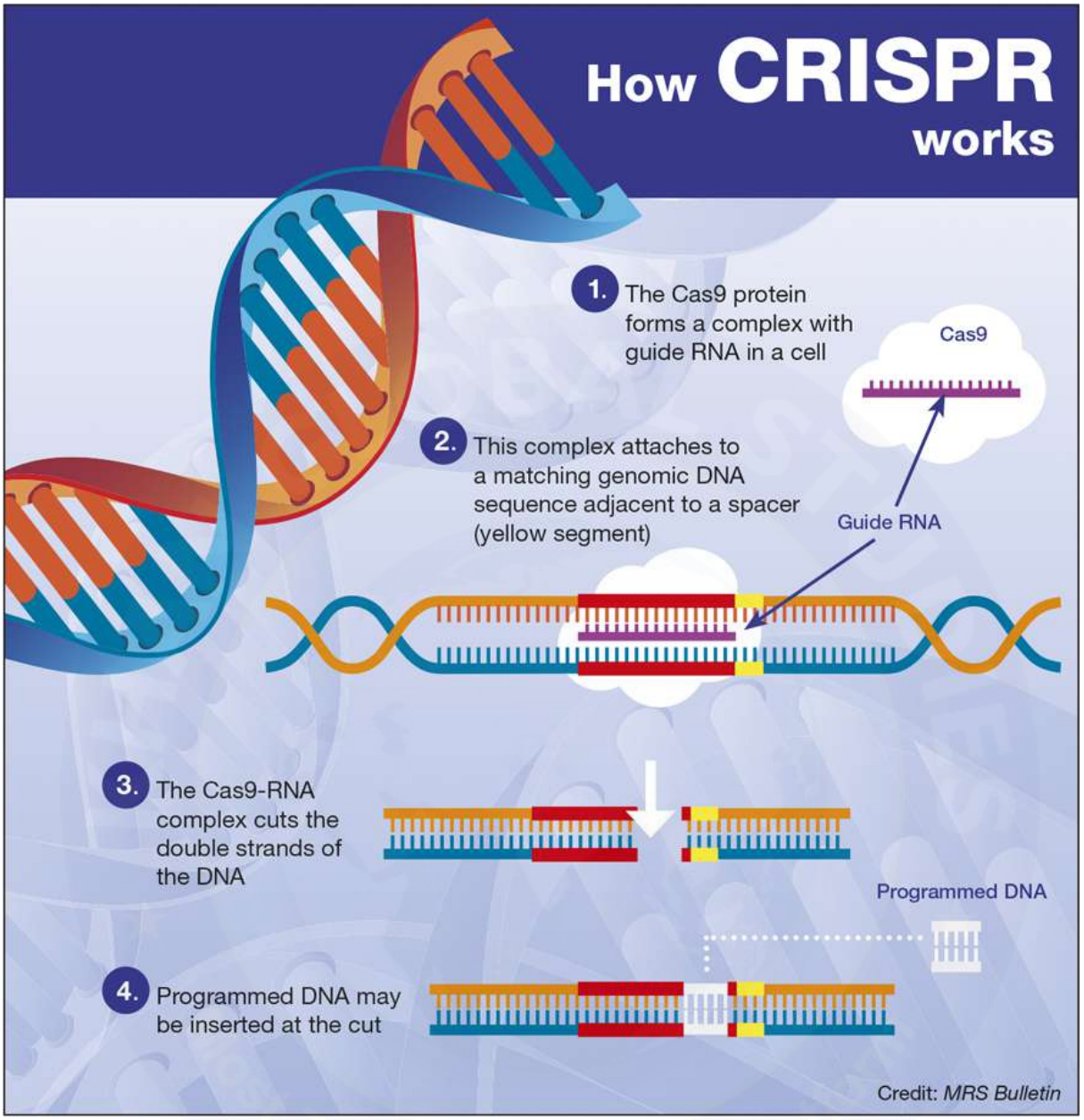
Overview of CRISPR Technology

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a revolutionary gene-editing technology enabling precise modifications to an organism's DNA.

Mechanism of Action

- **Adaptation from Bacteria**: CRISPR was adapted from genome editing systems naturally occurring in bacteria.
- **Role of RNA and Nuclease**: The technology uses RNA to guide a nuclease, such as **Cas9**, to a specific location in the genome.
- **DNA Modification**: At the targeted location, Cas9 cuts the DNA, allowing for the addition, removal, or alteration of genetic material.

CRISPR in Gene Editing Experiments



1. **Cas9 and Guide RNA Formation**: The **Cas9 protein**, which is an enzyme capable of cutting DNA, forms a complex with a **guide RNA** (gRNA). The guide RNA is specifically designed to match a particular DNA sequence in the genome that needs editing.
2. **DNA Targeting**: The **Cas9-gRNA complex** enters the cell's nucleus and the guide RNA binds to its matching DNA sequence. This sequence is located next to a **protospacer adjacent motif (PAM)**, which is essential for Cas9 to recognize and bind to the DNA.
3. **DNA Cutting**: Once the Cas9-gRNA complex has bound to the correct sequence, Cas9 performs a double-strand break in the DNA at this precise location.

4. **DNA Repair and Insertion**: The cell's natural DNA repair mechanisms then take over. There are two main pathways through which the DNA can be repaired:
    - **Non-homologous end joining (NHEJ)**, which can lead to insertions or deletions (indels) at the cut site. These indels can disrupt the gene, potentially "knocking out" its function.
    - **Homology-directed repair (HDR)**, which can be used to insert a new piece of DNA into the genome at the cut site. This process requires providing a piece of donor DNA with the desired sequence, which serves as a template for repair, resulting in the incorporation of new genetic information into the genome.

CRISPR-Cas9 technology has transformed genetic research by allowing precise and relatively easy modification of genes.

Applications of CRISPR Technology

Application Area	Principle	Examples
Disease Modelling	Utilising CRISPR to create cell and animal models with specific gene mutations to mimic human diseases for study.	Generating mice with specific cancer mutations, creating cellular models of cystic fibrosis.
Functional Genomics	Using CRISPR to knock out genes in cells or organisms to study their function.	Disabling genes in yeast to study gene function, creating loss-of-function mutants in model organisms.
Gene Therapy	Employing CRISPR to correct mutations in somatic cells to treat genetic disorders.	Treating inherited blindness caused by mutations in the RPE65 gene, correcting the ΔF508 mutation in cystic fibrosis.
Cancer Research	Applying CRISPR to modify genes to understand cancer progression and to develop new treatments.	Editing immune cells to target and destroy cancer cells, creating oncogene knockouts to study tumour suppressors.
HIV Research	Leveraging CRISPR to disrupt HIV-1 provirus in infected cells.	Removing the CCR5 receptor from T cells to prevent HIV entry, excising HIV DNA from infected cells.

Agricultural Improvements	Using CRISPR to enhance crop traits such as yield, disease resistance, and drought tolerance.	Creating rice strains with enhanced blast resistance, developing wheat with improved gluten quality.
Biological Research	Applying CRISPR for the study of gene function and interaction in biological pathways.	Investigating the CRISPR/Cas9 system itself, studying gene networks in development.
Drug Discovery	Utilising CRISPR to identify new drug targets by observing effects of gene knockouts.	High-throughput screening of gene knockouts to identify potential targets for cancer drugs.
Genome Engineering	Employing CRISPR to make precise alterations in DNA for various purposes.	Creating transgenic animals, developing gene drives for controlling pest populations.
Developmental Biology	Using CRISPR to study the roles of genes in embryonic development.	Knocking out genes in zebrafish to study developmental processes, editing genes to study organogenesis.
Microbiome Editing	Applying CRISPR to manipulate the genomes of bacteria within the microbiome.	Engineering bacteria to produce beneficial compounds, studying the role of specific bacteria in gut health.
Biomanufacturing	Leveraging CRISPR to engineer microorganisms for the production of pharmaceuticals and chemicals.	Modifying yeast to produce biofuels, engineering bacteria to produce insulin.

Limitations of CRISPR

- **Unintentional Edits**: CRISPR may inadvertently alter genes other than the intended ones, causing unwanted genetic changes.
- **Mixed Cell Populations**: Not all cells in an organism may carry the edit, resulting in a combination of modified and unmodified cells.
- **Immune Response**: The immune system could target the Cas9 protein or guide RNAs, affecting the treatment's effectiveness.
- **Technique Difficulties**: Properly delivering CRISPR components to target cells in living organisms remains a significant challenge.
- **Ethical Issues**: The ability to alter humans at a genetic level raises societal and ethical issues regarding the value of individuals with certain genetic traits.

- **Uncharted Risks**: The long-term consequences and potential hazards of CRISPR-Cas9 gene editing have yet to be fully understood.

CRISPR in Mosquito Control and Disease Prevention

Overview of CRISPR in Mosquito Population Management
CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is being explored for its potential in controlling mosquito populations.

This approach is critical in combating mosquito-borne diseases such as dengue, chikungunya, Zika, and malaria.

Strategies Employing CRISPR

1. **Precision-Guided Sterile Insect Technique (pgSIT)**: This technique uses CRISPR to sterilize mosquitoes, thereby reducing their population over time.
2. **Gene Drives**: Gene drives are designed to spread genetic modifications through mosquito populations rapidly. They are engineered to ensure that a specific trait is passed on to a high percentage of offspring, potentially altering entire populations. Gene drives have the potential to alter entire mosquito populations by spreading genetic modifications rapidly, which could lead to the suppression or even eradication of disease-carrying mosquito populations.

Advantages of CRISPR in Mosquito Control

- **Targeted Gene Editing**: CRISPR is valued for its precision in targeting specific mosquito genes.
- **Species-Specific Control Methods**: It offers the potential to create genetic control methods that are specific to mosquito species, thereby reducing unintended impacts on other organisms.

Ethical and Safety Considerations

- **Ethical Concerns**: The use of CRISPR-based gene drives has raised significant ethical issues, particularly about elimination of an entire species.
- **Safety Considerations**: The development of these methods also has safety concerns about their potentially irreversible nature and impact on ecosystems.

Cloning

The Meaning of a Clone

- **Definition**: A **clone** is an entity, be it a single cell, a multicellular organism, or nucleic acid, that is an exact genetic copy of another.
- In a biological context, a clone may range from single cells like bacteria or lymphocytes to multicellular organisms, all genetically identical to the original source organism.
- **Natural and Artificial Cloning**: Clones can arise naturally or can be created through deliberate human intervention.

The Meaning of Cloning

- **Cloning Process**: Cloning involves creating a genetically identical copy of an original cell, piece of DNA, or an organism.
- **Human Intervention**: Modern cloning includes deliberate human actions to achieve the process of cloning.
- **Historical Milestone**: The concept gained popularity with the creation of Dolly the sheep in 1997 by Ian Wilmut's team at the Roslin Institute in Edinburgh.

Types of Cloning

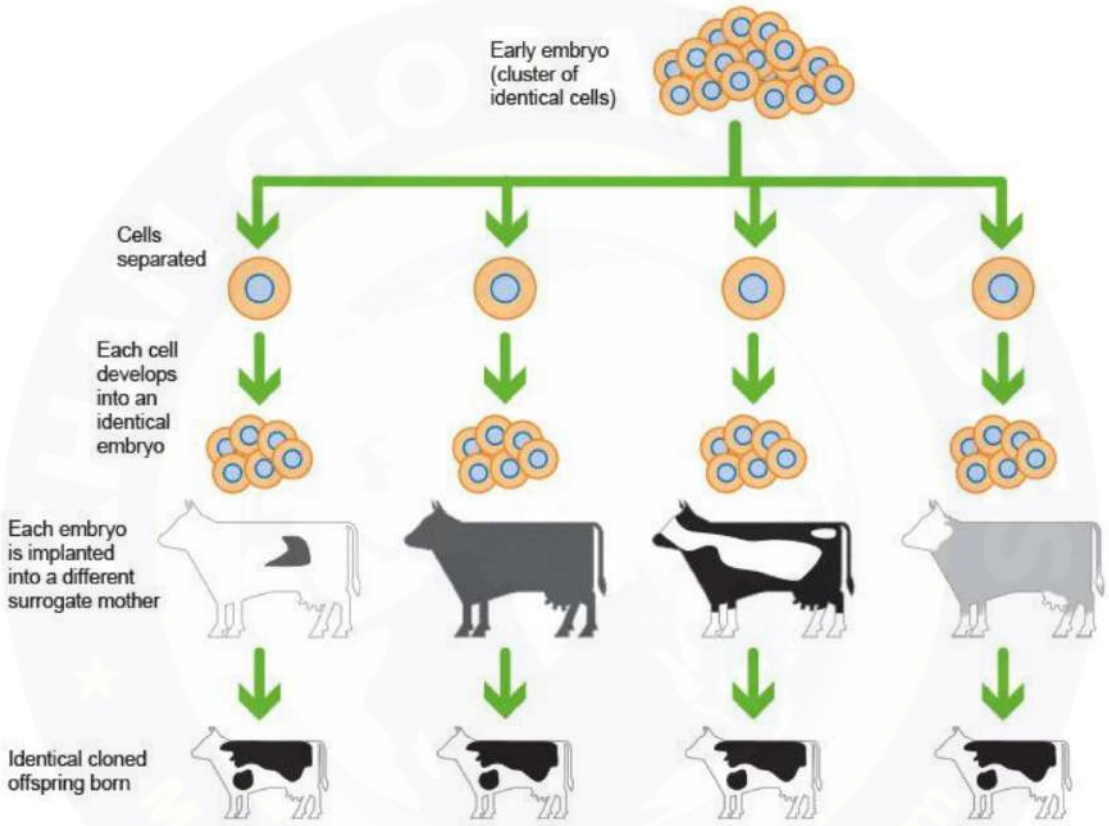
1. **Molecular Cloning**: Concerns the replication of DNA, performed in vivo or in vitro through methods like PCR.
2. **Cellular Cloning**: Conducted in culture conditions to derive a clonal population of cells from a single cell.
3. **Whole Organism Cloning**: Applicable to plants and animals, including:
    - **Therapeutic Cloning**: Generation of animal organs using stem cells.
    - **Reproductive Cloning**: Regeneration of the entire animal.

Methodology of Whole Animal Cloning

Principal Approaches

1. **Splitting of Early Embryos to Produce Clones**
    - Embryos are isolated at an early stage.

- Embryos are split at the 8 or 16 cell stage, with the split being symmetrical and non-damaging.
- Split parts are cultured to reach blastocyst stage before implantation into the mother using embryo transfer techniques.
- Resulting animals are clonal to each other but not to the parents.

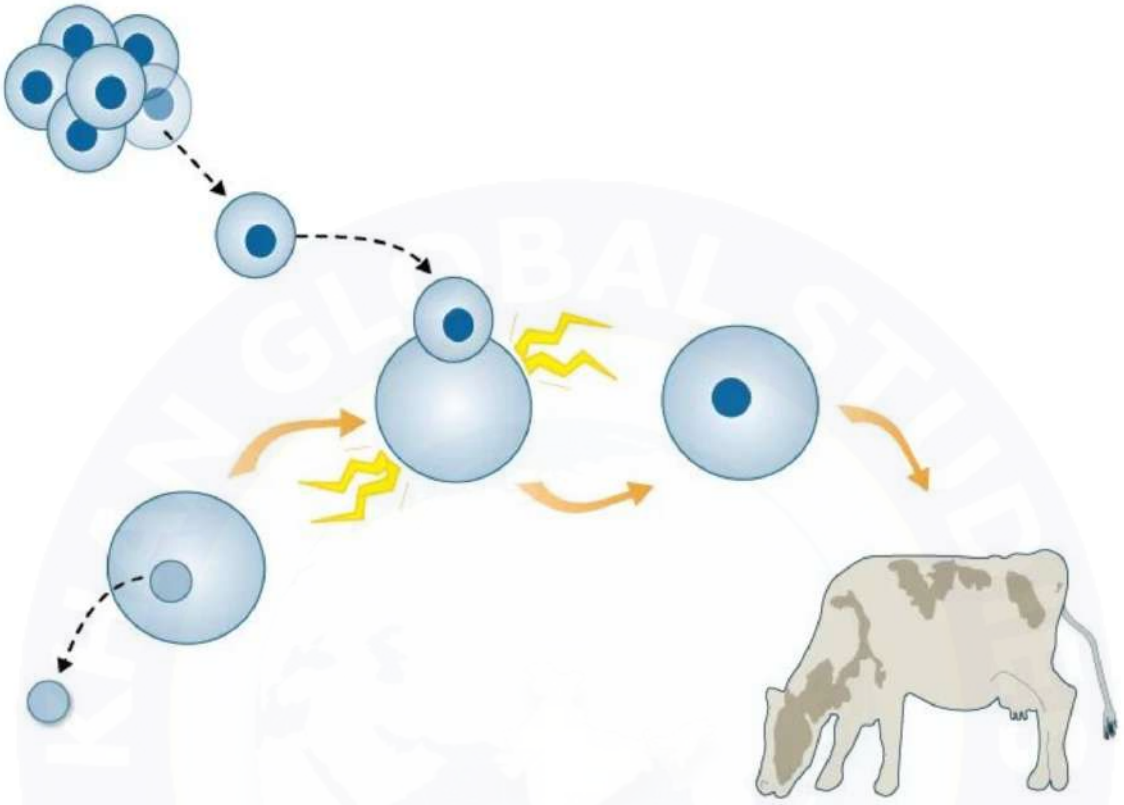


- **Advantages**
  - Simplest cloning method with no intra-cellular manipulation required.
  - Applicable to a large number of species.

2. **Nuclear Transplant Approach 1: Donor Nucleus from an Embryonic Cell**

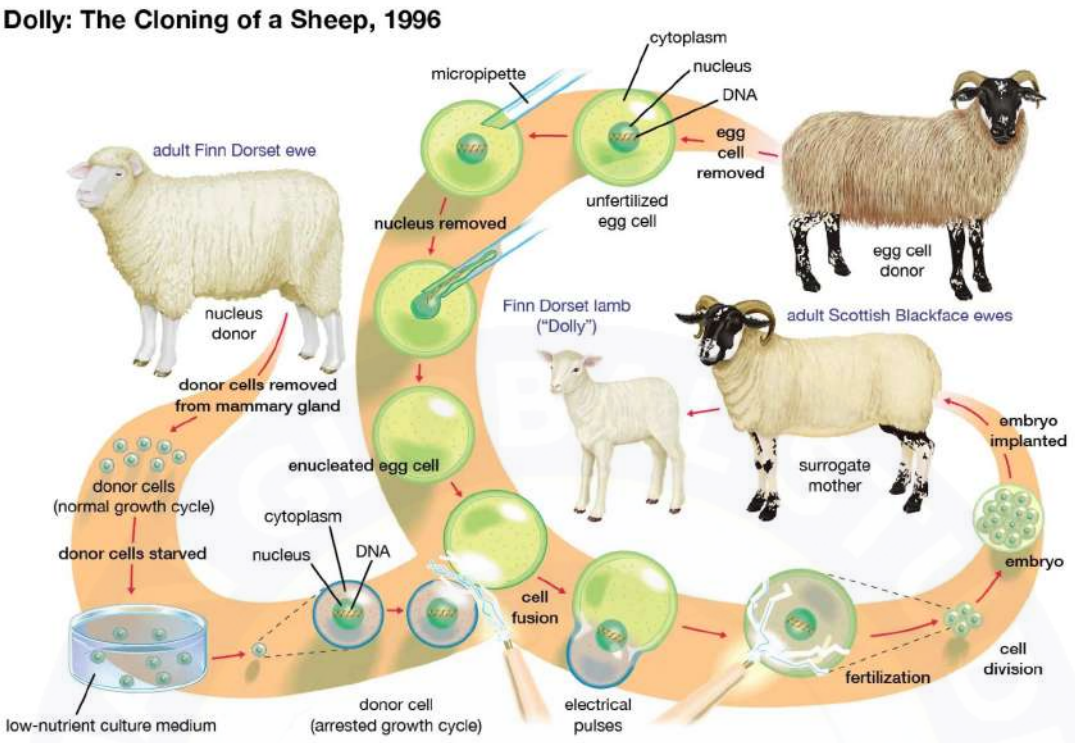
- Used since 1952 for developmental studies in frogs and later in cattle and sheep.
- Embryonic cells are used before blastocyst formation; their nuclei are undifferentiated and totipotent.
- The nucleus or whole embryonic cell is transferred to an activated enucleated oocyte, forming a reconstructed zygote.

- Post-electrical pulse activation, embryos are cultured and implanted into foster mothers.



- **Advantages**
  - High success rates
  - Enables efficient animal cell transgenesis by transplanting a transgenic nucleus into an enucleated egg.
- **Limitations**
  - Cannot clone adult animals.

3. **Nuclear Transplant Approach 2: Donor Nucleus from a Mature Somatic Cell**



- In 1997, Dolly the sheep was cloned from an adult somatic cell nucleus.
- Somatic cells from a mammary gland were cultured, made expressionally uncommitted, then fused with enucleated oocytes using electric pulses.
- Resulting embryos were implanted into sheep uteri, with Dolly being the sole survivor out of 434 attempts.
- **Advantages**
  - The only method to clone a mature animal.
- **Limitations**
  - Limited types of cells can serve as nuclear donors.
  - Success rates are low, with an average of 1% of reconstructed embryos leading to live births.
  - Not a pure parental clone due to mitochondrial DNA from the egg cell.

Major application areas

1. Rapid replication of valuable animals
2. Protecting/restoring a rare animal
3. For therapeutic cloning

4. Experimental purposes
5. Targeted genetic modifications can be made in donor cells before nuclear transfer, which can lead to:
    a. production of therapeutic proteins in the milk and blood of transgenic cloned animals
    b. use of cells, tissues, and organs from gene-modified animals for transplantation
    c. production of healthier and safer products in an environmentally friendly manner
    d. generation of genetically modified animals that accurately mimic human diseases for the purpose of developing new therapies

Limitations

1. Relatively a complex technology
2. High failure rates
3. Presently too expensive to be put to mainstream medical applications
4. Species restoration is a complex task and cannot be solely based on cloning
5. Biological complications such as progeria
6. Ethically questionable
7. May be biologically unsustainable due to genetic homogeneity

---

Mitochondrial Replacement Therapy

Overview of Mitochondrial Replacement Therapy (MRT)

- **Mitochondrial Replacement Therapy (MRT)**, also known as mitochondrial donation or **three-parent in vitro fertilisation (IVF)**, is a groundbreaking technique designed to prevent the inheritance of mitochondrial diseases.
- MRT involves **replacing mitochondria in cells, particularly in cases where mothers have genes for mitochondrial diseases**.
- MRT is **officially sanctioned in the United Kingdom** and **utilized in clinical research in the United States** for treating cardiac-compromised newborns.

- The method **involves generating new combinations of nuclear and mitochondrial genomes** and matching donor/recipient pairs according to mtDNA haplogroups.
- The potential benefits of MRT include the birth of healthy babies free from genetic disorders and the **prevention of defective mitochondrial DNA transmission to future generations**.

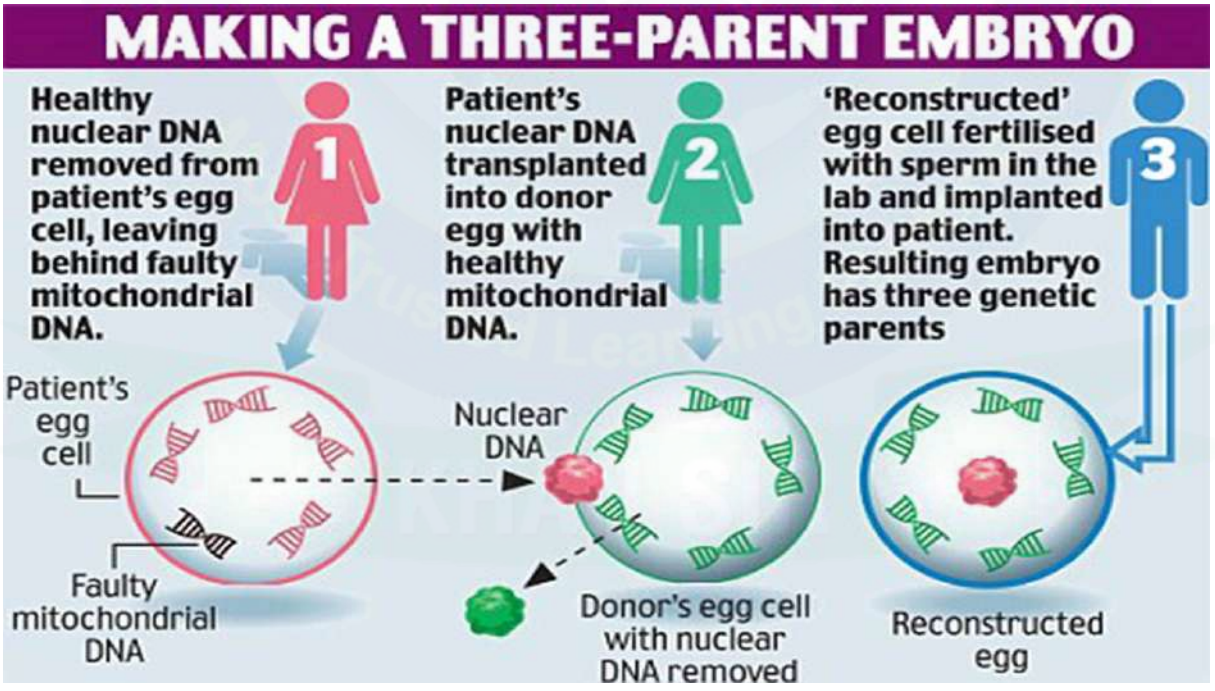
Mitochondrial Diseases and MRT Application

Mitochondrial diseases, though rare, can be severely debilitating and often fatal in infancy or childhood. They may result from mutations in mitochondrial DNA (mtDNA), nuclear DNA (nDNA), or environmental factors.

Examples of these diseases include **Mitochondrial myopathy**, **Maternally inherited diabetes mellitus and deafness (MIDD)**, **Mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS)**, **Leigh syndrome**, **Kearns-Sayre syndrome**, **Barth syndrome**, and **Friedreich's ataxia**.

MRT aims to prevent mtDNA disease transmission and incorporates techniques like **spindle transfer (ST)**, **pronuclear transfer (PNT)**, and **polar body transfer (PBT)**.

Concept of "Three-Parent Baby"



The term **"three-parent baby"** refers to a child conceived with the genetic material of two women and one man through **mitochondrial replacement therapy** and

three-person IVF.

This approach is intended to prevent mitochondrial diseases by replacing or modifying defective mitochondria with healthy ones from a donor, thus reducing the risk of passing mitochondrial DNA disease to the child.

While termed "three-parent baby", over 99.8% of the DNA in these babies originates from the mother and father, with a minimal amount from the donor.

The first baby born using the **"three-parent" technique** was a boy born in 2016. The doctor who led the team in the first "three-parent baby" experiment is **Dr. John Zhang**, a New York-based reproductive endocrinologist.

This groundbreaking technique, comprising **mitochondrial donation treatment (MDT)** or **mitochondrial replacement therapy (MRT)**, utilises DNA from three individuals: the nuclear DNA from the mother and father, and mitochondrial DNA from a donor.

This method is designed to assist women with mutated mitochondria in having healthy babies, mitigating the risk of passing on mitochondrial genetic disorders.

Limitations of Mitochondrial Replacement Therapy

MRT, while innovative, faces several challenges:

1. **Limited Availability**: MRT is not widely accessible and is only approved in select countries, like the UK.
2. **Ethical Concerns**: Ethical debates arise due to the creation of embryos with genetic material from three individuals and the potential for unforeseen consequences.
3. **Long-term Safety**: The long-term safety implications of MRT are yet to be fully comprehended, necessitating more research to understand potential risks and benefits.
4. **Cost**: The high cost of MRT may restrict its availability to those in need.
5. **Limited Scope**: MRT specifically targets the prevention of mitochondrial DNA disease and does not address other genetic disorders or diseases caused by environmental factors.

DNA Barcoding

Introduction to DNA Barcoding

DNA barcoding is a method used in molecular biology **to identify and classify species based on a short section of DNA from a specific gene or genes**.

The concept is analogous to how barcodes in shops identify products.

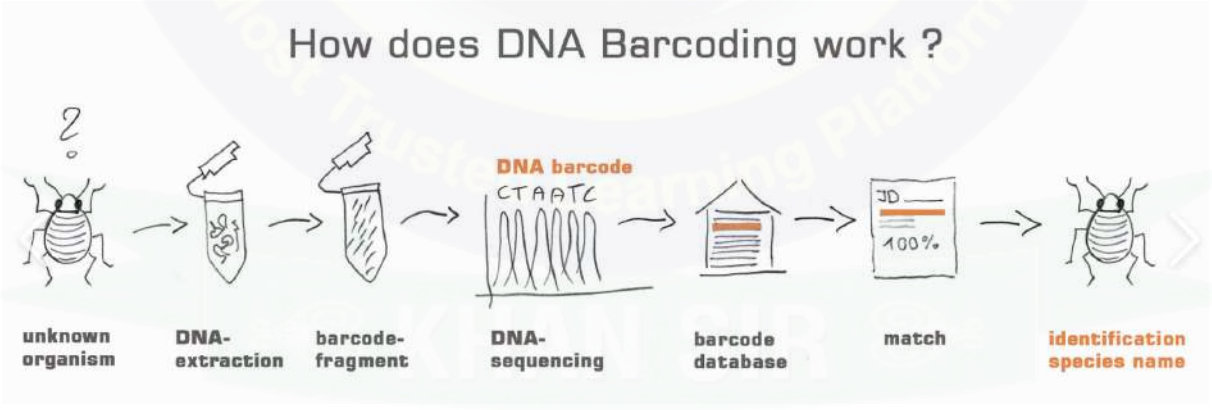
In DNA barcoding, **the 'barcodes' are short genetic sequences from a standardised region of the genome, unique to each species**.

Rationale for DNA Barcoding

DNA barcoding is needed for several reasons:

1. **An Accurate and Straightforward approach to Species Identification**: Traditional methods of species identification based on morphology can be challenging, especially for closely related species or in cases where the specimen is damaged or immature. DNA barcoding offers a more accurate and straightforward approach.
2. **Biodiversity Monitoring**: It facilitates the monitoring of biodiversity, enabling rapid assessment of ecosystem health.
3. **Detection of Illegal Trade**: It aids in the identification of species in illegal wildlife trade where traditional identification methods are not feasible.
4. **Conservation Efforts**: It is crucial in conservation biology for identifying endangered species and understanding their genetic diversity.

Process of DNA Barcoding



The process involves several steps:

1. **Sample Collection**: Obtaining a tissue sample from the organism.
2. **DNA Extraction**: Extracting DNA from the tissue.

3. **PCR Amplification**: Amplifying the specific barcode region of the DNA using Polymerase Chain Reaction (PCR).
4. **Sequencing**: Determining the sequence of the amplified DNA.
5. **Database Comparison**: Comparing this sequence to a reference database to identify the species.

Application Areas of DNA Barcoding

DNA barcoding has diverse applications:

1. **Taxonomy**: Assisting in the discovery and description of new species.
2. **Ecology**: Studying food webs and interactions between species.
3. **Forensics**: Identifying species from fragments of tissue in wildlife forensics.
4. **Agriculture**: Detecting and monitoring pest species in agriculture.
5. **Public Health**: Identifying vectors of human diseases and monitoring the spread of invasive species.
6. **Pharmacology**: Authenticating medicinal plants and detecting adulteration in herbal products.

In India, DNA barcoding has been applied in various fields, including the identification of medically important plant species, monitoring of fish stocks, and wildlife conservation efforts.

Conclusion

DNA barcoding is a powerful tool in modern biology, offering a rapid, accurate, and cost-effective means of species identification. Its applications span various fields, from ecology and conservation to public health and agriculture, making it an essential technique in the study of biodiversity and ecosystem management.