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Topic 1: Genome:

DNA Profiling

- Biometric database comprising information of individuals.
- Though 99.9% genetic material is similar in humans, 0.1% comprising **Variable Number Tandem Repeats (VNTR)** in unique to individuals DNA.

Genome Sequencing

- **Genome** →full set of DNA present in an individual.
- **DNA sequencing** → process of determining linear order of nucleotide bases in DNA (3 billion base pairs).
- Techniques used → Shotgun sequencing, Next generation sequencing.
- Dark Genome
- Dark DNA

Two methods:

- Whole exome sequencing
- Whole genome sequencing

Steps:

- DNA Shearing
- DNA Bar-coding
- DNA Sequencing

Advantages: Genetic disorders, Personalized medicines, Vaccines, Mutations,

Genome Projects

MANAV

- Human Atlas Initiative
- Construct a comprehensive map of every tissue of human body.

Genome India Project

- Carry out whole genome sequencing of Indian – study the diversity of Indians.
- Joint initiative of MoHFW and MoST

Indigen Initiative

- 1st of its kind whole genome sequencing of Indians – sequencing of 1000 representatives
- Precursor to Genome India project - By CSIR.

Earth Bio-Genome Project

International collaboration to sequence every eukaryotic biodiversity on earth over a period of 10 years.

Human Microbiome project

- Involves collection of saliva, stool, skin swab of 20,000 Indians
- Study human microbiome and its change during disease

Human Proteome Project

- Proteome: the complete set of proteins expressed by an organism
- International collaboration organized by Human proteome organization (HUPO)

National Genomic Grid

Collect sample from cancer patients through pan-India collection centres.

Z-DNA

Linked to genomes of bacteriophages.

Topic 2: DNA / Gene editing:

Techniques of Gene Editing

- rDNA
- SDN
- Crispr/CAS 9

SDN – site directed nuclease:

Enzymes

- **'Engineered nucleases'** - enzymes make double stranded cut in DNA sequences;
- DNA ligase:

Techniques

- ZFN: Zinc finger nucleases; targeted editing of the genome at user-specified locations;
- TALEN: Transcription activator-like effector nucleases;

Types:

- SDN1:
- SDN2: uses DNA template;
- SDN3: Foreign genes;

CRISPR-Cas9:

- Bacterial defence mechanism;
- Guide RNA (gRNA): crispr RNA (crRNA) - recognizes target DNA; direct Cas9 for editing;
- CAS9 nuclease: double stranded break;

Types of Gene editing

- **Germline editing**: Changing genes in eggs, sperms, or early embryo – heritable
- **Somatic cell gene editing**: Impacts targeted cells/tissues/organs in patients – not passed to subsequent generations.
- **IN-VIVO gene editing:** Editing carried out inside human body – 1st time to treat hunter's syndrome;

Gene Drive Technology

- Genetic elements that pass from parents to unusually high number of offspring's; Permanently change the traits of a population; E.g. Malaria

Topic 3: GM Crops

Bt Cotton

- Developed: Mahyco + Monsanto
- Alien Genes: Genes: Cry1Ab, Cry2Bc (Bacillus thuringiensis (Bt))
- Organism: Bacillus thoreineginesis
- Issues: yield stagnation, Pest attack;
- Working: Cry proteins -> Paralyze bollworms -> Death
- Timeline: Seeds: Bollgard1, Bollgard2
- Current Status: Only Transgenic crop approved for commercial cultivation
- Ht Cotton: Cp4-Epsps; Agrobacterium tumefaciens;
- Challenges: Pest resistance; HT - carcinogen;
- Illegal use: EPA 1986, BDA 20002, FSSAI 2006; Seed ACt 1966;

Glyphosate: broad spectrum herbicide; non-selective; Inhibits **ESPS** protein production; approved largely for weed control in tea gardens, playgrounds etc; MoA – restricted use - permitted through pest control operators;

Bt Brinjal

- Developed: Mahyco;
- Alien Genes: Cry Genes;
- Organism: Bacillus thoreineginesis
- Issues: Root and Shoot borer;
- Working: Cry proteins -> Paralyze bollworms -> Death

Timeline:

- Status: 2007-2009: Tests carried out
- 2009, Oct: GEAC granted approval
- 2010: SC TEC stayed; Govt adopted 10 year moratorium; More trials
- 2020: Moratorium ended

Current Status:

- 2020-23: trials in 8 states including PB, HR

GM Mustard:

- Seed: DMH 11, BRL-I and one year of BRL-II;
- Developed: Deepak Pental; Centre for Genetic Manipulation of Crop Plants (CGMCP)
- Alien Genes: Bar-Barnase-Barstar;
  - Barnase-Barstar: Bacillus amyloliquefaciens
  - Bar gene: herbicide tolerance
- Working: Heterosis - Cross between Indian mustard variety 'Varuna' and East European 'Early Heera-2' mustard;
- Advantages: Heterosis breeding; controlling inbreeding and promoting hybridization;
- Challenges: Effect on pollinators; Parliamentary Committee and the Supreme Court's TEC;

Timeline/Status:

- 2014-15: confined biosafety trials
- 2017: GEAC approval granted; later retracted;
- 2017: SC stayed commercial release
- 2022: Environmental release - PB and HR;

Topic 4: Immunity

Immunity

- **Innate**: Present at birth; first line of defense; Includes - physical barriers, proteins, special cells;
- **Adaptive**: acquired on exposure to pathogen; antibody mediated; specific; immunological memory;

Types:

Based on antibody generation:

- **Active**: Immune system of self makes antibodies; Creates immunological memory;
- **Passive**: Immunity, antibodies gained from others; fast acting - short lived; through - cytokines, antibodies, monoclonal antibodies; E.g. New born

Based on cell type:

- **Humoral immunity** - macromolecule mediated; extracellular fluids; antibodies(Immunoglobulins), proteins;
- **Cell mediated** - T cells; CAR-T : Cytotoxic therapy;

Lymphocytes:

- **B Cells**: B lymphocytes; Types - Plasma B Cells, Memory B cells;
- **T Cells**: types - CD8+ "killer" (cytotoxic) and CD4+ "helper" T cells;

Process

- Pathogen -> Cytokines-> Plasma cell : antibody secreting effector cell;
- Helper T Cells -> Mature B Cells into Plasma Cells; Memory B Cells; Activation of Cytotoxic T Cells; Memory T Cells;

- **Autoimmunity** : immune responses of an organism against its own healthy cells, tissues etc; **Immunological tolerance**: ability of an individual to ignore "self", while reacting to "non-self"
- **Regulatory T cells, Suppressor T cells**: Tolerance distinguish invading cells from "self"; Prevents immune cells from inappropriately reacting against one's own cells, known as an "autoimmune" response;

CAR-T Therapy:

Chimeric antigen receptors (CARs)- receptor proteins engineered to give T cells the new ability to target specific antigen; modify T cells to recognize cancer cells; cytotoxic;

Topic 5: Vaccines

Introduction

- **Vaccine** - made up of the antigens of the pathogen that cause the disease - the body is protected against the disease occurring in future.

Types of Vaccines

1. Whole Virus
    a. Live attenuated - MMR (Measles, mumps, rubella), Rotavirus, Smallpox, Chickenpox
    b. Inactivated vaccines - Hepatitis A, Polio, Rabies etc.
2. Subunit
    a. unit, recombinant, conjugate: Hepatitis B, HPV, Pneumococcal disease etc

3. Genetic material
    a. Viral vector: Adenovirus; Covishield, Sputnik;
    b. mRNA: Pfizer, Moderna; Nanolipds as delivery vehicle;

1. Live-attenuated vaccines

- Live vaccines use a weakened (or attenuated) form of the germ that causes a disease.
- E.g. MMR (Measles, mumps, rubella), Rotavirus, Smallpox, Chickenpox

2. **Inactivated vaccines**

- killed version of the germ that causes a disease;
- E.g. Hepatitis A, Polio, Rabies etc.

3. Subunit, recombinant, polysaccharide, and conjugate vaccines

- Subunit, recombinant, polysaccharide, and conjugate vaccines use specific pieces of the germ — like its protein, sugar, or capsid (a casing around the germ).
- Because these vaccines use only specific pieces of the germ, they give a very strong immune response that's targeted to key parts of the germ.
- Some examples are: Hepatitis B, HPV, Pneumococcal disease etc.

mRNA vaccine

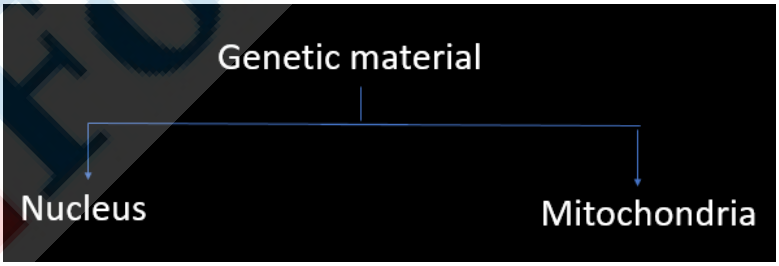
Delivers antigen-encoding mRNA into immune cells; stimulate adaptive immune response; encapsulated in lipid nanoparticles;

Diagnosis and Testing

- Antigen: RAT – Rapid antigen test;
- Genetic material: RT-PCR

Miscellaneous

3 Parent baby



- '**Maternal Spindle transfer'** - In this technique, maternal DNA is put into the egg of a donor woman, which is then fertilized using the father's sperm.
- **Pronuclear transfer:** Mother's egg is first fertilized with the father's sperm, producing a zygote; The pronuclei of the egg and sperm are then removed from the zygote and inserted into a donor egg that has been fertilized and has had its own nucleus removed;