Separation techniques are those techniques that can be used to separate two different states of matter such as liquids and solids.

Decantation is a process for the separation of mixtures of immiscible liquids or of a liquid and a solid mixture such as a suspension. Immiscible liquid separation:

Sedimentation: The tendency of particles in suspension to settle down in the fluid due to certain forces like gravity, centrifugal acceleration, or electromagnetism is called as sedimentation

Takes advantage of physical property of state of matter, its size and solubility in liquid. The substance which is left behind in the filtration medium is called **residue**.

The liquid which passes through the filtration medium is called **filtrate**. Mechanical, physical or biological operations that separates solids from fluids (liquids or gases) by adding a medium through which only the fluid can pass can be called as **filtration**.

Centrifugation is one of the techniques that scientists can use to separate mixtures **OR**

Centrifugation is a mechanical process which involves the use of the centrifugal force for the sedimentation of heterogeneous mixtures with a centrifuge.

This process is used to separate two immiscible liquids.

Centrifuge: Centrifuge is a device for separating particles from a solution according to their size, shape, density, medium viscosity of the medium.

Types of Centrifugation

1. **Differential Pelleting (differential centrifugation)**

2. Density Gradient Centrifugation

Two types of this:

(i) Rate-Zonal Density-Gradient Centrifugation

(ii) Isopynic Centrifugation

Ultracentrifugation

A high-speed centrifuge able to separate out colloidal and other small particles and used especially in determining the sizes of such particles or the molecular weights of large molecules

Types of Ultracentrifugation

1. **Analytical ultracentrifugation:-**
2. **2. Preparative ultracentrifugation:-**

Evaporation is type of vaporization that occurs on the surface of liquid as it changes into gas phase.

Distillation is a purification process where the components of a liquid mixture are vaporized and then condensed and isolated.

A **saturated solution** is a solution that contains the maximum amount of solute dissolved in a given volume of solvent at a particular temperature.

Sublimation is the transition of a substance directly from the solid to the gas phase, without passing through the intermediate liquid phase.

Chromatography is a separation technique used to separate the different components in a liquid mixture.

Types of chromatography

- Gas chromatography
- Liquid chromatography
- Thin layer chromatography
- Ion-exchange chromatography

Chromatography technique that uses paper sheets or strips as the adsorbent being the stationary phase through which a solution is made to pass is called **paper chromatography**

Gas chromatography : In this method stationary phase is a column which is placed in the device, and contains a liquid stationary phase which is adsorbed onto the surface of an inert solid. Gas chromatography is a "gas-liquid" chromatography

Thin Layer Chromatography can be defined as a method of separation or identification of a mixture of components into individual components by using finely divided adsorbent solid / (liquid) spread over a plate and liquid as a mobile phase.

Ion exchange chromatography (or ion chromatography) is a process that allows the separation of ions and polar molecules based on their affinity to ion exchangers

High performance liquid chromatography **(HPLC)** is a chromatographic technique that can separate a mixture of compounds.

Filtration is the process of separating substances based on their different physical and chemical qualities.

Types of filtration

- Vacuum Filtration
- Centrifugal filtration

- Gravity filtration
- Cold filtration
- Hot filtration
- Multilayer filtration

In **vacuum filtration**, a vacuum pump is used to rapidly draw the fluid through a filter.

A plant that performs distillation is called a **distillery**.

Types of Distillation

Fractional distillation, Destructive distillation, Steam distillation, Vacuum distillation

A **Soxhlet extractor** is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet.

It was originally designed for the extraction of a lipid from a solid material. Typically, Soxhlet extraction is used when the desired compound has a *limited* solubility in a solvent, and the impurity is insoluble in that solvent.

Rotary evaporator (or rotavap/rotovap) is a device used in chemical laboratories for the efficient and gentle removal of solvents from samples by evaporation.

Fractionation is a separation process in which a certain quantity of a mixture (gas, solid, liquid, enzymes, suspension, or isotope) is divided during a phase transition, into a number of smaller quantities (fractions) in which the composition varies according to a gradient.

Spectrophotometry is a method to measure how much a chemical substance absorbs light, by measuring the intensity of light, as a beam of light passes through sample solution .

A **weighing balance** is an instrument which is used to determine the **weight** or mass of an object

A **circular measure** was used in comparing circular cross-sections, e.g., of wires, etc. A circular unit of the Ares is the area of the circle whose diameter is one linear unit.

A **solution** is a homogeneous mixture created by dissolving one or more solutes in a solvent.

Molarity is defined as the number of moles of solute (the material dissolved) per liter of solution.

The strength of solution measured in terms of gram equivalent per litre is called **normality.**

There are three different temperature scales.

- Celsius scale of Temperature °C = 5/9(°F – 32)
- Fahrenheit Scale of Temperature °F =9/5°C + 32

- Kelvin Scale of Temperature K = °C + 273

Polymerase chain reaction (PCR) is a method widely used to rapidly make millions to billions of copies of a specific DNA sample, allowing scientists to take a very small sample of DNA and amplify it to a large enough amount to study in detail.

PCR consists of a series of 20–40 repeated temperature changes, called **thermal cycles**

PCR amplifies a specific region of a DNA strand (the DNA target). Most PCR methods amplify DNA fragments of between 0.1 and 10 kilo base pairs (kbp) in length, although some techniques allow for amplification of fragments up to 40 kbp.

Types

- Real-Time **PCR** (quantitative **PCR** or qPCR)
- Reverse-Transcriptase (RT-**PCR**)
- Multiplex **PCR**.
- Nested **PCR**.
- High Fidelity **PCR**.
- Fast **PCR**.
- Hot Start **PCR**.
- GC-Rich **PCR**

Real-time PCR also called quantitative PCR (qPCR) is a variant of standard polymerase chain reaction in which amplification and simultaneous quantitation of a target DNA is done in the same PCR machine, using commercially available fluorescence-detecting thermocyclers. Fluorescent dyes specifically label DNA of interest and the amount of fluorescence generated is proportional to the quantity of DNA present

Reverse Transcriptase PCR (RT-PCR) is a variation of the polymerase chain reaction that amplifies target RNA. Addition of reverse transcriptase (RT) enzyme prior to PCR makes it possible to amplify and detect RNA targets

Multiplex PCR is a variant of **PCR method** in which more than one target sequence is amplified using multiple sets of primers within a single PCR mixture. This enables amplification of several gene segments at the same time, instead of specific test runs for each. This technology was first used by Chamberlain et al. for the diagnosis of Duchenne muscular dystrophy (1988).

Nested PCR is a modification of PCR designed to increase the sensitivity and specificity of the assay reaction. It involves the use of two primer sets directed against the same target and two successive PCR reactions.

High Fidelity PCR .The fidelity of a polymerase refers to its ability to insert the correct base during PCR. Conversely, the rate of misincorporation is known as a polymerase's error rate

Enzymes capable of **fast PCR** can help in achieving faster turnaround times from sample to result and give improved throughput on existing thermocyclers.

Hot Start PCR is a technique that reduces non-specific amplification and offers the convenience of reaction set up at room temperature.

The **GC-RICH PCR** System is a blend of Taq DNA Polymerase and a proofreading polymerase for amplifying longer nucleic acid fragments.

DNA sequencing is the process of determining the sequence of nucleotide bases(As, Ts, Cs, and Gs)in a piece of DNA.Today, with the right equipment and materials, sequencing a short piece of DNA is relatively straightforward.

Maxam-Gilbert method of DNA Sequencing ; It is a method by which the sequence of a DNA fragment is identified by using chemicals, thats cut DNA at specific points. Also called Chemical degradation method of DNA sequencing.

DNA synthesis occurs by the process of replication. During replication, each of the two parental strands of DNA serves as a template for the synthesis of a Complementary strand

Pairing DNA; Attached to each sugar is one of four bases--adenine (A), cytosine (C), guanine (G), or thymine (T). The two strands are held together by hydrogen bonds between the bases, with adenine forming a base **pair** with thymine, and cytosine forming a base **pair** with guanine.

A DNA microarray (also commonly known as DNA chip or biochip) is a collection of microscopic DNA spots attached to a solid surface. Scientists use DNA microarrays to measure the expression levels of large numbers of genes simultaneously or to genotype multiple regions of a genome.

DNA microarrays can be used to detect DNA (as in comparative genomic hybridization), or detect RNA (most commonly as cDNA after reverse transcription) that may or may not be translated into proteins. The process of measuring gene expression via cDNA is called **expression analysis or expression profiling.**

Molecular hybridization of nucleic acids is the process in which two single-stranded nucleic acid molecules with complementary base sequences form a double-stranded nucleic acid molecule. Nucleic acid hybridization technology is a fundamental tool in molecular biology, and has been applied in various fields such as detection of gene expression, screening specific clone from cDNA or genomic library, determining the location of a gene in chromosome and diagnosis of diseases

Nucleic acid hybridization as a technique involves using a labeled nucleic acid probe, which is a **known DNA or RNA fragment**, to bind with the target nucleic acids, which is usually a poorly understood, heterogeneous population of nucleic acids. A **probe** labeled with detectable tracer is the prerequisite for determining a specific DNA sequence or gene in a sample or genomic DNA by nucleic acid hybridization.

Radioactive labeled known sequence of DNA is called **probe.**

The **probes** used in hybridization of nucleic acids include oligonucleotide(15-50 nucleotides), genomic DNA fragment, cDNA fragment and RNA.

Genomic DNA probes can be prepared from the cloned DNA fragment in plasmid.

cDNA probes can be prepared from the cloned cDNA in plasmid, or amplified directly from mRNA by RT-PCR.

RNA probes are usually transcribed in vitro from a cloned cDNA in a proper vector. The size of genomic DNA probes, cDNA probes and RNA probes may be 0.1 kb to 1 kb.

Southern blot hybridization is an assay for sample DNA by DNA-DNA hybridization which detects target DNA fragments that have been size-fractionated **by gel electrophoresis**

Is an enzyme that cuts DNA at or near specific recognition nucleotides sequences known as **Restriction site.** These enzymes are found in bacteria and provide a defense mechanism against invading viruses.

Genetic transformation is the incorporation of naked DNA from the extracellular environment.

Air-free techniques refer to a range of manipulations in the chemistry laboratory for the handling of compounds that are **air**-sensitive. These **techniques** prevent the compounds from reacting with components of **air**, usually water and oxygen; less commonly carbon dioxide and nitrogen.

Flow cytometry (FC) is a technique used to detect and measure physical and chemical characteristics of a population of cells or particles. In this process, a sample containing cells or particles is suspended in a fluid and injected into the **flow cytometer** instrument.

Western blot (Immunoblotting) : A technique for detecting specific proteins separated by electrophoresis by use of labeled antibodies.

The RNA molecules are then separated according to their sizes using a method called **gel electrophoresis.**

Southern blotting is a laboratory technique used to detect a specific DNA sequence in a blood or tissue sample. A restriction enzyme is used to cut a sample of DNA into fragments that are separated using gel electrophoresis.

Nitrocellulose typically has a binding capacity of about 100µg/cm, while nylon has a binding capacity of about 500 µg/cm. Many scientists feel nylon is better since it binds more and is less fragile.

A **northern blot** is a laboratory method used to detect specific RNA molecules among a mixture of RNA.

Sample Collection, such as handling, labeling, processing, aliquoting, storage, and transportation, may affect the results of the study.

A Sample is "a smaller (but hopefully representative) collection of units from a **population** used to determine truths about that population.

Random sampling is the purest form of probability sampling.

Systematic sampling is often used instead of random sampling. It is also called an Nth name selection technique.

Stratified sampling is commonly used probability method that is superior to random sampling because it reduces sampling error.

Cluster Sample: a probability sample in which each sampling unit is a collection of elements.

Convenience sampling is used in exploratory research where the researcher is interested in getting an inexpensive approximation.

Judgment sampling is a common nonprobability method.

Air Sampling Techniques;To investigate biological particles in the air, they have to be caught by a sampling device with a design format suitable for viewing particles under a microscope or other form of analysis.

Passive traps; This is the simplest way of collecting airborne biological particles.

Sampling from Soil;Soil microbial communities form the foundation of the terrestrial ecosystem and are critical drivers of biogeochemical processes.

Typically, the soil is collected using **a 1.5-2.5** in. diameter coring device, with the exact collection device being dictated by the local soil conditions such as rockiness or clay content. Soil sampling is conducted to a maximum depth of **30 cm** wherever possible

Evacuated tubes are commercially prepared with or without additives and with sufficient vacuum to draw a predetermined blood volume per tube.

Red-top tubes contain no additives. These tubes are used for tests performed on serum samples and DNA.

When you use the red-top tubes, the sample an be placed for 1-2 hours so that the serum and blood clots will be separated. Blood clots can be used for DNA analysis.

- Green-top tubes contain heparin
- Blue-top tubes contain sodium citrate and citric acid
- Black-top tubes contain sodium oxalate
- Yellow-top tubes contain acid-citrate-dextrose (ACD) solution.
- Grey-top tubes contain a glycolytic inhibitor.

Heparin is an anticoagulant. There are some reports of occasional problems with heparin in PCR assays, studies generally find that there are no major difference in the use of EDTA or heparin.

Citrate also works by calcium chelation and is used in coagulation studies and blood banking. It is optimal for assays conducted on lymphocytes and DNA.

Blood is a liquid tissue. Suspended in the watery plasma are seven types of cells and cell fragments.

Mononuclear leukocytes are the only cell type in blood capable of growth.

Lyophilization or freeze drying is a process in which water is removed from a product after it is frozen and placed under a vacuum, allowing the ice to change directly from solid to vapor without passing through a liquid phase.

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