



# Zoology Legends

# BT301

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# Zoology Legends

## Mcqs

- ✓ Erwin Chargaff work in... **(1940)**
- ✓ In -----, microbial population is studied by extracting DNA from environmental samples. **(Metagenomics)**
- ✓ One of the applications of chromosome walking and jumping is in the cloning of humans-----gene **(Cystic fibrosis)**
- ✓ Technique used to identify individuals by characteristics of their DNA sequences is called----**(DNA profiling and fingerprinting)**
- ✓ LTR consist of ----- bp **(250-600bp)**
- ✓ Somatostatin protein is prevented from degradation by----- **(Beta galactosidase)**
- ✓ cDNA lacks the ----- sequences which are present in genomic DNA. **(intrinsic)**
- ✓ The term RT-PCR is used for ----- PCR. **(Reverse Transcriptase)**
- ✓ Mitochondrial gene in human cell... **37**
- ✓ Techniques for DNA sequencing became available in the... Late **1970s**
- ✓ -----is a type of PCR reaction but the segments of DNA that are amplified are random **(RAPD)**
- ✓ Sequences that were read beyond 400 bp contained an average of \_\_\_\_\_ **3.2%**

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- ✓ The molecular weight of pBR322 vector is ----- **(4361 bp)**
- ✓ In case of mammals or fungi, the genome size of mitochondria ranges from 15- ----- kb. **(20)**
- ✓ Real time PCR also known as **(Q- PCR)**
- ✓ For the expression of foreign proteins----- must be present. **(Strong promoter)**
- ✓ Complementarity of Shine-Dalgarno (S-D) sequence with 16s rRNA can affect the... **Rate of translation**
- ✓ When the R groups are arranged in a beta pleated sheets. **(outer side)**
- ✓ Happy mapping in genome mapping is an entirely----- technique. **(in vitro)**
- ✓ When distances in genome mapping are calculated in terms of units then this map is known----- **(Physical map)**
- ✓ The term RT-PCR is used for ----- PCR. **(Reverse Transcriptase)**
- ✓ In -----, microbial population is studied by extracting DNA from environmental samples\_\_\_ **(Metagenomics)**
- ✓ The rate of synthesis of a particular protein will depend on the steady-rate of---- in the cell. **(mRNA)**
- ✓ The genome size of the nanoarchaeum equitans is ----- **(0.49)**
- ✓ A type of PCR reaction in which segments of DNA that are amplified are random is ----- **(RAPD)**

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- ✓ Particular transcript of mRNA in a cell is degraded by ----- to lower the expression of particular gene. (**mRNA**)
- ✓ Mitochondria generate in human cell (**13**)
- ✓ A shuttle vector is \_\_\_\_ (**A vector that can be used with two/more systems**)
- ✓ Sequences that are read beyond 400 bp contains an average of (**3.2%**)
- ✓ A term used to describe an approach which allows the isolation of gene sequences which is known as----- (**chromosome walking**)
- ✓ Phage display is the technology that allows the expression of ----- on the surface of phage particles. (**Exogenous polypeptides**)
- ✓ A modified procedure for cosmid is derived by **Ish-Horowitz and Burke**
- ✓ genome size of human  **$2.8 \times 10^6$**
- ✓ In -----two sets of primers are used in two successive PCRs (**Nested PCR**)
- ✓ Kuhne creates the term “enzymes” and discovered.....(**trypsin.** )
- ✓ Boyer brought in restriction enzymes ...(1973)
- ✓ The overall three-dimensional arrangement of all atoms in a protein is referred to as the protein’s ....(**tertiary structure**)
- ✓ mans have genome size of (**3,000 Mb**)

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- ✓ Those plasmids that do not contain tra genes they are called as **non-conjugate plasmids**.
- ✓ It is difficult to clone DNA segments longer than about ...when plasmids are used as vector..(**15000bp**)
- ✓ Charon 4A are ..... **replacement vector**
- ✓ types of DNA array ....(**two**)
- ✓ capsid is made of.....( **protein**)
- ✓ The Pribnow box ..... ( **TATAAT**)
- ✓ the loss of plasmid due to defective partitioning is called (**segregate instability**)
- ✓ process to change the genetic information of organisms is (**Mutagenesis**)
- ✓ genes in mitochondria is (**37**)
- ✓ happy mapping overcomes being an entirely in (**in vitro**)
- ✓ taq polymerase isolated from thermophilic bacterium (**thermus aquaticus**)
- ✓ phage display..... **Polypeptide** kuch is tarha ka option correct tha
- ✓ Total assets... **15**
- ✓ different methods have developed based on enzymatic..... **Solid phase**
- ✓ snps stand for.... **Single nucleotide polymorphism**

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- ✓ RT. Per stand for ... **Reverse transcriptase**
- ✓ prominent secondary structure.... **Alpha helix**
- ✓ The stop codon is----- . (**UAA**)
- ✓ Circular DNA molecule that replicates separately from the host chromosome are ----(**Plasmid**)
- ✓ The technique used for amplify the DNA is -----(**PCR**)
- ✓ The combination of chain terminator and ----- vectors to produce single stranded DNA is very powerful. (**M13**)
- ✓ The host organism that contains target clone is called as----- colonies. (**transferred or recombinant**)
- ✓ For the expression of foreign proteins----- must be present.(**Strong promoter**)
- ✓ Total assets of Biogen is **\$15 Billion**
- ✓ For construction of genomic libraries ----**vectors are required High capacity cloning**
- ✓ In place of phage- $\lambda$  derivatives, a number of high capacity cloning vectors such as **cosmids**, bacterial artificial chromosomes (**BACs**) and yeast artificial chromosomes (**YACs**) are available for construction of genomic libraries
- ✓ Different methods to detect **SNPs** have been developed that based on enzymatic, electrophoretic, solid phase or chromatographic analysis

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- ✓ DNA microarray was used for the first time in 1977 for the genome expression of the -----cells. (**Yeast**)
- ✓ The bacterial chromosome is a very long (**up to 1mm**).
- ✓ RP4 and **RSF1010** and many plasmids from Staphylococcus aureus have broad host range
- ✓ Sum of Purines residue equal to the **Pyrimidine**(AG=TC)
- ✓ Secondary structure of proteins refers to **the local conformation** of some part of a polypeptide

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## Subjective

### RAPD (Random Amplified Polymorphic DNA):

Principle: RAPD utilizes short, random oligonucleotide primers to anneal to multiple sites within the genomic DNA. During PCR, these primers amplify random DNA segments, resulting in a complex mixture of fragments.

#### Procedure:

- ✓ **DNA Extraction:** Genomic DNA is isolated from samples of interest.
- ✓ **PCR Amplification:** The RAPD reaction mixture contains template DNA, random primers, DNA polymerase, nucleotides, and buffer. PCR amplification involves denaturation, primer annealing, and extension steps.
- ✓ **Gel Electrophoresis:** Amplified DNA fragments are separated by size using agarose gel electrophoresis, followed by staining to visualize the bands.
- ✓ **Band Interpretation:** The presence or absence of bands in the gel indicates genetic variation among samples.

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**Applications:** RAPD finds applications in:

- ✓ Genetic fingerprinting for individual identification.
- ✓ Population genetics to study genetic diversity and structure.
- ✓ Phylogenetic analysis to infer evolutionary relationships.
- ✓ Genetic mapping for marker-assisted breeding.
- ✓ Disease diagnosis by identifying associated markers.

**Advantages:** RAPD is rapid, cost-effective, highly polymorphic, versatile, and requires minimal DNA input.

**Limitations:** Challenges include lack of reproducibility due to sensitivity to PCR conditions, dominant markers that cannot distinguish heterozygotes, and non-specific amplification leading to background noise.

## Comparative genomics of eukaryotes?

- The minimal eukaryotic genome is smaller than many bacterial genomes
- Comparative genomics can be used to identify genes and regulatory elements
- It gives insight into the evolution of key proteins

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## Screening strategies?

□ Major screening strategies involve

- 1). Genetic methods
- 2). Sequence-dependent screening
- 3). Screening expression libraries

## Multiplex and Real time PCR

Feature	Multiplex PCR	Real-time quantitative PCR (qPCR)
Purpose	Amplify multiple DNA sequences simultaneously	Measure the quantity of a target sequence
Targets	Amplifies several different DNA sequences	Measures starting amounts of DNA, cDNA, or RNA
Detection	End-point detection after PCR is completed	Real-time monitoring of PCR amplification during the reaction
Analysis	Usually requires gel electrophoresis for analysis	Data analysis can be done during or after the reaction
Sensitivity	Generally lower sensitivity compared to qPCR	Higher sensitivity, capable of detecting smaller amounts of target
Quantification	Not typically used for quantification	Used for absolute or relative quantification of target sequences
Applications	Genotyping, mutation analysis, pathogen detection	Gene expression analysis, viral load quantification, SNP genotyping

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## Four steps in Cloning With Lambda?

Cloning with lambda replacement vectors involves the following steps

- Isolation and cutting vector DNA with appropriate restriction enzymes
- Connecting the two lambda fragments to foreign DNA by using DNA ligase
- In vitro packaging of recombinant DNA
- Infection of E. coli cells and isolation of phage clones by picking plaques on a host strain
- Checking recombinant phage for the presence of foreign DNA

## CLONING STRATEGIES

Any cell based cloning procedure has four essential parts

- i). A method of generating DNA fragment for cloning
- ii). A reaction that inserts that fragment into the chosen cloning vectors
- iii). A means for introducing that recombinant vector into a host cell wherein it is replicated
- iv). A method of selecting recipient cells that have acquired the recombinant

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## Benefits of cDNA in recombinant DNA technology

Here are the benefits of using cDNA in recombinant DNA technology:

- **Simplified Cloning:** cDNA lacks non-coding regions, making it easier to clone genes into vectors for various applications.
- **Focused Gene Expression:** It represents actively expressed genes, allowing researchers to study specific gene functions more effectively.
- **Efficient Protein Production:** cDNA simplifies the process of producing proteins in host cells, as it contains only coding sequences.
- **Amplification of Low-Abundance Genes:** cDNA synthesis can amplify rare or low-abundance transcripts, aiding in the study of less common genes.
- **Discovery of New Genes:** cDNA libraries help in the discovery of new genes and understanding their roles in biological processes.

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## Define Genetic variation

Genetic variations are differences in DNA sequences among individuals within a population.

These variations can occur at the level of a single nucleotide (single nucleotide polymorphisms or SNPs), as well as larger scale variations such as insertions, deletions, or rearrangements of DNA segments.

Here's an example of genetic variation in real life:

**Example:** Blood Type, Humans Skin Color, Yellow Brown Hair Australian natives

## Requirement for expression in E. coli

To express a functional protein in E. coli, the gene of interest is cloned into an expression vector. Efficient transcription and translation of the gene are necessary, along with codon optimization for E. coli. Some proteins may require post-translational modifications, and compartmentalization within the cell may be needed for proper functionality.

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## What is primer Extension? Explain single method

Primer extension is a technique used in molecular biology to copy a specific segment of DNA

In the single primer method of primer extension, a short piece of chemically made DNA (7-20 base pairs long) is used to start copying a longer piece of DNA. This short piece has one base that's different from the original DNA. After putting this copied DNA into E. coli bacteria, we get two types of DNA: one that's exactly like the original and one with the changed base.

This copied DNA can be used for various molecular biology applications such as cloning or genetic analysis.

## Yeast libraries and prokaryotes libraries

Aspect	Yeast Libraries	Prokaryotic Libraries
Organism	Yeast ( <i>Saccharomyces cerevisiae</i> )	Bacteria (e.g., <i>Escherichia coli</i> )
Cell Type	Eukaryotic, with nucleus and complex cellular machinery	Prokaryotic, lacking nucleus and simpler cellular structure
Applications	Cloning, functional genomics, gene expression studies	Cloning, microbial genetics, protein production
Complexity	More complex due to eukaryotic cellular processes	Simpler due to absence of nucleus and organelles
Suitable for	Studying eukaryotic gene regulation, protein interactions	Studying microbial genomes, gene expression, and biotech

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**DNA is a polymer of deoxy ribonucleotide. Enlist and explain chemical composition of deoxyribonucleotide**

DNA (deoxyribonucleic acid) is composed of repeating units called nucleotides, which are made up of three main components: a phosphate group, a sugar molecule, and a nitrogenous base.

Here's the chemical composition of deoxyribonucleotide:

- **Phosphate:** Gives DNA its negative charge, linked to the 5' carbon of the sugar.
- **Sugar:** Deoxyribose, a five-carbon sugar with numbers 1' through 5', connects to both the phosphate and the base.
- **Base:** Adenine (A), cytosine (C), guanine (G), or thymine (T), linked to the 1' carbon of the sugar through a glycosidic bond.

**What is chromosomes jumping?**

Chromosome jumping involves identifying the DNA of interest, cutting it into fragments with restriction enzymes, and circularizing them. This method brings together DNA sequences that were originally distant in

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the genome. The cloned DNAs from these circularized fragments form a jumping library.

## What is biotechnology and its branches?

Biotechnology is using tiny organisms like microbes, or cells from animals or plants, along with their products, to make, break down, or change materials.

**Traditional biotechnology** refers to the conventional techniques that have been used for many centuries to produce beer, wine, cheese etc.

**Modern Biotechnology** embraces all methods of genetic modification by recombinant DNA & cell fusion techniques together with the modern developments of traditional biotechnological processes.

**White Biotechnology** Development of processes and microorganisms for Industrial processes.

**Example:** Enzyme production

**Red Biotechnology:** It is concerned with the discovery and development of innovative drugs and treatments

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**Green Biotechnology:** It is concerned with the modification of the genetic composition of plants to enhance existing traits or add new ones.

## Example

Bt corn from Syngenta

Bt cotton from Monsanto

## Why the product of PCR varying in quantity

The quantity of PCR product can vary due to factors like the amount of initial DNA template, PCR efficiency influenced by primer design and cycling conditions, potential PCR inhibition from contaminants, and variations in experimental technique and conditions.

## Why PCR is considered as alternative of DNA genome

PCR is considered an alternative to traditional methods for studying DNA genomes because it's fast, sensitive, specific, versatile, and can be automated.

It rapidly amplifies target DNA sequences, detects small amounts of DNA, and selectively amplifies specific regions without the need for cloning. PCR's efficiency and automation make it invaluable for various

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applications in genetics, molecular biology, and diagnostics.

## Spotted DNA arrays

Spotted DNA arrays are created by printing DNA samples onto specially treated microscope slides. This process involves transferring or spotting DNA clones or PCR products onto a solid support, where they become immobilized.

These arrays are used in functional genomics and proteomics studies for various applications, including analyzing DNA binding interactions and studying gene expression patterns.

## Cosmid

A cosmid vector is a type of DNA carrier that includes a specific segment from a bacteriophage called lambda ( $\lambda$ ). Including “Cos” site. These vectors, called cosmids, are useful for cloning large sections of foreign DNA.

They work well with an in vitro packaging system to insert DNA fragments. Cosmids are especially useful for

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creating libraries of genetic material from eukaryotic organisms.

## Maxam\_Gilbert DNA sequencing Method

The Maxam-Gilbert method is a DNA sequencing technique that uses chemical reagents to break the DNA at specific bases. By inducing base-specific cleavage, this method allows researchers to determine the sequence of nucleotides in a DNA molecule.

## ORI region

The "ori" region, short for origin of replication, is a specific DNA sequence where DNA replication begins.

It serves as the starting point for the replication process, where the enzymes responsible for copying the DNA molecule attach and initiate the synthesis of a new DNA strand.

The ori region is essential for ensuring accurate and efficient replication of the entire genome.

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## Chain termination or deoxy procedure

The chain termination or deoxy method, also known as the Sanger method, was developed by Sanger et al. (1977).

In this technique, DNA polymerase incorporates dideoxynucleoside triphosphates (ddNTPs) as substrates. These ddNTPs act as chain terminators, stopping DNA synthesis.

The procedure involves carrying out DNA synthesis in the presence of all four deoxynucleoside triphosphates (dNTPs), one of which may be labeled with  $^{32}\text{P}$ , in separate incubation mixes. As the ddNTPs randomly terminate DNA synthesis at different positions, a series of fragments with varying lengths are generated, allowing determination of the DNA sequence.

## How functional complementation restores the lost function in higher organism like animals?

Functional complementation is the process by which a particular DNA sequence compensates for a missing function in a mutant cell and thus restores the wild type phenotype.

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Functional complementation is also possible in transgenic animals and plants. It has been used for complementation in transgenic mice to isolate the Shaker-2 gene

## What is the role of Electroporation?

- Facilitates uptake of foreign molecules into cells.
- Allows for gene transfer in genetic engineering and gene therapy.
- Enables cell transformation to express new traits or proteins.
- Enhances drug delivery for medical treatments.
- Promotes microbial transformation for genetic manipulation.

## Application of PCR

PCR has widespread applications in various fields of life sciences including genetic engineering, medical, forensic, agriculture, environment etc.

### PCR-Gene cloning and expression.

PCR has been used in gene cloning and screening of genomic libraries

### PCR-Medicine

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PCR has major impact on medicine especially in the field of clinical microbiology or diagnosis. Molecular tools have also allowed to perform prenatal genetic diagnosis

## **PCR-Forensic sciences**

Forensic science is the application of scientific procedures to solve criminal and legal matters.

Molecular methods are used to established the filiations of a person or to obtain evidence from minimal samples of saliva, semen or other tissues

## **PCR-DNA profiling**

DNA profiling or DNA fingerprinting is a forensic technique used to identify individuals by characteristics of their DNA

## **PCR-Agricultural sciences and environment**

PCR has also facilitated research in detection of pathogens in plants, animals and environment

## **PCR-Molecular Paleontology**

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Molecular paleontology refers to the recovery and analysis of DNA and protein from ancient human, animal and plant remains

## Significant of PCR in medical field

PCR has major impact on medicine especially in the field of clinical microbiology or diagnosis. Molecular tools have also allowed to perform prenatal genetic diagnosis. PCR enables the detection of genetic disorders such as Down syndrome, cystic fibrosis, and sickle cell anemia during pregnancy,

## Automated DNA sequencing

To automate the process, it is desirable to acquire sequence data in real time by detecting the DNA bands within the gel during electrophoretic separation.

## Sequencing accuracy:

Sequences that were read beyond 400 base pairs contained an average of 3.2% error, while those less than 400 base pairs had 2.8% error.

## DNA sequence databases:

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Large amounts of sequence data accumulated since the development of current DNA sequencing technology are maintained in three databases:

- i) National Center for Biotechnology Information
- ii) DNA Databank of Japan
- iii) European Bioinformatics Institute-UK

## Nomenclature of restrictions enzyme Modification

After the discovery of large number of Type II endonucleases, scientists proposed that there must be a naming mechanism which we can call as the nomenclature of the restriction endonucleases.

A suitable system was proposed by Smith and Nathans (1973)

- The species name of host organisms is identified by the first letter of genus and first two letters of specific epithet. *E. coli* = Eco    *H. influenzae* = Hin    Strain identification is written as Ecok
- In case, host strain has several restriction and modification systems, these are identified by roman

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numerals, for example, in case of H. influenzae HindI, HindII, HindIII etc

- All restriction enzymes have general name endonuclease R and modification-methylase M followed by the system name, for example, in case of H. influenzae R. HindIII or M. HindIII

## Genomic and its types

The complete set of DNA found in each cell is known as the genome and study is called as genomics. Genomics particularly deals with genetic variants that affect health, disease or drug response.

### Types

- Structural Genomics
- Functional Genomics
- Comparative Genomics
- Evolutionary Genomics
- Population Genomics
- Epigenomics
- Metagenomics

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## Explain tertiary structure of protein

The tertiary structure of a protein refers to its overall three-dimensional arrangement, including longer-range aspects of its amino acid sequence. Within this structure, amino acids that are distant in the polypeptide chain can interact.

These interactions are held by various weak interactions and sometimes by covalent bonds between segments of the polypeptide chain. Proteins often fold into two or more globular clusters called domains, giving them a bi- or multilobal appearance.

## STS in physical maps?

STS stands for Sequence Tagged Site. In physical mapping, STSs are short DNA sequences with unique locations in the genome. They serve as landmarks to which other DNA sequences can be anchored.

By identifying the presence or absence of specific STSs in a set of clones, researchers can order and orient those clones along the chromosome. STSs are commonly used in constructing physical maps of genomes, helping to

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determine the order, distance, and organization of DNA sequences.

## Disadvantages of RT PCR

- It can be costly because of needing special equipment and materials.
- It's a bit complex to set up and analyze the data, requiring know-how.
- It's sensitive to stuff in the samples that can mess up the results.
- It might struggle with accurately measuring very low or high levels of stuff in the sample.
- Making sure the data is compared fairly across different samples can be tricky.
- It's not great at testing many things at once.
- Sometimes, it might give wrong results if there's contamination.

## What is DNA and its type?

DNA, or deoxyribonucleic acid, is a molecule found in all living organisms that carries genetic information. It consists of two long chains of nucleotides twisted into a double helix structure.

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Each nucleotide contains a sugar (deoxyribose), a phosphate group, and one of four nitrogenous bases: adenine (A), thymine (T), cytosine (C), or guanine (G). The sequence of these bases along the DNA molecule determines the genetic instructions for an organism's development, functioning, and traits.

## Types:

- Genomic DNA
- Mitochondrial DNA
- Chloroplast DNA

## FISH?

Fluorescence in situ hybridization (FISH) is a cytogenetic technique that employs fluorescent probes. These probes specifically bind to complementary sequences on chromosomes, allowing for the visualization and mapping of specific DNA sequences within the cell.

## Genome mapping and its type?

Assigning of a specific gene to particular region of a chromosome and determining the location of and relative distances between genes on the chromosome

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## Types of genome mapping:

- **Genetic Linkage Mapping:** Illustrates the order and relative distances between genes or genetic markers on chromosomes.
- **Physical Mapping:** Provides actual distances between landmarks on a chromosome, typically measured in base pairs.

## Chromosome walking?

Chromosome walking is an approach used to find gene sequences whose function is unknown but their genetic location is known. It starts with a cloned genomic fragment as a starting point.

For example, in the human genome, this starting point could be a sequence closely linked to a disease locus. One drawback of this method is that each DNA segment used must not be repeated elsewhere in the genome.

## Profiling in human disease

Profiling in human disease involves using arrays to study transcriptional profiles associated with various diseases.

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This approach helps identify novel disease markers and potential new drug targets.

## Hershey DNA experiment

In the Hershey-Chase experiment, conducted in 1952, bacteriophages containing either labeled DNA or proteins were used to infect bacteria. After infection and blending to remove the viral protein coats, centrifugation revealed that radioactive DNA entered the bacterial cells, while little protein did.

This definitive evidence established DNA as the genetic material responsible for heredity, revolutionizing our understanding of genetics and molecular biology.

The experiment paved the way for further research, including the elucidation of DNA's structure by Watson and Crick, and laid the foundation for modern molecular genetics.

## Shotgun sequencing

Shotgun sequencing is a method used to sequence long strands of DNA.

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- **Breaking Down DNA:** In shotgun sequencing, long DNA strands are broken into smaller fragments.
- **Individual Sequencing:** Each fragment is sequenced individually, allowing for efficient processing.
- **Reassembly Process:** After sequencing, the fragments are reassembled in their original order.
- **Efficient Genome Sequencing:** This method is effective for sequencing large genomes, aiding in the study of genetics and genetic disorders.

## Reverse transcriptase

Reverse transcriptases, found in retroviruses, are enzymes that convert RNA into DNA. They are crucial for retroviral replication, enabling the integration of viral RNA into the host cell's genome.

In addition to their role in viral infection, reverse transcriptases are widely used in molecular biology research. They power techniques like reverse transcription polymerase chain reaction (RT-PCR) and cDNA library construction, allowing scientists to study gene expression and RNA molecules.

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## RT-PCR

RT-PCR, or Reverse Transcription Polymerase Chain Reaction, is a technique used to amplify and detect RNA molecules.

It involves converting RNA into DNA using reverse transcriptase and then amplifying the DNA using PCR. RT-PCR is widely used in research, diagnostics, and forensic analysis for studying gene expression, detecting viruses, and genetic testing.

## AFLP\_PCR

AFLP-PCR, or Amplified Fragment Length Polymorphism Polymerase Chain Reaction, is a technique used to detect differences in DNA fragments caused by single nucleotide polymorphisms (SNPs) or insertions/deletions (INDELs).

AFLP is a PCR-based tool commonly utilized in genetic engineering and molecular biology research

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to analyze genetic variation and identify molecular markers associated with traits of interest.

**Importance of stability of plasmid and Effects of copy number of plasmid.**

## **Importance of Stability of Plasmid**

- Vital for maintaining genes of interest
- Ensures consistent gene expression
- Enables long-term storage
- Crucial for reliable experimental results
- Necessary for continuous bioproduction processes
- Important for biosafety considerations
- Prevents loss of genetic material during bacterial growth and replication
- Ensures integrity and functionality of genetic constructs

## **The effect of plasmid copy number**

□ The number of ribosomes in a cell far exceeds any one class of mRNA

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□ One way of increasing the expression of a cloned gene is to increase the number of the corresponding transcript

## DNA Sequencing: Benefits and Applications

DNA sequencing is the process of determining the precise order of nucleotides (adenine, cytosine, guanine, and thymine) within a DNA molecule

DNA sequence information is essential for planning any significant manipulation of the DNA molecule, such as genetic engineering or gene editing.

### Applications:

DNA sequencing information finds utility in various fields including molecular biology, evolutionary biology, metagenomics, medicine, forensics, and beyond.

### Benefits:

- Understanding genetic information
- Medical diagnostics and personalized medicine
- Biotechnological innovations
- Evolutionary studies and population genetics

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- Forensic investigations and human identification
- Environmental monitoring and biodiversity assessment
- Drug discovery and development
- Agricultural improvements and livestock breeding programs

## Work of Franklin & Wilkins (1950s)

Rosalind Franklin and Maurice Wilkins performed x-ray diffraction analysis of DNA fibers.

They demonstrated that DNA produces a characteristic x-ray diffraction pattern.

From this pattern, they made two important findings:

1. DNA molecules are helical.
2. The helices exhibit two periodicities along their long axis, with a primary one of  $3.4 \text{ \AA}$  and a secondary one of  $34 \text{ \AA}$ .

## What is meant by genome?

The genome is the complete set of genetic material present in an organism's DNA, including all of its genes, regulatory sequences, and non-coding regions. It serves as

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the blueprint for the development, functioning, and inheritance of an organism.

## Alpha beta sheet Common in secondary structure protein?

Alpha helix and beta sheet are common secondary structures in proteins. The alpha helix is a coiled structure formed by twisting the polypeptide chain, while the beta sheet is a sheet-like structure formed by extended segments of the chain. These structures are stabilized by hydrogen bonds and contribute to the overall shape and stability of proteins.

## Amino acid and give name

Amino acids are organic compounds that serve as the building blocks of proteins. There are 20 standard amino acids commonly found in proteins, each with a unique chemical structure and side chain.

Here are the names of the Some standard amino acids:

Alanine (Ala), Arginine (Arg), Glycine (Gly), Leucine (Leu), Isoleucine (Ile), Proline, Tyrosine (Tyr), Valine (Val)

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## RNA and it's types

RNA, or ribonucleic acid, is a molecule essential for various biological processes, including protein synthesis, gene regulation, and genetic information transfer.

Here are the main types of RNA:

- Messenger RNA (mRNA)
- Transfer RNA (tRNA)
- Ribosomal RNA (rRNA)
- MicroRNA (miRNA)
- Small interfering RNA (siRNA)
- Long non-coding RNA (lncRNA)

## DNA Microarrays

DNA Microarrays are utilized to simultaneously study the expression of numerous genes. The process involves immobilizing thousands of gene sequences on a glass slide, which are then detected through complementary base pairing between the sample and the gene sequences on the chip.

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- **Spotted DNA arrays:** In this type of DNA array, gene sequences are manually spotted or deposited onto the surface of the microarray slide.
- **Printed oligonucleotide chip:** In this variation, oligonucleotide probes are synthesized directly onto the microarray chip using printing techniques.

## Amplification of DNA sequencing

DNA sequencing amplification refers to the process of increasing the amount of DNA fragments before they are sequenced. This amplification step is often necessary because the DNA sample obtained from biological material may be insufficient for direct sequencing.

Common methods of DNA amplification include polymerase chain reaction (PCR) and multiple displacement amplification (MDA). These techniques enable researchers to generate enough DNA copies for sequencing, allowing for the accurate determination of the DNA sequence.

## Protein Trafficking in Bacteria

Proteins synthesized in the cytoplasm of bacteria often need to be transported to other cellular locations, such as

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the inner membrane, periplasmic space, or outer membrane.

This process involves several mechanisms:

**General Secretion Pathway:** Moves proteins from the cytoplasm across the cytoplasmic membrane into the periplasmic space for secretion outside the cell.

**ABC Pathway for Protein Export:** Relies on ATP-binding cassette (ABC) transporters to move proteins across the cytoplasmic membrane using energy from ATP hydrolysis.

**Type II Secretion Pathway:** Translocate proteins from the periplasmic space across the outer membrane. Proteins are first translocated into the periplasmic space by the Sec system before being exported through the outer membrane.

**What is Mutagenesis? Describe its methods.**

Mutagenesis is a process to change the genetic information of an organism.

It can occur naturally, or as a result of exposure to mutagens or induced experimentally in laboratory

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□ Three different methods of site-directed mutagenesis have been devised

- i). Cassette mutagenesis
- ii). Primer extension
- iii). Procedures based on PCR

## What is the function of RNA

- mRNA: Carries genetic instructions from DNA to ribosomes for protein synthesis.
- tRNA: Transports amino acids to ribosomes during protein synthesis.
- rRNA: Structural component of ribosomes, facilitating protein synthesis.
- miRNA: Regulates gene expression by binding to mRNA molecules.
- siRNA: Mediates RNA interference, leading to gene silencing.
- lncRNA: Regulates gene expression and cellular processes without coding for proteins.
- RNA is crucial for genetic information transfer and gene expression regulation in cells.

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## DNA sequence databases

Since the development of current DNA sequencing technology large amount of sequence data has accumulated that is maintained in 3 data bases

- i). National center for Biotechnology Information
- ii). DNA Databank of Japan
- iii). European Bioinformatic institute-UK

## Chargaff work on DNA structure

Erwin Chargaff and his colleagues discovered important clues to DNA's structure in the late 1940s.

### Chargaff's rules state:

- DNA's base composition varies between species.
- Different tissues of the same species have the same base composition.
- DNA's base composition remains constant regardless of an organism's age, diet, or environment.
- In DNA, the number of adenine (A) residues equals the number of thymine (T) residues, and the number of guanine (G) residues equals the number of cytosine (C) residues.

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This implies that the sum of purine residues equals the sum of pyrimidine residues ( $A+G = T+C$ ).

## Benefits of vector

- Vectors are essential tools in genetic engineering.
- They facilitate the cloning and insertion of DNA fragments into host organisms.
- Vectors can deliver foreign DNA into target cells for gene therapy and research.
- They enable stable inheritance and replication of inserted DNA.
- Vectors often contain selectable markers for identifying transformed cells.
- Overall, vectors play a crucial role in advancing scientific understanding and practical applications in various fields.

## Difference between pBR 322 and pBR 325 vectors?

Difference	pBR322	pBR325
Size	Smaller (Approx. 4.4 kb)	Larger (Approx. 7.4 kb)
Antibiotic Resistance	Ampicillin (amp), Tetracycline (tet)	Ampicillin (amp), Tetracycline (tet), Kanamycin (kan)

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## Goucher disease?

Goucher disease, or glycogen storage disease type IX (GSD IX), is a rare genetic disorder impacting glycogen metabolism due to a deficiency of the enzyme phosphorylase kinase.

Various subtypes exist, each linked to mutations in different genes. Symptoms vary but commonly include hypoglycemia, enlarged liver, poor growth, and muscle weakness. Management typically involves dietary adjustments to stabilize blood sugar levels and regular medical supervision.

## What is biopanning?

The primary screening method involves enriching phage clones with binding affinity for the target through a process called biopanning.

Biopanning is a process used to find specific molecules, like antibodies, by enriching them from a mixture.

## Eukaryotic DNA types

- ✓ Protein Coding Genes
- ✓ Tandemly Repeated Genes

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- ✓ Repeated DNA
- ✓ Unclassified Spacer DNA

Protein Coding Genes can further be classified as:

- ✓ Duplicated and Diverged Genes
- ✓ Functional Gene Families and Non-functional Pseudogenes

Tandemly Repeated Genes encode:

- ✓ rRNA
- ✓ 5sRNA
- ✓ tRNA
- ✓ Histones

## Gene Cloning

- ✓ PCR is utilized to amplify target DNA sequences for cloning into vectors.
- ✓ The amplified DNA fragments can be ligated into cloning vectors to generate recombinant DNA molecules.
- ✓ This enables the replication and propagation of the target genes in host organisms, such as bacteria.

**Functional Genomics:**

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Functional genomics is a branch of molecular biology that focuses on understanding the functions and interactions of genes within an organism's genome.

It involves studying how genes are expressed, regulated, and interact with each other to determine their roles in biological processes and disease.

## mRNA Stability

mRNA stability refers to how long mRNA molecules persist within a cell before they are degraded. It is regulated by RNA-binding proteins and degradation pathways, including exonucleolytic and endonucleolytic cleavage.

mRNA stability elements, such as AU-rich elements, and post-transcriptional modifications also play roles.

Changes in mRNA stability can influence gene expression dynamics and are associated with various diseases

- ✓  feature of D loop of human mitochondrial genome
- ✓  termination method
- ✓  Hershey and chase experiment
- ✓  belton and davis lift procedure

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# Zoology Legends

✓ □ SNPs

- 1) Genomic sequence of eukaryotic?
- 2) Define Palisade sequence?
- 3) Why PCR used in Genomic libraries sequence Analysis?

Palindromic sequence

Steps of screen sequencing

YACs

Primary structure of protein long

Steps of colonial strategy long.

1. What is principal strategy used in siRNA technology?  
(3)

3. What may be advantages of cloning in high capacity vector?  
(3)

6. What is purpose of using Northern blotting technique?  
(3)

8. How can PCR help in improving quality of environment?  
(5)

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9. Describe significance of inverse PCR? (5)

1. Write the length of major and minor groove and number of nucleotides on major groove.
2. What is use of RH mapping?
3. What is interrupted matting?
3. Describe chain termination method.

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**Remember me in you Precious Prayer**

**Jazak Allah**

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