Chapter 3
Gene Cloning

EXERCISES

1. Describe the methods used for isolation of DNA.

Ans: Isolating DNA is a fundamental step in many molecular biology and biotechnology techniques. Here are some commonly used methods for DNA isolation:

Phenol-Chloroform Extraction:

- This method involves breaking open cells using a lysis buffer and then extracting DNA with a mixture of phenol and chloroform. Phenol denatures proteins, allowing them to be separated from DNA. Chloroform helps in the phase separation. DNA is then precipitated with ethanol or isopropanol, washed, and rehydrated for further use.

Spin Column-Based Kits:

- Commercial DNA isolation kits use silica-based spin columns for efficient purification. In these kits, cell lysates are applied to the column, and DNA binds to the silica membrane while contaminants are washed away. Finally, DNA is eluted in a low-salt buffer or water.

Salting Out Method:

- This traditional method involves cell lysis followed by precipitation of proteins and other contaminants by adding a high concentration of salt (usually ammonium acetate or sodium chloride). The DNA is then precipitated with ethanol or isopropanol, washed, and resuspended in an appropriate buffer.

Chelex Resin Method:

- Chelex resin chelates metal ions, preventing them from participating in the breakdown of DNA. In this method, a cell suspension is mixed with Chelex resin, and after heating to denature proteins, DNA is released into the solution. The Chelex-DNA mixture is then heated again to denature the DNA and remove proteins and other contaminants.

CTAB (Cetyltrimethylammonium Bromide) Method:

- This method is suitable for DNA extraction from plant tissues. CTAB, a cationic detergent, is used to disrupt cell membranes, and phenol-chloroform extraction is often employed afterward. DNA is then precipitated with isopropanol, washed, and resuspended.

Commercial DNA Extraction Kits:

- Various companies offer DNA extraction kits that provide pre-packaged reagents and spin columns or magnetic beads for quick and efficient DNA isolation. These kits are designed for different sample types, including blood, tissues, and cultured cells.

Proteinase K Digestion:

- Proteinase K is an enzyme that digests proteins. In this method, cells are lysed, and proteinase K is added to digest proteins, releasing DNA. After digestion, phenol-chloroform extraction or other purification methods are applied.

Automated DNA Extraction Systems:

- Automated systems, such as those using robotic workstations or magnetic bead-based platforms, are available for high-throughput DNA extraction. These systems streamline the process and minimize manual labor.

The choice of DNA isolation method depends on factors such as the sample type, the downstream application, and the required purity and yield of DNA. Researchers often choose the most appropriate method based on their specific needs and the characteristics of the samples they are working with.

2. What is the role of biological detergent in the process of isolation of nucleic acid?

Ans: Biological detergents play a crucial role in the process of isolating nucleic acids by facilitating the disruption of cell membranes and cellular structures. Here are the main roles of biological detergents in the isolation of nucleic acids:

Cell Membrane Disruption:

- Biological detergents help break down cell membranes by solubilizing lipids and disrupting the lipid bilayer structure. This is crucial for releasing cellular components, including nucleic acids, into the solution.

Protein Denaturation:

- Detergents aid in the denaturation of proteins, especially membrane proteins. This helps in the solubilization of proteins and prevents them from interfering with the isolation of nucleic acids.

Cell Lysis:

- Detergents contribute to the lysis of cells, breaking them open to release intracellular contents. This is a critical step in the nucleic acid isolation process, as it allows access to the genetic material present within the cells.

Inactivation of Nucleases:

- Some biological detergents have the ability to inactivate nucleases, enzymes that can degrade nucleic acids. By inhibiting nucleases, detergents help protect the integrity of the nucleic acids during the isolation process.

Emulsification:

- Detergents aid in the emulsification of cellular components, promoting the even distribution of lipids, proteins, and nucleic acids in the lysate. This facilitates subsequent steps in the isolation process, such as extraction and purification.

Enhanced Homogenization:

- Detergents help in achieving a homogenous cell lysate by promoting the uniform dispersion of cellular components. This homogenization is important for obtaining a representative sample for downstream applications.

Commonly used biological detergents in nucleic acid isolation include sodium dodecyl sulfate (SDS), Triton X-100, and cetyltrimethylammonium bromide (CTAB). The choice of detergent depends on the nature of the sample, the type of nucleic acid being isolated (DNA or RNA), and the downstream applications for which the nucleic acids will be used.

In summary, biological detergents play a crucial role in breaking down cellular barriers, solubilizing cellular components, and facilitating the isolation of nucleic acids from a variety of biological samples.

3. How does DNA isolation from plant tissue differ from that of bacterial cell?

Ans:

DNA isolation from plant tissue and bacterial cells differs primarily due to the structural and compositional differences between eukaryotic plant cells and prokaryotic bacterial cells. Here are the key distinctions in the DNA isolation process for plant tissue and bacterial cells:

DNA Isolation from Plant Tissue:

Cell Wall Disruption:

- Plant cells have a rigid cell wall made of cellulose, hemicellulose, and pectin. DNA isolation from plant tissue requires breaking down or digesting the cell wall. This can be achieved through enzymatic methods (using enzymes like cellulase or pectinase) or mechanical methods (such as grinding or mortar and pestle).

Secondary Metabolites:

- Plant tissues may contain secondary metabolites, such as polyphenols and polysaccharides, that can co-precipitate with DNA. Special precautions, such as the addition of polyvinylpyrrolidone (PVP) or beta-mercaptoethanol, are taken to remove or neutralize these compounds.

Use of CTAB (Cetyltrimethylammonium Bromide):

- CTAB is a detergent that is commonly used in plant DNA extraction. It helps to solubilize membranes and proteins and is effective in removing contaminants from plant tissues.

Phenol-Chloroform Extraction:

- Phenol-chloroform extraction is often employed to remove proteins and other contaminants. This step is crucial for obtaining high-purity DNA from plant tissues.

Genomic DNA Isolation:

- The target for DNA isolation from plant tissues is often genomic DNA. The goal is to obtain high-molecular-weight DNA suitable for various molecular biology applications.

DNA Isolation from Bacterial Cells:

Cell Wall Simplicity:

- Bacterial cells lack a rigid cell wall like that found in plant cells. DNA isolation from bacterial cells involves direct cell lysis using detergents (e.g., SDS) to solubilize membranes and denature proteins.

Removal of RNA and Proteins:

- RNase treatment is commonly used during bacterial DNA isolation to remove RNA contaminants. Proteinase K is also employed to digest proteins, facilitating the separation of DNA from cellular proteins.

Phenol-Chloroform Extraction:

- Similar to plant DNA isolation, phenol-chloroform extraction is often used for bacterial DNA isolation to remove proteins and achieve high-purity DNA.

Plasmid DNA Isolation:

- Bacterial DNA isolation may specifically target plasmid DNA when extracting from strains carrying plasmids. Plasmid DNA can be isolated using alkaline lysis or other methods specific to plasmid purification.

In summary, DNA isolation methods are adapted to the unique characteristics of plant tissues and bacterial cells. Differences in cell structure, the presence of secondary metabolites, and the specific goals of DNA isolation (genomic DNA or plasmid DNA) necessitate distinct protocols for each type of sample.

4. How many types of restriction enzymes (REs) are there? Can all REs be used in rDNA technology? Give justification.

Ans: Restriction enzymes (REs), also known as restriction endonucleases, are enzymes that recognize specific DNA sequences and cleave the DNA at or near these sequences. There are three main types of restriction enzymes based on their cleavage patterns:

Type I Restriction Enzymes:

- Type I enzymes cleave DNA at sites that are remote from their recognition sequences. They are multifunctional enzymes that possess both endonuclease and methyltransferase activities. The cleavage occurs at a significant distance from the recognition site and results in random DNA fragments.

Type II Restriction Enzymes:

- Type II enzymes are the most commonly used in recombinant DNA technology. They recognize specific DNA sequences and cleave the DNA at or near these recognition sites. Type II enzymes are widely employed for generating DNA fragments with defined ends and for various molecular biology applications.

Type III Restriction Enzymes:

- Type III enzymes, like Type I enzymes, have both endonuclease and methyltransferase activities. Similar to Type I enzymes, Type III enzymes cleave DNA at sites remote from their recognition sequences.

Can all REs be used in rDNA technology?

Not all restriction enzymes can be used in recombinant DNA (rDNA) technology. Here's the justification:

Recognition Site Specificity:

- For a restriction enzyme to be useful in rDNA technology, it must recognize specific DNA sequences that are strategically chosen for DNA manipulation. Type II restriction enzymes are preferred because they cleave DNA at or near their recognition sequences, allowing precise control over DNA fragment ends. Type I and Type III enzymes, with their cleavage sites located at a distance from the recognition site, are less suitable for the controlled generation of DNA fragments required in rDNA technology.

DNA Ends Produced:

- Type II restriction enzymes typically produce DNA fragments with cohesive (sticky) ends or blunt ends. These ends are valuable for DNA ligation and the construction of recombinant DNA molecules. The ability to generate fragments with compatible cohesive ends is crucial for the successful insertion of a foreign gene into a vector during cloning.

Availability and Characterization:

- Type II restriction enzymes are the most well-characterized and widely available. Their recognition sequences are known, and they have been extensively studied, making them the enzymes of choice in rDNA technology. Additionally, databases like REBASE (Restriction Enzyme Database) provide comprehensive information on Type II restriction enzymes.

In conclusion, while Type I and Type III restriction enzymes have their roles in different contexts, Type II restriction enzymes are the most commonly used in rDNA technology due to their specificity, ability to produce cohesive ends, and extensive characterization. Researchers choose specific Type II enzymes based on their recognition sequences and the desired DNA manipulation outcomes in recombinant DNA work.

5. What are the challenges faced during the process of nucleic acid extraction?

Ans: The process of nucleic acid extraction, whether it's DNA or RNA, can present several challenges. Overcoming these challenges is crucial to obtain high-quality, pure nucleic acids for downstream applications. Some common challenges during nucleic acid extraction include:

Sample Quality and Integrity:

- The quality of the starting material (tissue, cells, blood, etc.) is critical. Degraded or contaminated samples can lead to poor nucleic acid quality, affecting downstream applications such as PCR, sequencing, or cloning.

Contaminants:

- Contaminants such as proteins, polysaccharides, phenols, or secondary metabolites can co-purify with nucleic acids and interfere with downstream processes. Contamination can affect the accuracy and efficiency of molecular biology applications.

RNase Contamination:

- Ribonucleases (RNases) are enzymes that degrade RNA. Contamination with RNases can lead to the degradation of RNA during the extraction process, impacting the quality and quantity of isolated RNA.

DNA Shearing:

- Mechanical forces, harsh handling, or enzymatic activity during the extraction process can lead to the shearing of DNA molecules. This is

particularly relevant when isolating high-molecular-weight DNA for applications such as genomic library construction.

Inhibitors:

- Some samples may contain substances that inhibit enzymatic reactions, such as PCR inhibitors. These inhibitors can carry over during nucleic acid extraction, affecting downstream applications.

Low Yield:

- Obtaining a low yield of nucleic acids can be a challenge, especially when working with samples with low DNA or RNA concentrations. This is particularly relevant for rare or precious samples.

Difficult Sample Types:

- Certain sample types, such as bones, hair, or formalin-fixed paraffin-embedded tissues, can pose challenges due to the presence of inhibitors, cross-linking, or degradation.

Automation Issues:

- In automated nucleic acid extraction systems, technical issues, errors in liquid handling, or equipment malfunctions can impact the reliability and reproducibility of the extraction process.

Incomplete Lysis:

- Incomplete lysis of cells or tissues can result in insufficient release of nucleic acids. Achieving complete lysis while minimizing DNA or RNA degradation can be challenging.

Sample Size and Homogeneity:

- Small sample sizes or heterogeneous samples can make it challenging to obtain representative and reliable results, especially in situations where sample variability is high.

Batch-to-Batch Variation:

- Variability in reagents, extraction kits, or instruments between different batches can lead to inconsistent results, affecting the reproducibility of nucleic acid extraction.

To address these challenges, researchers often optimize protocols, choose appropriate extraction methods for specific sample types, and employ quality control measures. Quality assurance steps, such as using RNase-free reagents and performing mock extractions as negative controls, are also crucial to ensure the reliability of nucleic acid extraction procedures.

6. Write the role of alkaline phosphatase, DNA ligase, terminal transferase in rDNA technology.

Ans: In recombinant DNA (rDNA) technology, various enzymes play essential roles in manipulating and creating recombinant DNA molecules. Here are the roles of alkaline phosphatase, DNA ligase, and terminal transferase in rDNA technology:

Alkaline Phosphatase:

- **Role:** Alkaline phosphatase (AP) is used to dephosphorylate DNA molecules in rDNA technology.
- **Justification:**
- Dephosphorylation of DNA is crucial when creating recombinant DNA molecules. Many vectors used in cloning procedures have multiple cloning sites with unique restriction enzyme recognition sequences, which allow the insertion of DNA fragments in a specific orientation. However, to prevent self-ligation of the vector without the insert (vector recircularization), it is essential to remove the 5' phosphate groups from the vector ends.
- Alkaline phosphatase catalyzes the removal of these phosphate groups, converting the 5'-phosphorylated ends of the vector into 5'-hydroxyl ends. This prevents the vector from ligating to itself and favors the ligation of the vector with an insert.

DNA Ligase:

- **Role:** DNA ligase is used to join DNA fragments together by catalyzing the formation of phosphodiester bonds between adjacent nucleotides.
- **Justification:**
- In rDNA technology, DNA ligase is employed during the cloning process to ligate a DNA insert (e.g., a gene of interest) into a vector. The vector and insert are typically cleaved by restriction enzymes, resulting in complementary ends that can be ligated together.
- DNA ligase seals the nicks in the phosphodiester backbone, creating a continuous, covalently linked DNA molecule. This ligation step is crucial for the creation of recombinant plasmids or other vectors containing the desired gene.

Terminal Transferase:

- **Role:** Terminal transferase is used to add nucleotides to the 3' ends of DNA molecules in a template-independent manner.
- **Justification:**
- Terminal transferase is often employed in the addition of homopolymeric tails to the 3' ends of linearized vectors. These tails, consisting of a single type of nucleotide (e.g., poly-A or poly-T), provide complementary overhangs that can enhance the efficiency of ligation with a complementary insert.
- This technique, known as tailing, facilitates the joining of a DNA insert with a vector, especially when the insert has blunt ends or ends that are not compatible with the vector. The addition of homopolymeric tails promotes hybridization between the vector and insert, increasing the likelihood of successful ligation.

In summary, alkaline phosphatase, DNA ligase, and terminal transferase are important enzymes in rDNA technology, each playing a specific role in the manipulation and construction of recombinant DNA molecules. These enzymes contribute to the success of cloning and other molecular biology applications in which the precise joining of DNA fragments is required.

7. Describe the role of chelating agent in the process of DNA extraction.

Ans: Chelating agents play a crucial role in the process of DNA extraction, specifically in removing divalent cations, such as magnesium (Mg^2+) and calcium (Ca^2+), from the DNA extraction buffer. One commonly used chelating agent in this context is ethylenediaminetetraacetic acid (EDTA). Here is a description of the role of chelating agents in DNA extraction:

Role of Chelating Agents (e.g., EDTA):

Inhibition of Nucleases:

- Nucleases are enzymes that can degrade nucleic acids, including DNA. Many nucleases require divalent cations as cofactors for their activity. By chelating or sequestering these divalent cations, chelating agents, such as EDTA, inhibit the activity of nucleases present in the cell lysate during the DNA extraction process.

Prevention of DNA Degradation:

- The removal of divalent cations helps prevent the activity of nucleases that could otherwise degrade the genomic DNA present in the sample. This is particularly important for obtaining intact and high-quality DNA for downstream applications.

Inhibition of Enzymatic Reactions:

- Some enzymatic reactions, such as those involving enzymes that degrade or modify DNA, are dependent on the presence of divalent cations. Chelating agents inhibit these reactions by sequestering the metal ions required for enzymatic activity.

Preservation of DNA Integrity:

- Chelating agents contribute to the preservation of DNA integrity by preventing the action of metal-dependent enzymes that might otherwise cause fragmentation or damage to the DNA molecules during the extraction process.

Minimization of Metal-Induced Oxidation:

- Metal ions can induce oxidative damage to DNA through the generation of reactive oxygen species. Chelating agents help minimize metal-induced oxidation, which could compromise the quality of the isolated DNA.

Stabilization of DNA:

- Chelating agents contribute to the stabilization of DNA by preventing the degradation of DNA molecules during the extraction procedure. This is especially important when working with samples that are prone to enzymatic degradation.

Prevention of Metal-Induced Inhibition:

- Some downstream applications, such as PCR (polymerase chain reaction), are sensitive to the presence of metal ions that can inhibit the activity of DNA polymerases. Chelating agents help prevent such inhibition by sequestering metal ions that could interfere with enzymatic reactions.

In summary, chelating agents like EDTA are included in DNA extraction buffers to inhibit nucleases, preserve DNA integrity, and minimize metal-induced degradation or oxidation. Their presence helps ensure the isolation of high-quality, intact DNA suitable for various molecular biology applications.

8. Briefly describe the modes of DNA transfer into the host.

Ans: DNA transfer into the host is a fundamental process in genetic engineering and recombinant DNA technology. There are several modes of DNA transfer, each designed to introduce foreign DNA into a host organism. The choice of a specific method depends on the nature of the host organism, the type of genetic modification desired, and the goals of the experiment. Here are some commonly used modes of DNA transfer into the host:

1. **Transformation:**
    - **Description:** Transformation is a process in which a host cell takes up naked DNA from its environment and incorporates it into its own genome. In bacterial transformation, for example, competent bacterial cells are treated

with calcium chloride to make their membranes more permeable, allowing them to take up foreign DNA.

2. **Transfection:**
    - **Description:** Transfection is the introduction of foreign DNA into eukaryotic cells. It is commonly used in cell culture systems. Transfection methods include chemical transfection (using liposomes or other chemical agents to deliver DNA), electroporation (using electrical pulses to create temporary pores in cell membranes), and viral transfection (using viral vectors to deliver DNA).
3. **Conjugation:**
    - **Description:** Conjugation involves the transfer of genetic material from one bacterial cell to another through direct cell-to-cell contact. This process is mediated by a conjugative plasmid, and the transfer can occur between cells of the same or different bacterial species.
4. **Transduction:**
    - **Description:** Transduction is a process in which bacterial DNA is transferred from one bacterium to another by a bacteriophage (a virus that infects bacteria). During the lytic cycle, a bacteriophage may mistakenly package bacterial DNA instead of its own, and this DNA can be transferred to another bacterium upon subsequent infection.
5. **Microinjection:**
    - **Description:** Microinjection involves the direct injection of DNA into the nucleus of a eukaryotic cell using a fine glass needle. This method is commonly used in the creation of transgenic organisms, such as genetically modified mice.
6. **Biolistics (Particle Bombardment):**
    - **Description:** Biolistics involves the delivery of DNA-coated particles (usually gold or tungsten) into target cells using a gene gun. The particles are propelled into the cells by a burst of high-pressure helium or nitrogen gas. This method is particularly useful for transforming plant cells.
7. **Agrobacterium-Mediated Transformation:**
    - **Description:** Agrobacterium tumefaciens is a bacterium that naturally transfers DNA (T-DNA) into plant cells, causing the formation of crown gall tumors. In biotechnology, this natural process is harnessed to introduce

foreign genes into plants. The T-DNA region of the Ti plasmid is modified to carry the desired genes, and the modified Agrobacterium is used to infect plant tissues.

8. **Electroporation:**
   - **Description:** Electroporation involves the use of electrical pulses to create temporary pores in cell membranes, allowing DNA to enter the cell. This method is commonly used for bacterial and eukaryotic cell transformation.

Each of these modes of DNA transfer has its advantages and limitations, and the choice of method depends on the characteristics of the host organism and the specific goals of the genetic engineering experiment.

9. Identify the correct statement for blue-white selection method.

(a) A specific dye is used to stain bacterial colony.

(b) It is based on the expression of lacZ gene.

(c) The recombinant bacterial colony remains blue.

(d) lacZ gene is inserted in an antibiotic resistant gene.

Ans: (b) It is based on the expression of lacZ gene.

10. Identify the correctly matched pair from the following options.

(a) Northern blot: Detect specific sequence of DNA

(b) Southern blot: Detect specific sequence of RNA

(c) Western blot: Detect specific proteins

(d) Eastern blot: Detect transcriptional modifications in RNA

Ans: The correctly matched pair is:

(c) Western blot: Detect specific proteins

11. Identify the incorrect matched pair from the following options.
(a) Taq polymerase: Thermus aquaticus

(b) Pfu polymerase: Pyrococcus furiosus

(c) HindIII: Haemophilus influenza

(d) PstI: Pyrococcus stuartii

Ans: The incorrect matched pair is:

(d) PstI: Pyrococcus stuartii

PstI is a restriction enzyme derived from Providencia stuartii, not Pyrococcus stuartii.

12. How are recombinants screened? Describe the methods in detail.

Ans: Recombinant screening involves identifying and selecting organisms or cells that have successfully incorporated the desired recombinant DNA into their genome or plasmids. The screening methods vary based on the specific objectives and the type of recombinant DNA being used. Here are several common methods for screening recombinants:

1. **Selection Based on Phenotypic Markers:**
   - **Description:** This method involves introducing a selectable marker, such as an antibiotic resistance gene, along with the gene of interest into the host organism. Recombinants that have successfully incorporated the desired DNA will also express the selectable marker, allowing for their selection under specific conditions (e.g., growth on a medium containing the antibiotic).
2. **Blue-White Screening (for Cloning in Plasmids):**
   - **Description:** In this method, a plasmid vector contains a selectable marker (often an antibiotic resistance gene) and a reporter gene, such as the lacZ gene. The lacZ gene encodes beta-galactosidase, an enzyme that cleaves X-gal (a colorless substrate) into a blue product. The insertion of foreign DNA disrupts the lacZ gene. Recombinants with the insert will not produce

functional beta-galactosidase and will form white colonies, while non-recombinants form blue colonies.

3. **PCR Screening:**
   - **Description:** PCR (Polymerase Chain Reaction) can be used to screen for the presence of specific DNA sequences in recombinants. Primers are designed to amplify the region of interest, and PCR is performed using DNA from the potential recombinant. Successful amplification indicates the presence of the desired DNA sequence.
4. **Southern Blotting (for Genomic DNA):**
   - **Description:** Southern blotting involves the transfer of DNA fragments from a gel to a membrane, followed by hybridization with a labeled probe specific to the gene of interest. This method is useful for screening recombinants with genomic DNA modifications, insertions, or deletions.
5. **Northern Blotting (for RNA):**
   - **Description:** Similar to Southern blotting, Northern blotting is used for screening RNA molecules. RNA is separated on a gel, transferred to a membrane, and probed with a labeled nucleic acid sequence complementary to the target RNA.
6. **Western Blotting (for Proteins):**
   - **Description:** Western blotting involves the separation of proteins on a gel, transfer to a membrane, and subsequent detection using antibodies specific to the protein of interest. This method is commonly used for screening protein expression in recombinant systems.
7. **Fluorescence-Based Screening:**
   - **Description:** Recombinants may be engineered to express fluorescent proteins, such as green fluorescent protein (GFP). Fluorescence microscopy or flow cytometry can then be used to screen for the presence of the fluorescent marker, indicating successful incorporation of the recombinant DNA.
8. **Enzyme Assays:**
   - **Description:** Enzyme assays can be used to screen for the expression of specific enzymes encoded by the recombinant DNA. For example, if the recombinant DNA includes a gene encoding an enzyme, the activity of that enzyme can be measured.

The choice of screening method depends on the nature of the recombinant DNA, the host organism, and the specific goals of the experiment. Combining multiple methods may also provide more comprehensive verification of successful recombinant DNA incorporation.

13. Differentiate between the Southern, Northern and Western blotting.

Ans: Southern blotting, Northern blotting, and Western blotting are molecular biology techniques used to analyze DNA, RNA, and proteins, respectively. Here are the key differences between Southern, Northern, and Western blotting:

1. **Southern Blotting:**
    - **Target Molecule:** DNA
    - **Purpose:** Detection of specific DNA sequences in a sample.
    - **Procedure:**
        - DNA fragments are separated by gel electrophoresis based on size.
        - The DNA is then transferred from the gel to a solid support membrane (usually a nitrocellulose or nylon membrane) through capillary or vacuum transfer.
        - The membrane is then hybridized with a labeled DNA probe that is complementary to the target DNA sequence.
        - After hybridization, the membrane is washed to remove unbound probe, and the labeled bands are visualized using autoradiography or other detection methods.
2. **Northern Blotting:**
    - **Target Molecule:** RNA
    - **Purpose:** Detection of specific RNA molecules in a sample, often used to study gene expression.
    - **Procedure:**
        - RNA molecules are separated by gel electrophoresis based on size.
        - The separated RNA is transferred to a solid support membrane.
        - The membrane is then hybridized with a labeled RNA or DNA probe that is complementary to the target RNA sequence.

- After hybridization, the membrane is washed to remove unbound probe, and the labeled bands are visualized.

3. **Western Blotting:**
   - **Target Molecule:** Proteins
   - **Purpose:** Detection and analysis of specific proteins in a sample.
   - **Procedure:**
     - Proteins are separated by gel electrophoresis based on size (SDS-PAGE).
     - The separated proteins are transferred to a solid support membrane (usually nitrocellulose or PVDF).
     - The membrane is then incubated with specific antibodies that recognize the target protein.
     - After antibody binding, the membrane is washed to remove unbound antibodies, and the presence of the target protein is detected using secondary antibodies labeled with enzymes or fluorophores.
     - The labeled bands are visualized using chemiluminescence, colorimetry, or fluorescence.

In summary, Southern blotting is used for DNA detection, Northern blotting is used for RNA detection, and Western blotting is used for protein detection. Each technique involves the separation of target molecules, transfer to a membrane, hybridization with specific probes or antibodies, and detection of the labeled bands. The choice of blotting method depends on the type of molecule under investigation.

14. What is PCR? Describe in detail.

Ans: Polymerase Chain Reaction (PCR) is a molecular biology technique that amplifies DNA exponentially, producing millions to billions of copies of a specific DNA sequence. Developed by Kary Mullis in 1983, PCR has become a fundamental tool in various biological and medical applications, including DNA cloning, genetic analysis, forensics, and diagnostic testing.

Principle of PCR:

PCR relies on the enzymatic amplification of a specific DNA target region using a heat-stable DNA polymerase enzyme, synthetic oligonucleotide primers, and nucleotide triphosphates (dNTPs). The reaction is carried out in a thermal cycler, which allows for precise control of temperature.

Components of PCR:

1. **Template DNA:**
   - The DNA template contains the region of interest that needs to be amplified. This can be genomic DNA, cDNA, or plasmid DNA.
2. **Primers:**
   - Short single-stranded oligonucleotides (primers) are designed to be complementary to the sequences flanking the target region. Two primers, one for each strand of the DNA, define the boundaries of the target sequence.
3. **DNA Polymerase:**
   - A heat-stable DNA polymerase enzyme is essential for the synthesis of new DNA strands. Taq polymerase, derived from the bacterium Thermus aquaticus, is commonly used in PCR due to its ability to withstand the high temperatures used in the denaturation step.
4. **Nucleotide Triphosphates (dNTPs):**
   - The building blocks for DNA synthesis, including adenine (A), thymine (T), cytosine (C), and guanine (G).
5. **Buffer Solution:**
   - The reaction buffer provides optimal conditions for DNA denaturation, primer annealing, and DNA extension.

PCR Procedure:

PCR involves a series of temperature cycles, each consisting of three steps: denaturation, annealing, and extension.

1. **Denaturation (94-98°C):**

- The reaction mixture is heated to a high temperature to denature the double-stranded DNA template into two single strands. This step typically lasts for 20-30 seconds.

2. **Annealing (50-65°C):**
    - The temperature is lowered to allow the primers to anneal to their complementary sequences on the single-stranded DNA template. This step lasts for 20-40 seconds.
3. **Extension (72°C):**
    - The temperature is raised, and the DNA polymerase synthesizes a new DNA strand by extending from the primers. The enzyme adds nucleotides in a 5' to 3' direction, and this step's duration depends on the length of the target sequence.
    - The denaturation, annealing, and extension steps are repeated in multiple cycles (usually 20-40 cycles), leading to an exponential increase in the number of DNA copies.

Final Extension:

- After the last cycle, a final extension step is performed at 72°C for an extended period (5-10 minutes) to ensure complete synthesis of any remaining DNA strands.

Final PCR Product:

- The end result is an amplified DNA fragment that is identical to the target region flanked by the primer sequences.

PCR has revolutionized molecular biology by providing a rapid and efficient method for amplifying specific DNA sequences. Its applications range from basic research to clinical diagnostics, forensic analysis, and genetic engineering. Multiple variations of PCR, such as quantitative PCR (qPCR) and reverse transcription PCR (RT-PCR), have been developed to address different experimental needs

15. Write a comparative account of the genomic and cDNA libraries.

Ans: Genomic libraries and cDNA libraries are two types of libraries used in molecular biology for the storage and analysis of genetic information. Here's a comparative account of genomic libraries and cDNA libraries:

1. Definition:

- **Genomic Library:**
  - A genomic library is a collection of clones that represent the entire genome of an organism. It includes both coding and non-coding regions of DNA.
- **cDNA Library:**
  - A cDNA library is a collection of clones that represent the transcribed and expressed genes in a cell. It is derived from complementary DNA (cDNA) synthesized from mRNA.

2. Source of DNA:

- **Genomic Library:**
  - Genomic DNA, which includes both coding and non-coding regions, is used to construct genomic libraries.
- **cDNA Library:**
  - cDNA libraries are constructed from complementary DNA synthesized from the mRNA of a specific cell type or tissue.

3. Representation of the Genome:

- **Genomic Library:**
  - Represents the entire genome, including introns, exons, regulatory regions, and non-coding DNA.
- **cDNA Library:**
  - Represents only the transcribed and expressed portions of the genome, excluding non-coding regions.

4. Cloning Process:

- **Genomic Library:**
  - Genomic DNA is cleaved into fragments using restriction enzymes, and these fragments are ligated into cloning vectors to create a library.
- **cDNA Library:**
  - mRNA is reverse transcribed into cDNA using reverse transcriptase. The cDNA is then cloned into a vector to create the library.

5. Intron-Exon Structure:

- **Genomic Library:**
  - Preserves the intron-exon structure of genes, as it includes the entire genomic DNA.
- **cDNA Library:**
  - Represents only the exonic sequences, as it is derived from reverse transcription of mature mRNA.

6. Complexity:

- **Genomic Library:**
  - More complex due to the inclusion of non-coding regions and repetitive elements.
- **cDNA Library:**
  - Less complex as it focuses on expressed genes and lacks non-coding regions.

7. Applications:

- **Genomic Library:**
  - Used for studying the entire genome, identifying genes, mapping, and understanding genetic diversity.
- **cDNA Library:**
  - Used for studying gene expression, identifying and isolating specific genes, and analyzing transcripts.

8. Cell or Tissue Specificity:

- **Genomic Library:**
  - Represents the entire genomic DNA of an organism, irrespective of tissue or cell type.
- **cDNA Library:**
  - Represents the gene expression profile of a specific cell type or tissue from which the mRNA was isolated.

9. Size of Inserts:

- **Genomic Library:**
  - Can have large inserts, including entire genes and regulatory elements.
- **cDNA Library:**
  - Contains smaller inserts corresponding to the transcribed regions of genes.

10. Complexity Reduction:

- **Genomic Library:**
  - Does not involve complexity reduction steps as it aims to capture the entire genome.
- **cDNA Library:**
  - Involves complexity reduction by excluding non-coding regions and focusing on transcribed genes.

In summary, genomic libraries represent the entire genome, while cDNA libraries focus on expressed genes. The choice between the two depends on the research goals, such as studying the entire genomic content or specifically analyzing gene expression profiles in a particular cell type or tissue.

16. Diploid human genome contains:

(a) 3.2 × 109 base pairs

(b) 6.4 × 108 base pairs

(c) 3.2 × 108 base pairs

(d) 6.4 × 109 base pairs

Ans: (a) 3.2 × 10^9 base pairs

17. Select the incorrectly matched pair from the following.

(a) Nucleases : Hydrolyse phosphodiester bond

(b) Restriction enzymes: Cleave DNA at specific sequence

(c) Palindromic sequence: Read same backwards and forward

(d) EcoRI: Type I Restriction Enzyme

Ans: The incorrectly matched pair is:

(d) EcoRI: Type I Restriction Enzyme

EcoRI is a Type II Restriction Enzyme, not Type I.

18. Assertion: PCR can be used to amplify very small amount of DNA using DNA modifying enzymes. Reason: PCR uses Taq Polymerase.

(a) Both assertion and reason are true and the reason is the correct explanation of the assertion.

(b) Both assertion and reason are true but the reason is not the correct explanation of the assertion.

(c) Assertion is true but reason is false.

(d) Both assertion and reason are false.

Ans: The correct answer is:

(a) Both assertion and reason are true and the reason is the correct explanation of the assertion.

Explanation: PCR (Polymerase Chain Reaction) can indeed amplify very small amounts of DNA, and it relies on the use of DNA-modifying enzymes, including Taq Polymerase. Taq Polymerase is a heat-stable DNA polymerase derived from the bacterium Thermus aquaticus, and it plays a crucial role in the amplification process during PCR. The reason provided is a correct explanation of the assertion.

19. Assertion: Foreign gene can be introduced into host bacterium by transformation techniques like electroporation. Reason: Bacteria have cell wall/membrane.

(a) Both assertion and reason are true and the reason is the correct explanation of the assertion.

(b) Both assertion and reason are true but the reason is not the correct explanation of the assertion.

(c) Assertion is true but reason is false.

(d) Both assertion and reason are false.

Ans: The correct answer is:

(a) Both assertion and reason are true and the reason is the correct explanation of the assertion.

Explanation: Foreign genes can indeed be introduced into a host bacterium by transformation techniques like electroporation. The reason is that bacteria have a cell wall/membrane that can be made transiently permeable by techniques such as electroporation, allowing the uptake of foreign DNA. The reason provided is a correct explanation of the assertion.

SUMMARY

• Isolation of nucleic acids from different organisms is the most essential requirement for any molecular biology experiment. There are four steps in the process of extraction of nucleic acids i.e., disruption of biological samples, protection of nucleic acids from its degrading enzymes, separation of nucleic acids from other molecules and assessment of purity and quality of the isolated nucleic acids.

• Various enzymes play an important role in recombinant DNA (rDNA) technology. These are nucleases, DNA ligase, alkaline phosphatase, polynucleotide kinase, poly A polymerase, etc.

• The major task of the manipulation of DNA involves cutting and ligation of the gene of interest into the vector DNA.

• Nucleases are the enzymes that cleave nucleic acids by hydrolysing the phosphodiester bond that joins the sugar residues of adjacent nucleotides. Two major types of nuclease enzymes depending on its action on the phosphodiester bonds of polynucleotide chains have been identified, which are exonuclease and endonuclease.

• Exonuclease enzymes can remove mononucleotide either from the 3′ or 5′ end of the DNA molecule.

• Endonuclease enzymes cleave DNA molecules at a specific sequence, hence called restriction endonucleases or restriction enzymes (REs). REs are mainly categorised into three groups (i.e., Types I, II and III) based on their cofactor requirement and the position of their DNA cleavage site relative to the target sequence. Type II REs find application in rDNA technology.

• DNA ligase can join two DNA strands together by catalysing the formation of a phosphodiester bond in the duplex form.

• DNA polymerases are a group of enzymes that catalyse the synthesis of new DNA strand by using dNTPs on a template strand.

• Alkaline phosphatase is used to remove the terminal phosphate group from 5′ end of DNA strands.

• Reverse transcriptase is used to generate complementary DNA (cDNA) strand from an RNA template, a process called reverse transcription.

• In rDNA technology, the recombinant DNA is introduced (transferred) in host cells by a number of methods, such as chemical based transfection (calcium

chloride, lipofection etc.) and physical transfection (electroporation, microinjection and biolistic) methods.

• Selection of transformed bacteria is the most essential step for a successful cloning experiment i.e., to identify the transformed cells having recombinant vector (with gene of interest) from a mixture of transformed and non-transformed cells. These selection methods may be direct or through insertional inactivation.

• In direct selection, the transformed cells are distinguished from non-transformed cells on the basis of expression of certain traits, such as resistance to antibiotics.

• In insertional inactivation method, a vector is used having two markers (either two antibiotic resistant genes or one antibiotic resistant gene and lacZ gene).

• Blue-white selection method is another example of insertional inactivation to select recombinant transformed cells in which the expression of lacZ gene can directly be observed in bacterial colonies.

• Blotting techniques are widely used to separate and identify DNA, RNA and proteins from a mixture of molecules.

• Southern blotting technique is used to detect specific sequence of DNA in DNA samples.

• Northern blotting technique is used to detect specific RNA molecules in a mixture of RNA.

• Western blotting is used to detect specific proteins in a sample of tissue homogenate or extract.

• Polymerase Chain Reaction (PCR) is used to amplify a small amount of DNA into thousands to millions of copies, which involves three steps i.e., denaturation, annealing and extension. The amplified product of PCR can be analysed by gel electrophoresis at the end of reaction (end point analysis).

• The latest advancement in PCR technology is real-time quantitative PCR (qPCR), in which the fluorescent markers are used that have specific binding affinity to double stranded DNA. In qPCR, gel electrophoresis is not needed as in the case of conventional PCR.

• DNA libraries are constructed by collecting DNA fragments that have been cloned into vectors so that specific DNA fragments of interest can be identified and isolated. There are basically two types of DNA libraries — genomic and cDNA library.

• A genomic library is a collection of clones of small fragments of DNA that

together represent complete genome of an organism.

• The cDNA library constitutes cDNA clones of all the genes expressed in a specific cell type or tissue of an organism.