جامعة الملك سعود
King Saud University

Practical Note Book

BOT 621
Advance Experimental Taxonomy

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Lab Activity No 01
Title of the Activity- DNA Extraction from plant tissue

Learning Objectives:

- Understand the process of extracting DNA from plant tissue.
- Learn about the role of different reagents in DNA extraction.
- Observe the physical appearance of DNA.

Aim:

To extract and observe DNA from plant tissue using a simple extraction method.

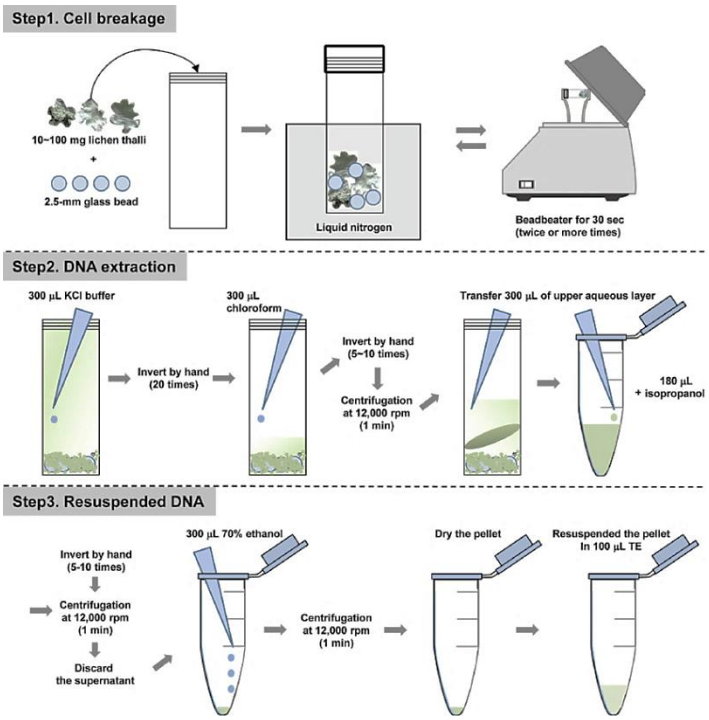
Apparatus & Materials:

- Fresh plant tissue (e.g., banana, spinach, or strawberry)
- Mortar and pestle
- 10% dishwashing liquid or shampoo (detergent solution)
- 5% salt solution (NaCl)
- Ice-cold ethanol or isopropanol
- Distilled water
- Beaker (100 mL)
- Glass rod or toothpick
- Filter paper and funnel
- Test tube

Theory:

DNA (deoxyribonucleic acid) is a genetic material present in all living cells. It can be extracted by breaking open the cells, dissolving the cell membranes, and precipitating the DNA using alcohol. The detergent helps in breaking down the lipid membranes, while the salt stabilizes the DNA molecules. Alcohol is used to precipitate the DNA as it is insoluble in ethanol.

Diagram:



Procedure:

1. Take fresh plant tissue (about 10 g) and grind it with a small amount of saltwater using a mortar and pestle to break open the cells.
2. Transfer the crushed tissue to a beaker and add 10 mL of detergent solution. Stir gently for 5–10 minutes to break the cell membranes.
3. Filter the mixture using filter paper and a funnel to remove solid debris. Collect the filtrate in a test tube.
4. Slowly add an equal volume of ice-cold ethanol along the sides of the test tube without mixing.
5. Allow the tube to stand for a few minutes. DNA will appear as white, stringy precipitate at the interface of the alcohol and filtrate.
6. Use a glass rod or toothpick to spool out and observe the DNA.

Observations Table:

Step	Observation
Grinding plant tissue	Mixture becomes thick and greenish
Adding detergent	Solution becomes frothy and slightly clearer
Filtering	Clear liquid is obtained
Adding ethanol	White, thread-like DNA appears

Result:
DNA was successfully extracted from plant tissue and appeared as a white, stringy precipitate.

Conclusion:
The experiment demonstrates that DNA can be isolated from plant cells using simple chemical treatments. The detergent breaks cell membranes, salt stabilizes DNA, and alcohol helps in precipitation.

Precautions:

- Use fresh plant tissue for better results.
- Do not vigorously shake after adding ethanol to avoid breaking DNA strands.
- Use ice-cold ethanol for better precipitation.
- Handle chemicals carefully to avoid spillage.

Lab Activity No 02
Title of the Activity- PCR Primer and Polymerase Chain Reaction (PCR)

Learning Objectives:

- Understand the principles of Polymerase Chain Reaction (PCR).
- Learn the function and design of primers in PCR.
- Perform PCR and analyze the amplification of DNA.

Aim:
To amplify a specific DNA sequence using Polymerase Chain Reaction (PCR).

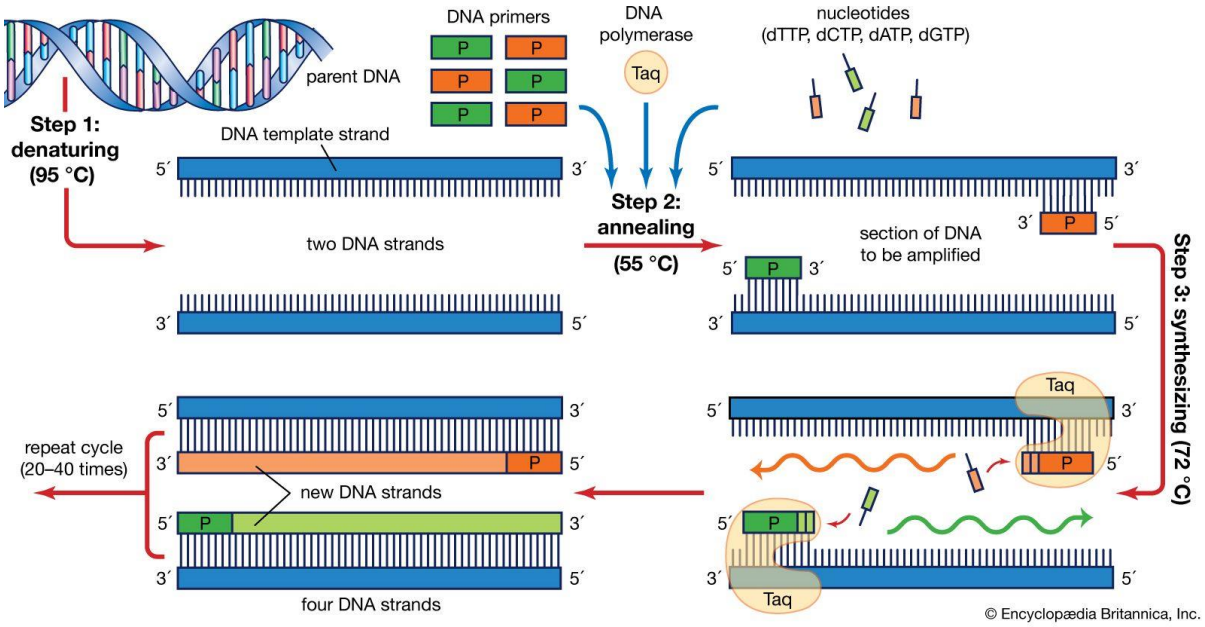
Apparatus & Materials:

- DNA template (target DNA sample)
- Forward and reverse primers

- Taq DNA polymerase
- dNTPs (deoxynucleotide triphosphates)
- PCR buffer with Mg^{2+} ions
- PCR tubes
- Thermal cycler (PCR machine)
- Micropipettes and tips
- Agarose gel and electrophoresis apparatus (for analysis)
- UV transilluminator

Theory:
Polymerase Chain Reaction (PCR) is a molecular biology technique used to amplify a specific DNA sequence. It involves repeated cycles of denaturation, annealing, and extension. Primers are short DNA sequences that define the region to be amplified. Taq polymerase synthesizes new DNA strands using the template. The exponential amplification of DNA makes PCR an essential tool in genetics, forensic science, medical diagnostics, and research.

Diagram:



Procedure:

1. **Preparation of PCR Master Mix:**
   - In a PCR tube, mix the following components:
     - 10–50 ng of template DNA
     - 0.5–1 µM of forward and reverse primers
     - 200 µM of dNTPs
     - 1X PCR buffer (with Mg^{2+})
     - 1–2 U of Taq DNA polymerase
     - Adjust the final volume with nuclease-free water.
2. **Placing the Tube in the Thermal Cycler:**
   - Set up the PCR conditions as follows:

Step	Temperature	Time	Function
Initial Denaturation	94-98°C	2-5 min	Breaks double-stranded DNA into single strands

Denaturation	94-98°C	30 sec	Separates DNA strands
Annealing	50-65°C	30 sec	Primers bind to complementary sequences
Extension	72°C	30-60 sec	DNA polymerase extends the new strand
Final Extension	72°C	5-10 min	Ensures complete DNA synthesis
Hold	4°C	Indefinite	Stores the PCR product

- Repeat **denaturation, annealing, and extension** steps for **25-40 cycles**.

3. **Agarose Gel Electrophoresis (for visualization):**
    - Prepare a **1-2% agarose gel** and load the PCR product.
    - Run electrophoresis at **100V for 30 minutes**.
    - Visualize DNA bands under a **UV transilluminator**.

Observations Table:

Step	Observation
Sample loaded in PCR machine	No visible change
Post-PCR (before gel electrophoresis)	Clear liquid in the tube
After gel electrophoresis	DNA bands visible under UV light

Result:
A distinct DNA band corresponding to the amplified target sequence is observed on the agarose gel, confirming successful PCR amplification.

Conclusion:
The experiment successfully demonstrates the amplification of a specific DNA sequence using PCR. The results confirm the efficiency of primers in targeting the desired DNA region, and the process can be applied in various molecular biology studies.

Precautions:
- Use **sterile** micropipette tips and PCR tubes to prevent contamination.
- Keep **Taq polymerase and dNTPs on ice** to maintain enzyme stability.
- Accurately set the **thermal cycler parameters** for optimal amplification.
- Use **ice-cold ethanol or isopropanol** for DNA precipitation if required.
- Properly handle the UV transilluminator to avoid exposure.

Lab Activity No 03
Title of the Activity- Gel Electrophoresis for DNA Quality

Learning Objectives:
- Understand the principle of gel electrophoresis and its role in DNA analysis.
- Learn how to assess DNA quality based on band patterns.
- Gain hands-on experience in preparing and running an agarose gel electrophoresis experiment.

Aim:
To analyze the quality of DNA using agarose gel electrophoresis by observing the integrity, purity, and size of DNA fragments.

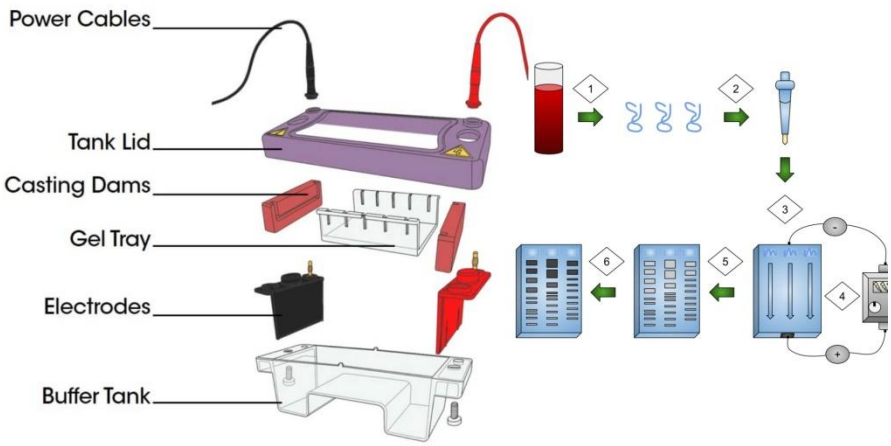
Apparatus & Materials:
- DNA sample (extracted or PCR product)
- Agarose powder
- 1X TAE (Tris-Acetate-EDTA) or TBE (Tris-Borate-EDTA) buffer
- Ethidium bromide (EtBr) or SYBR Green (for DNA staining)

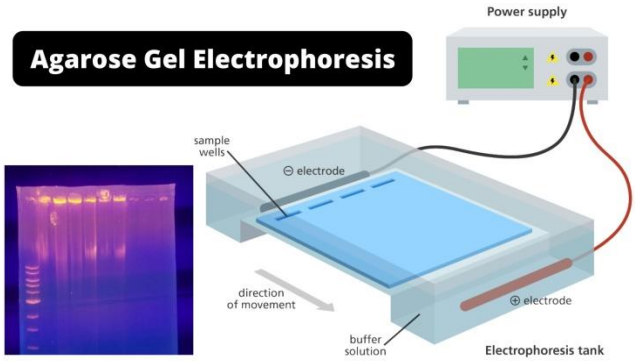
- Loading dye (e.g., bromophenol blue)
- DNA ladder (molecular weight marker)
- Gel casting tray and comb
- Electrophoresis chamber and power supply
- Micropipettes and tips
- UV transilluminator or gel documentation system

Theory:
Gel electrophoresis is a technique used to separate and analyze DNA fragments based on their size. DNA molecules are negatively charged due to their phosphate backbone and migrate towards the positive electrode when an electric field is applied.

- **Agarose Gel Concentration:** Affects resolution; lower % gels (0.8-1%) separate larger fragments, while higher % gels (2-3%) separate smaller fragments.
- **DNA Migration:** Smaller DNA fragments move faster and farther, while larger fragments move slower.
- **Staining:** DNA is visualized under UV light after binding with EtBr or SYBR Green.
- **DNA Quality Indicators:**
    - Intact DNA appears as a high-molecular-weight band.
    - Degraded DNA appears as a smear.
    - Contaminated DNA may show additional unexpected bands.

Diagram:





Procedure:

1. **Prepare the Gel:**

- Dissolve **0.8-2% agarose** in **1X TAE/TBE buffer** by heating.
- Cool slightly, add **EtBr/SYBR Green**, and pour into a gel tray with a comb.
- Allow the gel to solidify (20-30 minutes).

2. **Prepare DNA Samples:**
   - Mix **5 µL of DNA sample** with **1 µL of loading dye**.
   - Prepare a **DNA ladder** in a similar way.
3. **Load the Gel:**
   - Place the solidified gel in the **electrophoresis chamber** and cover it with **1X TAE/TBE buffer**.
   - Load the **DNA samples and ladder** into separate wells using a micropipette.
4. **Run the Electrophoresis:**
   - Connect the electrodes (negative at the well side, positive at the opposite end).
   - Set voltage to **80-120V** and run for **30-45 minutes** (depending on gel size).
5. **Visualize DNA:**
   - Place the gel on a **UV transilluminator** or **gel documentation system**.
   - Capture an image to analyze the bands.

Observations Table:

Sample	Observation under UV Light	Interpretation
High-quality DNA	Single, sharp, high-molecular-weight band	Good integrity
Degraded DNA	Smear instead of distinct bands	DNA degradation
Contaminated DNA	Additional unexpected bands	RNA/protein contamination
DNA ladder	Clear bands at expected sizes	Gel running properly

Result:
Based on the gel electrophoresis results, the DNA quality was assessed. High-quality DNA appeared as intact bands, while degraded or contaminated DNA showed smearing or extra bands.

Conclusion:
Gel electrophoresis is an effective method for evaluating DNA quality. The integrity and purity of DNA can be determined by analyzing band patterns. Proper DNA extraction and storage methods help in obtaining high-quality DNA for further experiments.

Precautions:
- Use gloves and goggles when handling **EtBr** or **SYBR Green** (carcinogenic substances).
- Ensure the gel is properly set before loading samples to prevent leakage.
- Load samples carefully to **avoid cross-contamination** between wells.
- Do not run electrophoresis at excessively high voltage, as it may cause **DNA degradation**.
- Dispose of used gels and chemicals **as per lab safety protocols**.

Lab Activity No 04
Title of the Activity- Sanger Sequencing

Learning Objectives:

- Understand the principle of Sanger sequencing and its role in DNA sequencing.
- Learn how chain termination using dideoxynucleotides (ddNTPs) helps determine DNA sequences.
- Gain knowledge of the components, procedure, and interpretation of Sanger sequencing results.

Aim:

To determine the nucleotide sequence of a given DNA sample using the Sanger sequencing method.

Apparatus & Materials:

- **DNA Template:** The single-stranded DNA to be sequenced.
- **DNA Polymerase:** Taq or another thermostable enzyme for DNA synthesis.
- **Primers:** Short oligonucleotide sequences that initiate DNA synthesis.
- **dNTPs (Deoxynucleotide Triphosphates):** Standard nucleotides (A, T, G, C) for chain elongation.
- **ddNTPs (Dideoxynucleotide Triphosphates):** Modified nucleotides that terminate DNA synthesis.
- **PCR Tubes:** To set up the sequencing reactions.
- **Thermal Cycler:** For amplification and chain termination reactions.
- **Capillary Electrophoresis System or Polyacrylamide Gel:** For fragment separation.
- **Fluorescent Labeling Dye or Radioactive Labeling:** For visualization of the DNA sequence.
- **Computational Software:** For automated sequence analysis.

Theory:

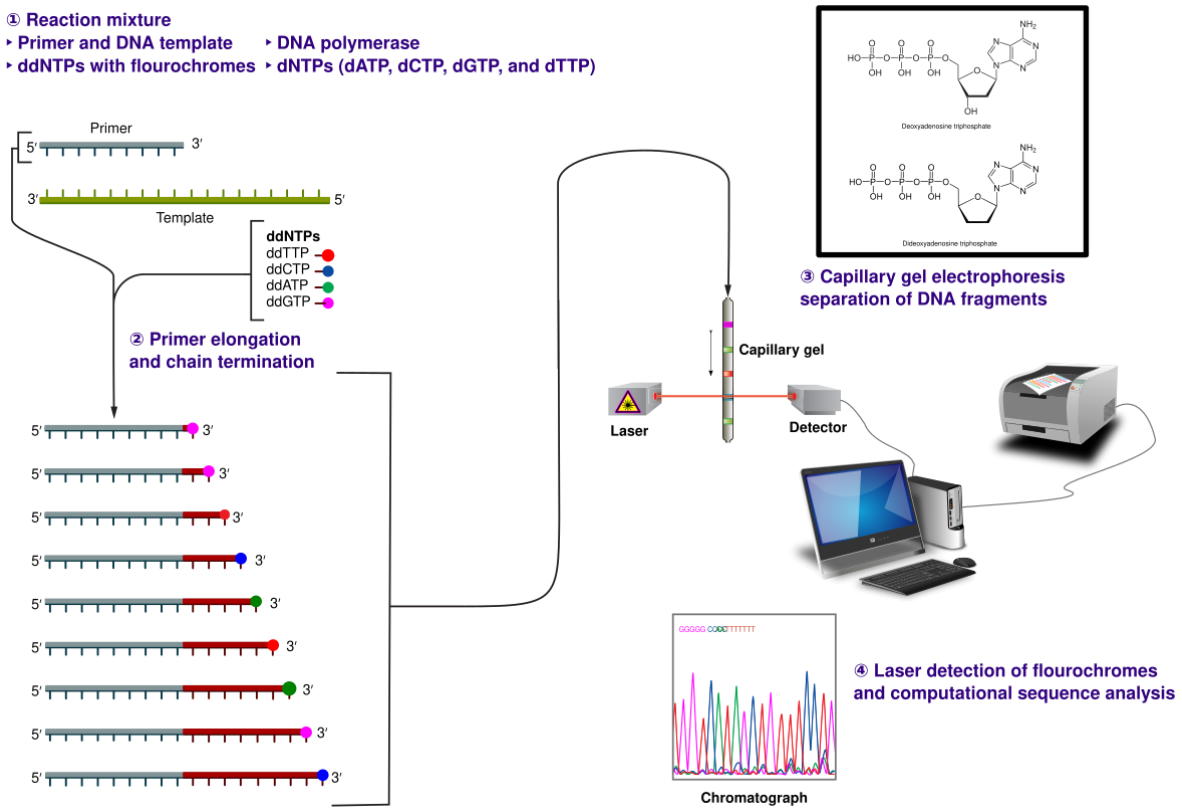
Sanger sequencing, also called the **chain termination method**, is a DNA sequencing technique developed by Frederick Sanger. The process involves DNA synthesis using a polymerase enzyme in the presence of both **normal dNTPs** (which extend the DNA strand) and **ddNTPs** (which cause termination). Since ddNTPs lack a 3'-OH group, their incorporation results in **premature termination** of strand elongation.

By running separate reactions for each nucleotide (A, T, G, C) or using **fluorescently labeled ddNTPs**, the resulting DNA fragments of different lengths can be separated via **capillary electrophoresis or gel electrophoresis** to determine the sequence.

Diagram:

Procedure:

1. **Reaction Setup:**
    - Prepare four separate reaction tubes, each containing:
        - Template DNA
        - DNA polymerase
        - Primers
        - dNTPs
        - A small amount of one of the four **ddNTPs** (ddATP, ddTTP, ddGTP, or ddCTP)



2. **DNA Amplification via PCR:**
    - Run a thermal cycling program:
        - **Denaturation (95°C, 30 sec):** DNA strands separate.
        - **Annealing (50-60°C, 30 sec):** Primers bind to the template.
        - **Extension (72°C, 1 min):** DNA polymerase incorporates nucleotides until a **ddNTP** is added, terminating the strand.
        - Repeat for 25-30 cycles.
3. **Fragment Separation:**
    - Load the amplified DNA into a **capillary electrophoresis system** or **polyacrylamide gel electrophoresis** to separate fragments by size.
4. **Detection and Analysis:**
    - If using **radioactive or fluorescently labeled ddNTPs**, detect fragment sizes using **autoradiography** (for radioactive sequencing) or **laser detection in a sequencer** (for fluorescent sequencing).
    - The banding pattern in electrophoresis corresponds to the **complementary DNA sequence**.

Observations Table:

Step	Observation	Interpretation
DNA denaturation	Single-stranded DNA formation	Ready for primer binding
Primer annealing	Primers attach to the template	DNA polymerase can initiate synthesis
DNA extension with dNTPs & ddNTPs	DNA fragments of varying lengths	Chain termination occurring
Electrophoresis result	DNA bands or peaks of	DNA sequence can be read

	different sizes	

Result:
The sequence of the given DNA template is determined based on the band pattern or **fluorescent peak readings** obtained after electrophoresis. The **shortest fragments represent the start of the sequence**, and the **longest fragments represent the end**.

Conclusion:
Sanger sequencing is a reliable method for determining DNA sequences. It uses **ddNTP-mediated termination**, and by separating the fragments based on size, the **order of nucleotides in the DNA strand can be deduced**. The method is widely used in **genetic analysis, mutation detection, and genome sequencing**.

Precautions:
- Ensure **accurate pipetting** of dNTPs and ddNTPs to prevent sequencing errors.
- Maintain **sterile conditions** to prevent contamination.
- Use **high-purity DNA** for accurate sequencing results.
- Optimize **PCR cycling conditions** to ensure proper amplification.
- Handle **fluorescent dyes and radioactive materials** with proper safety measures.

Lab Activity No 05
Title of the Activity- Retrieval of DNA sequence data from NCBI

Learning Objectives:
- Understand how to access and retrieve DNA sequence data from the **National Center for Biotechnology Information (NCBI)** database.
- Learn the use of **GenBank, FASTA format, and BLAST (Basic Local Alignment Search Tool)** for sequence analysis.
- Gain experience in navigating the **NCBI website** for DNA sequence retrieval.

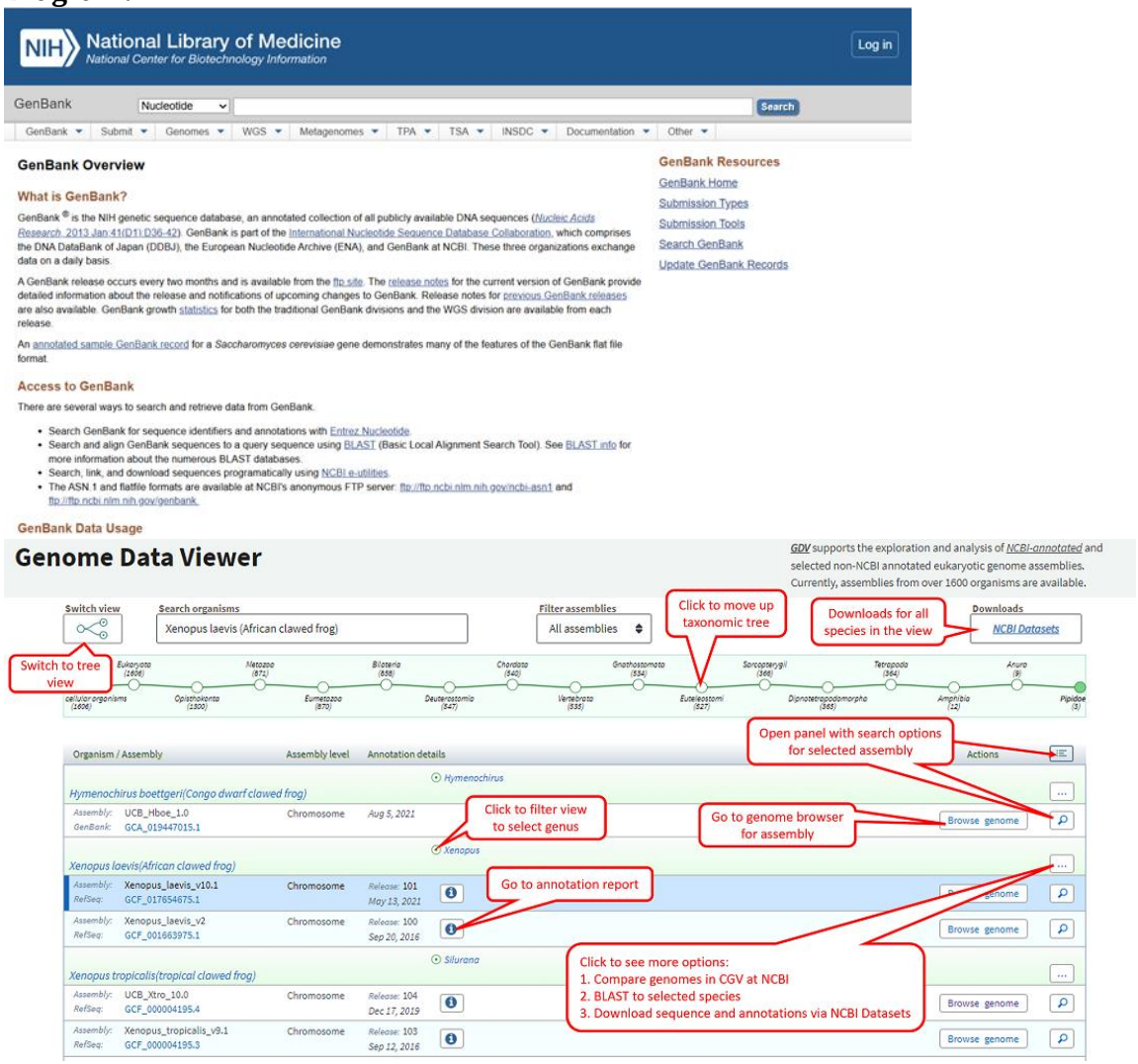
Aim:
To retrieve and analyze a DNA sequence from the **NCBI GenBank database** using an accession number or a keyword search.

Apparatus & Materials:
- **Computer with Internet access**
- **NCBI website (https://www.ncbi.nlm.nih.gov/)**
- **Web browser (Google Chrome, Mozilla Firefox, etc.)**
- **Accession number or search term**
- **Text editor (Notepad, MS Word, or BioEdit for FASTA files)**

Theory:
The **National Center for Biotechnology Information (NCBI)** provides a public database containing DNA, RNA, and protein sequences. The key features include:
1. **GenBank Database:** A comprehensive collection of publicly available genetic sequences.
2. **FASTA Format:** A standard format for representing DNA and protein sequences.
3. **Accession Number:** A unique identifier for each sequence entry in GenBank.
4. **BLAST (Basic Local Alignment Search Tool):** A tool to compare a query sequence with known sequences in the database.

NCBI allows users to **search, retrieve, and analyze** DNA sequences, making it a vital resource for bioinformatics research.

Diagram:



Procedure:

Step 1: Access NCBI Website

- Open a web browser and go to NCBI GenBank.

Step 2: Search for a DNA Sequence

- In the search bar, enter a **gene name, organism name, or an accession number** (e.g., **NM_001301717.2** for the human BRCA1 gene).
- Click **Search** to retrieve matching records.

Step 3: Select a Sequence

- Click on the desired entry from the search results.
- The sequence information, including **source organism, gene location, coding region, and references**, will be displayed.

Step 4: Retrieve the DNA Sequence

- Scroll down to find the **FASTA format** option.
- Click **"FASTA"** to display the sequence in a text format.
- Copy the sequence for further analysis.

Step 5: Download the Sequence File

- Click **"Send to" → "File" → Select FASTA format → Download**.

Step 6: Analyze the Sequence using BLAST (Optional)

- Go to the **BLAST tool** (NCBI BLAST).
- Paste the copied sequence into the input box.
- Click **"BLAST"** to find similar sequences in the database.

Observations Table:

Step	Observation	Interpretation
Search results	Multiple sequence entries appear	NCBI contains many related sequences
Selection of entry	Detailed sequence and metadata displayed	Correct sequence identified
FASTA sequence retrieved	A text file containing A, T, G, C bases	DNA sequence successfully extracted
BLAST result (if used)	Similar sequences with % identity appear	Sequence similarity analysis completed

Result:

A DNA sequence was successfully retrieved from **NCBI GenBank**, displayed in **FASTA format**, and stored for further analysis.

Conclusion:

The NCBI database is an essential tool for retrieving and analyzing DNA sequences. By using search queries and accession numbers, researchers can access genetic information for various applications in **bioinformatics, evolutionary studies, and genetic research**.

Precautions:

- Ensure correct spelling and scientific names while searching.
- Use **official accession numbers** for accurate sequence retrieval.
- Check the **date and version** of the sequence to get the latest update.
- Verify the source organism before using the data in further analysis.
- Avoid **editing** the retrieved sequence accidentally before saving.

Lab Activity No 06
Title of the Activity- Multiple Sequence Alignment (MSA) Using ClustalX

Learning Objectives:

- Understand the principle and importance of Multiple Sequence Alignment (MSA).
- Learn how to perform MSA using **ClustalX**, a widely used bioinformatics tool.
- Interpret sequence alignment results for evolutionary and functional analysis.

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Aim:

To perform **Multiple Sequence Alignment (MSA)** of DNA or protein sequences using **ClustalX** and analyze sequence conservation and evolutionary relationships.

Apparatus & Materials:

- **Computer with Windows/Linux/Mac OS**
- **ClustalX software (Download from: EBI Website)**
- **FASTA format sequence files**
- **Internet access (optional, for database searches and downloads)**

Theory:

Multiple Sequence Alignment (MSA) is a method used to align three or more biological sequences (DNA, RNA, or proteins) to identify regions of similarity. These similarities may indicate **evolutionary relationships, functional regions, or conserved motifs**.

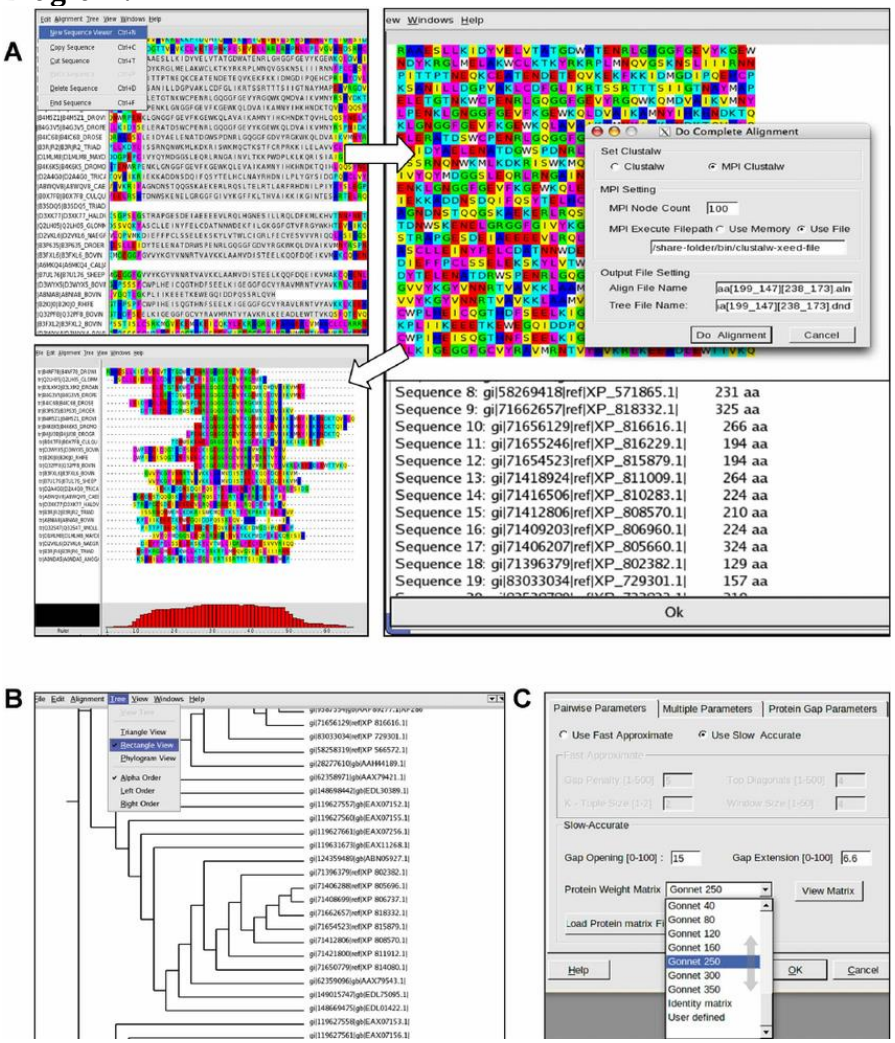
ClustalX is a graphical version of the Clustal algorithm that performs MSA using:

1. **Progressive Alignment Method** – Sequences are aligned stepwise based on similarity scores.
2. **Scoring Matrices** – Used to evaluate alignment quality (e.g., BLOSUM for proteins, PAM for DNA).
3. **Phylogenetic Tree Construction** – Helps understand evolutionary relationships.

Applications of MSA:

- Identifying **conserved domains** in genes and proteins.
- Constructing **phylogenetic trees** for evolutionary studies.
- Comparing **functional sites** in homologous sequences.

Diagram:



Procedure:

Step 1: Install ClustalX

- Download **ClustalX** from the EBI website and install it on your system.

Step 2: Prepare the Input Sequences

- Obtain DNA/protein sequences in **FASTA format**.
- Save all sequences in a **single text file** with .fasta or .txt extension.

Step 3: Load Sequences into ClustalX

- Open **ClustalX** and click **File → Load Sequences**.
- Select the prepared **FASTA file** and open it.

Step 4: Perform Multiple Sequence Alignment

- Click **Alignment → Do Complete Alignment**.
- Adjust parameters if needed (gap penalties, scoring matrix, etc.).
- Wait for the software to process the alignment.

Step 5: View and Analyze the Alignment

- The aligned sequences will be displayed with **conserved regions marked**.
- Save the results by clicking **File → Save Alignment As**.

Step 6: Construct a Phylogenetic Tree (Optional)

- Click **Trees → Draw Tree** to generate a phylogenetic tree.
- Save the tree as an image or text file for analysis.

Observations Table:

Step	Observation	Interpretation
Loading sequences	All sequences appear in ClustalX	Proper sequence input
Alignment process	Aligned sequences with gaps appear	Successful MSA
Conserved regions	Identified by asterisks (*) in the alignment	Highly conserved sequences
Phylogenetic tree	Dendrogram/tree displayed	Evolutionary relationships inferred

Result:

The given DNA/protein sequences were successfully aligned using **ClustalX**, and **conserved regions** were identified. The **phylogenetic tree** showed relationships between sequences.

Conclusion:

ClustalX is an effective tool for performing **Multiple Sequence Alignment (MSA)**, revealing conserved regions and evolutionary relationships among DNA/protein sequences. This alignment can be used for **functional analysis, comparative genomics, and phylogenetics**.

Precautions:

- Ensure **FASTA format** is correctly maintained to avoid errors.
- Choose the **appropriate scoring matrix** (BLOSUM for proteins, PAM for DNA).
- Set **gap penalties correctly** to prevent misalignment.
- Verify **sequence quality** before running MSA.
- Save results **frequently** to prevent data loss.

Lab Activity No 07
Title of the Activity- NJ Phylogenetic Tree Construction Using MEGA

Learning Objectives:

- Understand the **Neighbor-Joining (NJ) method** for constructing phylogenetic trees.

- Learn how to use **MEGA (Molecular Evolutionary Genetics Analysis)** software for tree construction.
- Analyze the evolutionary relationships between different DNA/protein sequences.

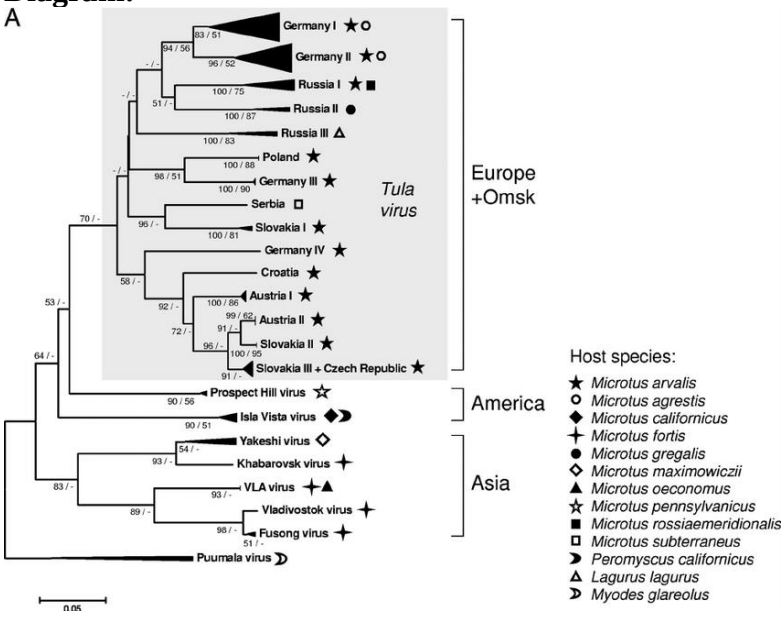
Aim:
To construct a **Neighbor-Joining (NJ) phylogenetic tree** using **MEGA** software to study the evolutionary relationships between sequences.

Apparatus & Materials:
- **Computer with Windows/Linux/Mac OS**
- **MEGA software (Download from: https://www.megasoftware.net/)**
- **FASTA format sequence file**
- **Internet access (optional, for sequence retrieval from NCBI)**

Theory:
A **phylogenetic tree** represents the evolutionary relationships among species or genes. The **Neighbor-Joining (NJ) method**, developed by Saitou and Nei (1987), is a widely used algorithm for tree construction.
Principle of Neighbor-Joining (NJ) Method:
1. **Calculate a distance matrix** based on sequence similarity.
2. **Iteratively join the two closest taxa** (sequences) into a common ancestor.
3. **Reduce the distance matrix** and repeat until a single tree is formed.

Applications of NJ Trees:
- Understanding **evolutionary relationships** among species.
- Identifying **common ancestors** and divergence points.
- Analyzing **genetic similarities** in different populations.

Diagram:



Procedure:
Step 1: Install and Open MEGA Software
- Download and install **MEGA** from the official website.
- Open the software and select **Phylogeny → Construct/Analyze Phylogenetic Tree**.

Step 2: Prepare the Input Sequences

- Obtain DNA or protein sequences in **FASTA format**.
- Open **MEGA → Align → Edit/Build Alignment** to import sequences.
- Save the alignment as a **MEGA (.meg) file**.

Step 3: Create a Distance Matrix

- Click **Phylogeny → Compute Pairwise Distance**.
- Choose an appropriate substitution model (e.g., **Kimura 2-parameter** for DNA).

Step 4: Construct the NJ Phylogenetic Tree

- Go to **Phylogeny → Construct NJ Tree**.
- Select the **NJ method** and choose the substitution model.
- Click **OK** to generate the tree.

Step 5: View and Interpret the Tree

- The phylogenetic tree will be displayed with **branch lengths and bootstrap values**.
- Save the tree by clicking **File → Export Tree as Image**.

Observations Table:

Step	Observation	Interpretation
Sequence alignment	Aligned sequences displayed in MEGA	Ready for tree construction
Distance matrix calculation	Pairwise distances generated	Sequence relationships quantified
NJ tree construction	Tree structure appears with branches	Evolutionary connections established
Bootstrap values	Support values displayed on branches	Confidence level of relationships

Result:

A **Neighbor-Joining (NJ) phylogenetic tree** was successfully constructed using **MEGA software**, revealing the **evolutionary relationships** among the input sequences.

Conclusion:

The NJ method in MEGA efficiently constructs **phylogenetic trees** based on sequence similarity. It is widely used in **molecular evolution, comparative genomics, and phylogenetics** to study the relationships among species or genes.

Precautions:

- Ensure sequences are **properly aligned** before tree construction.
- Choose the **appropriate substitution model** for accurate distance calculations.
- Use **bootstrap analysis** (≥1000 replications) for statistical reliability.
- Verify sequence quality to avoid misinterpretation.
- Save results and **export the tree image** for documentation.

Lab Activity No 08
Title of the Activity- MP Phylogenetic Tree Construction Using MEGA

Learning Objectives:

- Understand the **Maximum Parsimony (MP) method** for constructing phylogenetic trees.
- Learn how to use **MEGA (Molecular Evolutionary Genetics Analysis) software** for MP tree construction.

- Analyze **evolutionary relationships** among DNA/protein sequences using the MP approach.

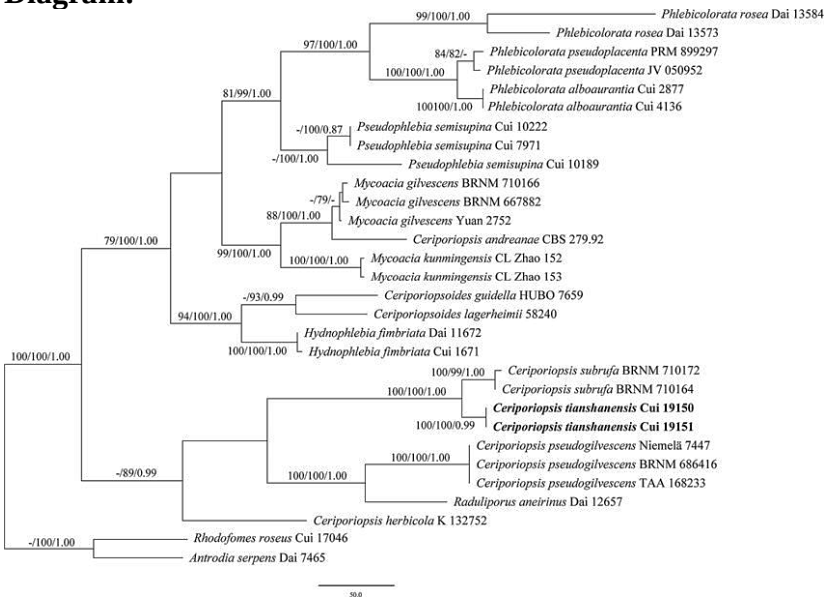
Aim:
To construct a **Maximum Parsimony (MP) phylogenetic tree** using **MEGA software** and analyze sequence relationships.

Apparatus & Materials:
- **Computer with Windows/Linux/Mac OS**
- **MEGA software (Download from: https://www.megasoftware.net/)**
- **FASTA format sequence file**
- **Internet access (optional, for sequence retrieval from NCBI)**

Theory:
A **phylogenetic tree** visually represents evolutionary relationships among species or genes. The **Maximum Parsimony (MP) method** seeks to find the simplest tree (with the fewest evolutionary changes) that explains the given sequence data.
Principles of Maximum Parsimony (MP):
1. **Minimizes total evolutionary changes** (mutations) in the tree.
2. **Uses character states (nucleotide/amino acid positions)** to determine the most likely ancestral relationships.
3. **Ideal for closely related sequences** but computationally intensive for large datasets.

Applications of MP Trees:
- Understanding **evolutionary history** of species or genes.
- Identifying **ancestral traits and lineage divergence**.
- Studying genetic variation and **conserved regions** in sequences.

Diagram:



Procedure:
Step 1: Install and Open MEGA Software
- Download and install **MEGA** from the official website.
- Open the software and select **Phylogeny → Construct/Analyze Phylogenetic Tree**.

Step 2: Load and Align Sequences

- Obtain DNA or protein sequences in **FASTA format**.
- Open **MEGA → Align → Edit/Build Alignment** to import sequences.
- Perform **Multiple Sequence Alignment (MSA)** and save as a **MEGA (.meg) file**.

Step 3: Select Maximum Parsimony Method

- Click **Phylogeny → Construct Maximum Parsimony Tree**.
- Choose **MP search criteria**, such as **Close-Neighbor-Interchange (CNI)** for branch swapping.
- Select the **substitution model** (e.g., Parsimony for nucleotides or proteins).

Step 4: Perform Bootstrap Analysis (Optional for Reliability)

- In the MP settings, enable **Bootstrap Test (≥1000 replicates)**.
- Higher bootstrap values indicate **stronger support** for evolutionary relationships.

Step 5: View and Interpret the Tree

- The MP tree will display **branch lengths and bootstrap values**.
- Save the tree by clicking **File → Export Tree as Image**.

Observations Table:

Step	Observation	Interpretation
Sequence alignment	Aligned sequences loaded into MEGA	Ready for tree construction
MP tree calculation	Tree structure appears with branches	Evolutionary relationships inferred
Bootstrap values	Support values displayed on branches	Confidence in evolutionary history

Result:

A **Maximum Parsimony (MP) phylogenetic tree** was successfully constructed using **MEGA software**, illustrating the **evolutionary relationships** among the input sequences.

Conclusion:

The **Maximum Parsimony (MP) method** provides a simple and effective way to infer evolutionary relationships. It is particularly useful for closely related species but may be computationally challenging for large datasets.

Precautions:

- Ensure sequences are **properly aligned** before tree construction.
- Use **bootstrap analysis** to confirm tree reliability.
- Select appropriate **parsimony criteria (CNI, SPR, TBR)** based on dataset size.
- Verify sequence quality to prevent misinterpretation.
- Save results and **export the tree image** for documentation.

Lab Activity No 09
Title of the Activity- ML Phylogenetic Tree Construction Using MEGA

Learning Objectives:

- Understand the **Maximum Likelihood (ML) method** for phylogenetic tree construction.
- Learn how to use **MEGA (Molecular Evolutionary Genetics Analysis)** software to build ML trees.
- Analyze **evolutionary relationships** between DNA/protein sequences using the ML approach.

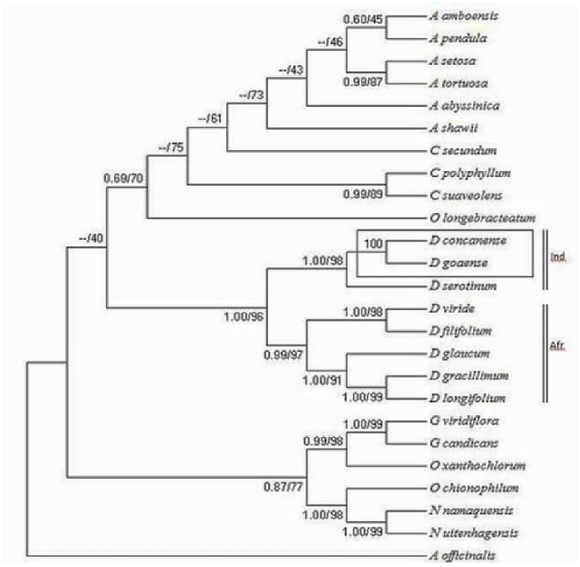
Aim:
To construct a **Maximum Likelihood (ML) phylogenetic tree** using **MEGA software** and interpret the evolutionary relationships among sequences.

Apparatus & Materials:
- **Computer with Windows/Linux/Mac OS**
- **MEGA software (Download from: https://www.megasoftware.net/)**
- **FASTA format sequence file**
- **Internet access (optional, for sequence retrieval from NCBI)**

Theory:
A **phylogenetic tree** is a diagram representing the evolutionary relationships among organisms or genes. The **Maximum Likelihood (ML) method** is a statistical approach that finds the **best tree** by **calculating probabilities of different evolutionary events**.
Principles of Maximum Likelihood (ML) Method:
1. Uses a **statistical model of evolution** (e.g., Jukes-Cantor, Kimura 2-parameter).
2. Evaluates multiple tree topologies to **find the most likely tree**.
3. Computes **branch lengths** based on **likelihood scores**.
4. Computationally intensive but **more accurate than NJ or MP**.

Applications of ML Trees:
- Studying **species evolution and genetic divergence**.
- Inferring **ancestral relationships** in complex datasets.
- Comparing **mutation rates and selection pressure** in genes.

Diagram:



Procedure:
Step 1: Install and Open MEGA Software
- Download and install **MEGA** from the official website.
- Open the software and select **Phylogeny → Construct/Analyze Phylogenetic Tree**.

Step 2: Load and Align Sequences

- Obtain DNA or protein sequences in **FASTA format**.
- Open **MEGA → Align → Edit/Build Alignment** to import sequences.
- Perform **Multiple Sequence Alignment (MSA)** and save as a **MEGA (.meg) file**.

Step 3: Select Maximum Likelihood Method

- Click **Phylogeny → Construct Maximum Likelihood Tree**.
- Choose an **evolutionary model** (e.g., **Kimura 2-parameter, JTT for proteins**).

Step 4: Perform Bootstrap Analysis (Optional for Reliability)

- In the ML settings, enable **Bootstrap Test (≥1000 replications)**.
- Higher bootstrap values indicate **stronger support** for evolutionary relationships.

Step 5: View and Interpret the Tree

- The ML tree will display **branch lengths and bootstrap values**.
- Save the tree by clicking **File → Export Tree as Image**.

Observations Table:

Step	Observation	Interpretation
Sequence alignment	Aligned sequences displayed in MEGA	Ready for tree construction
ML tree calculation	Tree structure appears with branches	Evolutionary relationships inferred
Bootstrap values	Support values displayed on branches	Confidence in evolutionary history

Result:

A **Maximum Likelihood (ML) phylogenetic tree** was successfully constructed using **MEGA software**, revealing **evolutionary relationships** among the input sequences.

Conclusion:

The **Maximum Likelihood (ML) method** is a powerful approach for constructing accurate phylogenetic trees. It provides **statistically robust evolutionary relationships** based on sequence data.

Precautions:

- Ensure sequences are **properly aligned** before tree construction.
- Choose an **appropriate substitution model** for accurate distance calculations.
- Use **bootstrap analysis** to confirm tree reliability.
- Verify sequence quality to avoid misinterpretation.
- Save results and **export the tree image** for documentation.

Lab Activity No 10

Title of the Activity- Construction of chloroplast genome map using GeSeq

Learning Objectives:

- Understand the process of **chloroplast genome annotation** using **GeSeq (Gene Sequence Annotation Server)**.
- Learn how to use **GeSeq** to predict and visualize **chloroplast genome features** such as protein-coding genes, rRNAs, and tRNAs.
- Construct a **chloroplast genome map** and analyze its structure.

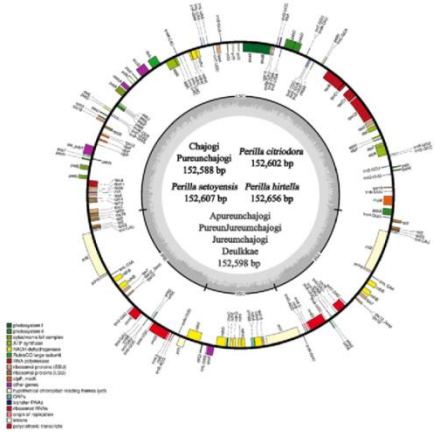
Aim:
To annotate and construct a **chloroplast genome map** using **GeSeq**, identifying genes and structural elements within the chloroplast genome.

Apparatus & Materials:
- **Computer with Internet access**
- **GeSeq (Available at: https://chlorobox.mpimp-golm.mpg.de/geseq.html)**
- **FASTA file of chloroplast genome sequence**
- **NCBI or other sequence repositories for reference genomes**

Theory:
The **chloroplast genome** is a circular, double-stranded DNA molecule found in plant cells, primarily responsible for **photosynthesis and other essential metabolic processes**.
Annotation of the chloroplast genome is crucial for studying **gene functions, evolutionary relationships, and genetic engineering applications**.
What is GeSeq?
- **GeSeq (Gene Sequence Annotation Server)** is an online tool used for **annotating chloroplast genomes**.
- It identifies **protein-coding genes, rRNAs, tRNAs, and conserved regions** based on sequence comparison with reference databases.
- It also generates **graphical genome maps**, which are useful for **genome visualization and comparative genomics**.

Applications of Chloroplast Genome Annotation:
- Understanding **chloroplast gene functions**.
- Studying **plant phylogenetics and evolution**.
- Assisting in **genetic modification and synthetic biology**.
- Detecting **mutations and structural variations** in chloroplast genomes.

Diagram:



Procedure:
Step 1: Access GeSeq Online
- Open **GeSeq** by visiting: https://chlorobox.mpimp-golm.mpg.de/geseq.html.

Step 2: Upload the Chloroplast Genome Sequence
- Click **"Upload FASTA file"** and select the chloroplast genome sequence file.
- Alternatively, enter the **NCBI accession number** to fetch an existing sequence.

Step 3: Select Annotation Parameters

- Choose databases for comparison: **NCBI RefSeq, EMBL, or BLAST against existing chloroplast genomes**.
- Enable **tRNA and rRNA annotation** using **tRNAscan-SE and RNA-BLAST**.
- Set the **annotation output format** (e.g., **GFF, GenBank, or graphical genome map**).

Step 4: Run the Annotation Process

- Click **"Start Annotation"** and wait for the tool to process the genome.
- The process may take a few minutes, depending on genome size.

Step 5: View and Download Annotated Genome

- Check the **list of identified genes (protein-coding, tRNA, rRNA, and non-coding regions)**.
- Click **"Generate Genome Map"** to visualize the **circular chloroplast genome structure**.
- Download results in **GenBank format** for further analysis.

Observations Table:

Step	Observation	Interpretation
Genome upload	FASTA file successfully loaded	Ready for annotation
Annotation process	Genes, rRNAs, and tRNAs identified	Chloroplast genome elements detected
Genome map generation	Circular genome map displayed	Visualization of genome structure
Exported annotation	Gene list and sequence data available	Data ready for comparative analysis

Result:

A **chloroplast genome map** was successfully constructed using **GeSeq**, identifying key genetic elements such as **protein-coding genes, rRNAs, and tRNAs**.

Conclusion:

The **GeSeq tool** effectively annotates **chloroplast genomes** by comparing sequences with reference databases. This process provides a comprehensive view of **chloroplast gene organization**, useful for **phylogenetic studies, evolutionary biology, and genetic research**.

Precautions:

- Ensure the **input sequence is high quality** and in **FASTA format**.
- Select appropriate **reference databases** for accurate annotation.
- Verify **gene predictions** with other annotation tools if needed.
- Use **graphical visualization tools** to confirm gene positions.
- Save and backup **annotation results and genome maps** for further analysis