On-Job Training Report:
National Facility for Biopharmaceuticals, Matunga, Mumbai

Submitted by:

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M. Sc Bioinformatics (Part 1)

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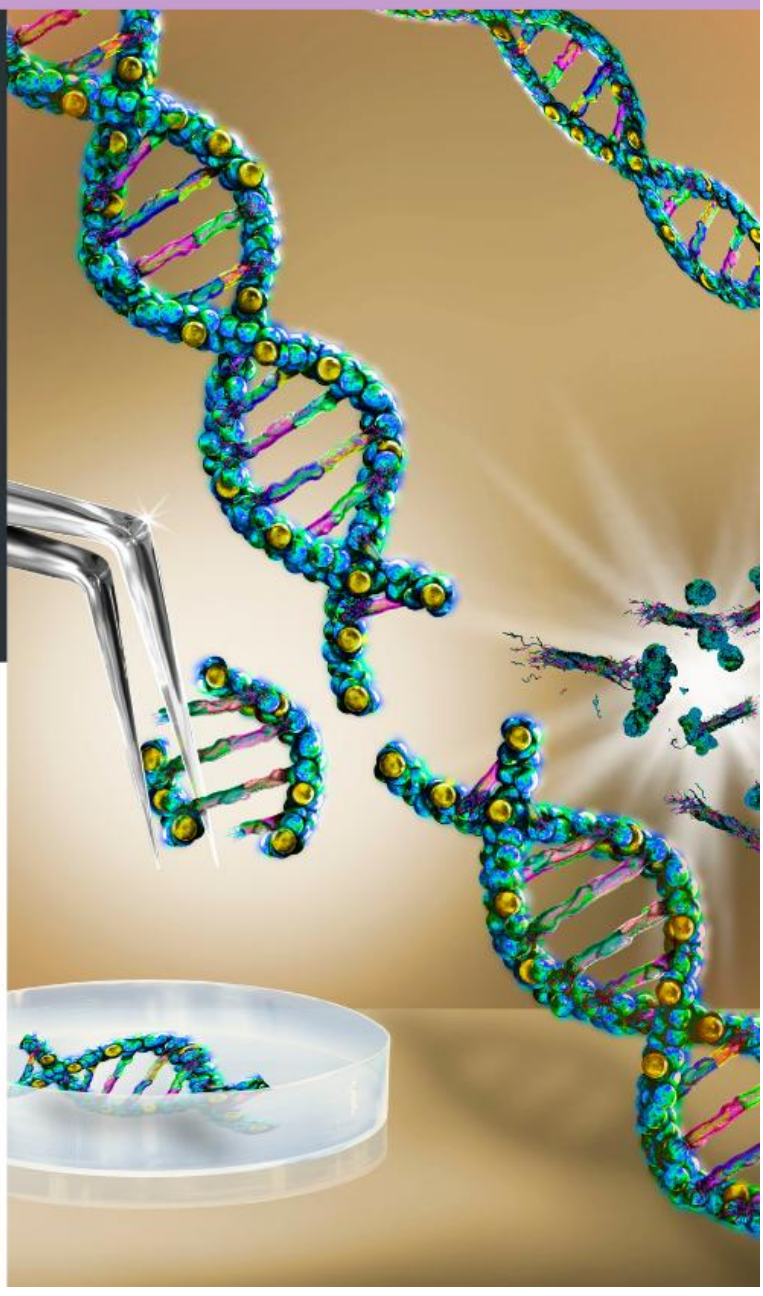

Submitted to:

Department of Bioinformatics,
Guru Nanak Khalsa College,
Matunga

ACKNOWLEDGEMENT

I would like to convey our profound appreciation to the exceptional group of instructors at the National Facility for Biopharmaceuticals (NFB) for all of their assistance and encouragement during our practical training program.

My sincere appreciation extends towards Mr. Vikas Jha, the Head of the Molecular Biology Lab, for his amazing teaching approach and encouraging mentoring. He has guided the lab to become a cutting-edge center that nourishes our passion for molecular biology and offers excellent resources for our development.

I would like to express our sincere gratitude to Mr. Dinesh Kumar, Head of the Proteomics & Protein Characterization Lab, for his invaluable contributions to the field of proteomics research.

I sincerely thank Ms. Valencia D'Souza, Senior Research Fellow, for her understanding in molecular biology, especially with regard to real-time PCR. Her instruction and examples have been really helpful to us. We particularly value the efforts made byMs. Sakshi Padawe, Ms. Ira Kode and Mr. Jaydeep Yadav, whose devoted assistance has made it possible for a variety of facility operations to run smoothly, improving our educational experience.

I express my gratitude to Dr. Gursimran Kaur Uppal, who oversees the Bioinformatics Department at Guru Nanak Khalsa College of Arts, Science, and Commerce, for providing me with unwavering support and motivation. Their guidance has been really helpful in helping me get beyond the obstacles I've had in my academic career.

I would like to express my gratitude to my teachers, Mrs. Aparna Patil Kose and Mrs. Sermarani Nadar, for their insightful advice and unwavering support during my master's program. My educational experience has been greatly enhanced by their knowledge and assistance.

Finally, I would want to sincerely thank my friends, family, and coworkers for their steadfast understanding and support during my studies. Their inspiration and support have helped me stay motivated and focused on achieving my objectives.

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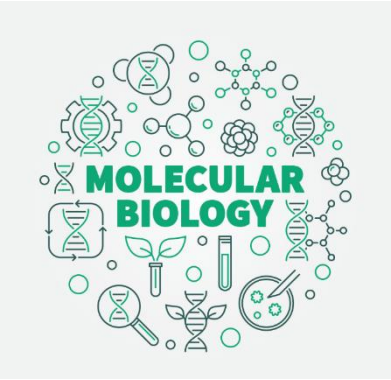
CHAPTER 1 – INTRODUCTION TO MOLECULAR BIOLOGY

Molecular biology is a branch of biology that focuses on studying the molecular basis of biological activity. It involves understanding how molecules interact within living organisms to carry out life functions, with a particular emphasis on DNA, RNA, and proteins.



The term 'molecular biology' was first used in 1945 by physicist William Astbury. This field emerged in the mid-20th century, with key milestones like the discovery of the DNA double helix model in 1953 as proposed by Francis Crick, James Watson, Rosalind Franklin, and colleagues. Molecular biologists use various techniques to investigate molecular processes, enabling advancements in drug development, disease diagnosis, and understanding cell physiology, and developing medical therapies such as gene therapy.

Advantages and Disadvantages of Molecular Biology –

The key advantages of molecular biology include:

1. Enables a deeper understanding of biological processes at the molecular level, leading to new scientific discoveries and technological advancements.
2. Provides powerful tools for genetic engineering, drug development, and personalized medicine, with the potential to address major global challenges in healthcare, agriculture, and the environment.
3. Facilitates the characterization of microorganisms and the development of new antimicrobial treatments, which is crucial for combating the growing threat of antimicrobial resistance.
4. Supports the development of innovative biotechnologies, such as mRNA vaccines and monoclonal antibody treatments, as demonstrated during the COVID-19 pandemic.

While molecular biology offers many benefits, it also faces some challenges and potential drawbacks:

1. Ethical concerns surrounding genetic engineering, stem cell research, and the potential misuse of biotechnology for biological warfare.
2. Potential negative impacts on biodiversity due to the widespread use of genetically modified organisms in agriculture.
3. Complexity in managing and integrating the vast amounts of biological data generated by molecular biology techniques, such as DNA sequencing.
4. Difficulties in translating molecular biology discoveries from the laboratory to real-world applications, particularly in resource-limited or off-the-grid scenarios.

Applications and Future Prospects of Molecular Biology –

Molecular biology has a wide range of applications and a promising future:

1. Advancements in genetic engineering and biotechnology, enabling the development of new drugs, therapies, and agricultural products.
2. Improved understanding and treatment of diseases, including personalized medicine approaches targeting specific molecular pathways.
3. Enhanced capabilities in microbiology and virology research, leading to better detection, prevention, and treatment of infectious diseases.
4. Innovative applications in forensics, diagnostics, and environmental monitoring, leveraging molecular techniques like DNA sequencing.
5. Continued progress in regenerative biology and tissue engineering, with the potential to address a wide range of medical conditions.
6. Emerging applications in areas like bioproduction, biosensing, and closed-loop therapeutic delivery, enabled by advancements in synthetic biology.

CHAPTER 2 – TRAINING DETAILS

Dates – 20th December, 2023 to 15th January, 2024

Location – The National Facility for Biopharmaceuticals (NFB), Mumbai

The National Facility for Biopharmaceuticals (NFB) located in Mumbai is dedicated to providing hands-on training in various aspects of the biopharma industry, with a focus on molecular biology, protein purification, fermentation, and mammalian cell culture techniques.

Designed and constructed to international standards required for developing and manufacturing recombinant biopharmaceutical products, the NFB has GLP and ISO certifications for the services it offers. The facility is equipped with state-of-the-art infrastructure and high-end sophisticated instruments, enabling students, colleges, and universities to conduct reproducible experiments in specialized areas like molecular biology, proteomics, microbiology, and cell culture.

The NFB's vision is to "Transform Education into Expertise" by offering best-in-class infrastructure and training solutions to biology students and professionals working in the biopharmaceutical, biotechnology, and research sectors. With over 10 years of experience, the facility aims to empower trainees to maximize their potential and contribute to the growth of the biopharmaceutical industry in India.

Purpose of Hands-On Training program –

1. To equip trainees with the basic knowledge and skills required to function effectively in a molecular biology laboratory.
2. The training aims to provide students with an understanding of how they can use molecular techniques to address a variety of questions and problems in their research.
3. The hands-on course is designed to make research trainees in physiology comfortable with the tools of molecular biology, addressing a growing need to prepare them for using these techniques.
4. The combination of comprehensive background lectures and extensive hands-on experience allows students to gain an appreciation for proper technique, accuracy, and the importance of each step in molecular biology procedures.

Overall, the hands-on training is intended to give trainees the practical skills and conceptual understanding needed to apply molecular biology methods in their work, whether it is in research, diagnostics, or other areas.

Mentors

Mr. Vikas Jha

Role: Head and Laboratory In-charge - Molecular Biology Laboratory

Mr. Vikas Jha sir is a dedicated and accomplished molecular biologist who serves as the laboratory in-charge at the National Facility for Biopharmaceuticals (NFB).

1. **Laboratory Leadership:** He oversees the daily operations of the laboratory, ensuring that all experiments and research projects are conducted efficiently and effectively.
2. **Technical Expertise:** He leads operations in Molecular Biology and Fermentation Technology in addition to overseeing research and development initiatives.
3. **Research & Development:** With a focus on innovation, Mr. Jha contributes to proof of concepts for scientific studies, applying his expertise in molecular biology to design experiments that advance scientific understanding.
4. **Training & Skill Development:** He orchestrates training programs to enhance laboratory skills and scientific methodologies.
5. **Data Analysis & Quality Control:** He analyses samples using cutting-edge techniques to ensure data accuracy and reliability, aiming to uncover transformative discoveries.
6. **Project Management:** Collaborating with interdisciplinary teams, he drives research and development efforts, from conceptualization to implementation, fostering a culture of innovation.
7. **Mentorship:** He mentors bachelor's and master's students, guiding them through scientific inquiries and nurturing the next generation of scientific leaders.

Mr. Dinesh Kumar

Role: Head of the Proteomics and Protein Characterization Laboratory

Dinesh Kumar, the Head of the Proteomics and Protein Characterization Laboratory at the National Facility for Biopharmaceuticals (NFB), offers significant expertise and outstanding leadership to his position. His extensive knowledge of proteomics and protein characterisation propels the lab's research efforts toward innovation and excellence.

His team's use of state-of-the-art tools and techniques allows them to decipher the complicated relationships between protein structure and function. Sir engages with academia, business, and the larger scientific community to make significant change in proteomics by fostering a collaborative atmosphere that encourages multidisciplinary research.
His leadership is distinguished by his continuous commitment to furthering protein science research in this exciting subject. By providing his laboratory's experts the opportunity to push the limits of science, he establishes the NFB lab as a pioneer in novel discoveries and cutting-edge technical developments. Dinesh Kumar sir's contributions to the field of proteomics are noteworthy due to his vision, competence, and collaborative approach.

Mr. Valencia D'Souza

Role: In-charge – Mammalian Cell Culture Department

Valencia D'Souza ma'am is a passionate bioresearcher and science communicator, whose role at the National Facility for Biopharmaceuticals (NFB) in India is multifaceted.

Her expertise ranges in the following domains:

1. **Senior Research Fellow and In-charge of the Cell Culture Department:** She leads and manages the cell culture lab, ensuring its efficient operation and successful research projects.
2. **Student Coordinator:** Valencia plays a crucial role in coordinating various training modules offered by NFB, ensuring a smooth learning experience for students.
3. **Social Media Manager:** She manages NFB's social media presence, keeping the audience informed and engaged through various platforms.
4. **Event Organizer:** Valencia contributes to organizing workshops and events, fostering a dynamic learning environment at NFB.

Valencia D'Souza's path exemplifies the importance of pursuing one's research curiosity. Her combined expertise in research, technical skills, and communication makes her a valuable asset to the NFB team. She plays a key role in advancing cell culture research, training future scientists, and promoting public awareness about biopharmaceuticals.

Ms. Sakshi Padawe, Ms. Ira Kode and Mr. Jaydeep Yadav

Role: Laboratory Assistants

At the National Facility for Biopharmaceuticals (NFB), Ms. Sakshi Padawe, Ms. Ira Kode, and Mr. Jaydeep Yadav play crucial roles in ensuring smooth operations. Ms. Padawe and Ms. Kode manage laboratory administration, overseeing supplies and equipment for research, ensuring efficient experiments. Mr. Jaydeep Yadav supports the Proteomics lab with technical expertise and organization, aiding in resource management, scheduling, and team communication. Together, they are integral to the NFB's success, contributing significantly to research and educational endeavors.

Techniques covered –

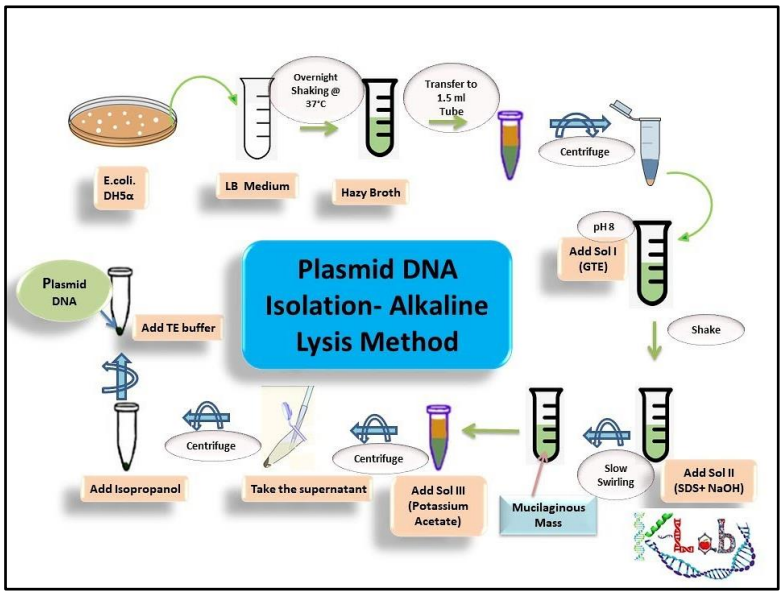
1. Plasmid Isolation
2. DNA Isolation
3. Polymerase Chain Reaction
4. Restriction Enzyme Digestion
5. DNA Quantification
6. Ligation
7. Agarose Gel Electrophoresis
8. Protein Characterization by SDS-PAGE

CHAPTER 3 – WET LAB PRACTICALS

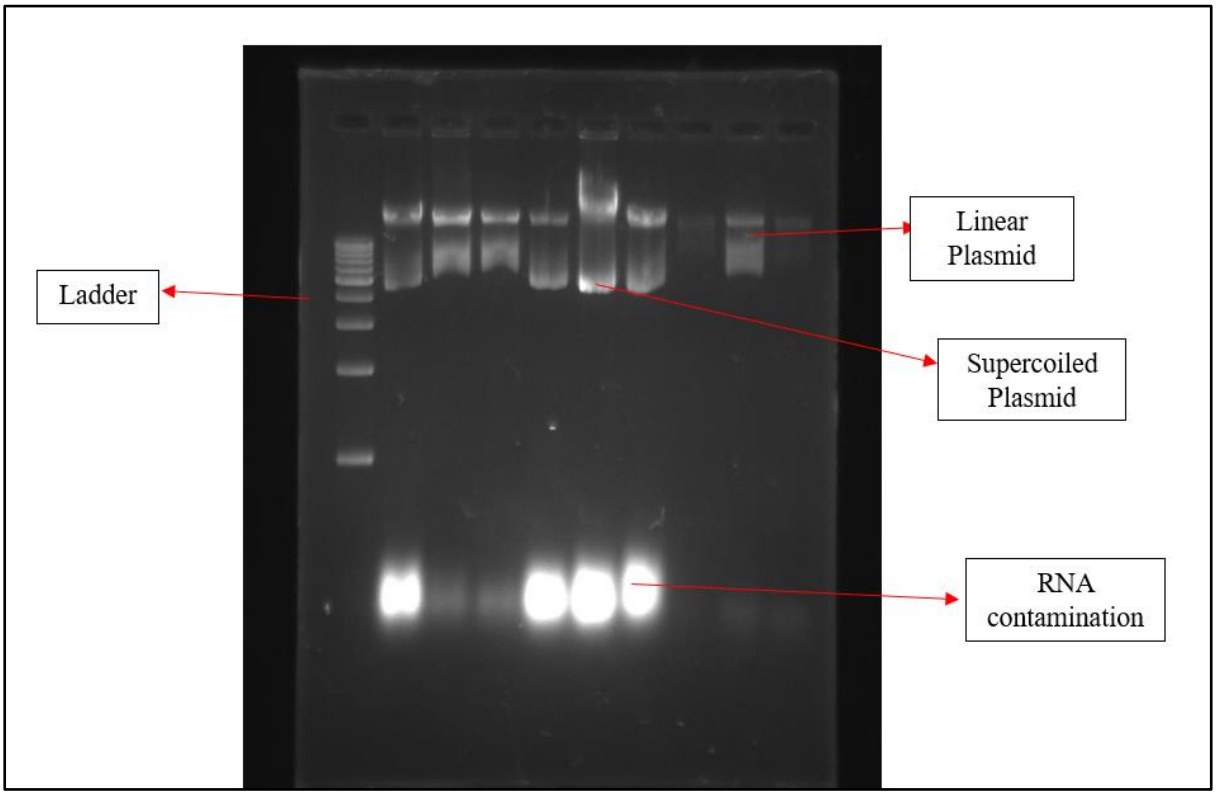
1. Isolation of Plasmid DNA

Category	Description
Objective	To isolate plasmid pET21b from *E. coli* culture using the alkaline lysis method.
Method	**Alkaline Lysis Method –** Involves raising the pH to a high level to achieve cell lysis and DNA denaturation, followed by neutralization.
Materials	**Chemicals and Reagents**
	1. Solution 1 (Re-suspension buffer): 40% glucose + 0.5 molar EDTA + 1 molar tris + Sterile water **2. Solution 2 (Alkaline Lysis buffer):** 0.4 N NaOH + 10% Triton X **3. Solution 3:** 5 M potassium acetate + GAA – glacial acetic acid + Water **4.** Chloroform: isoamyl alcohol (24:1) **5.** IPA – isopropyl alcohol **6.** Chilled 70% alcohol **7.** Absolute alcohol
	Agarose Gel Preparation
	Gel: 0.6 g Agarose + 60 mL TAE buffer **Dyes:** EtBr (Ethidium Bromide), Bromophenol blue
	Instruments, Glassware and Miscellaneous
	1. Micro-pipette 2. Tips of the micropipette 3. Oven 4. Beaker 5. Weighing scale 6. Comb 7. Electrodes 8. Gel casting tray
Results	1. Isolated plasmid DNA exhibited distinct migration patterns on agarose gel. 2. Linear DNA migrated slowest, followed by partially coiled and supercoiled forms. 3. This confirms separation based on size and conformation of DNA molecules.

Analysis/Discussion	1. Successful isolation allows for downstream applications like cloning and gene expression studies. 2. Established techniques effectively separate plasmid DNA for further analysis or manipulation. 3. This experiment highlights the importance of plasmids in genetic engineering and biotechnology.



Alkaline Lysis Method

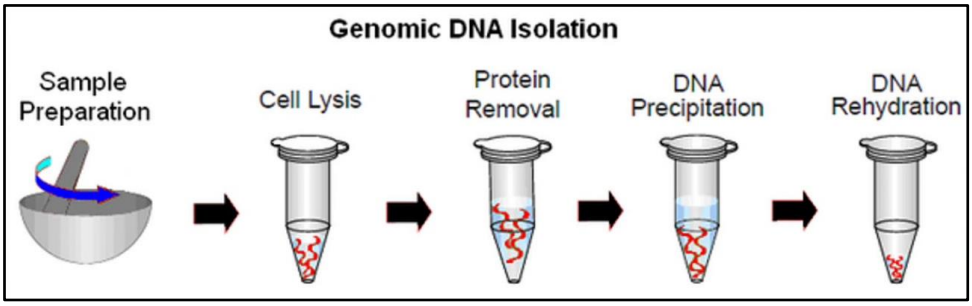


Gel image for Plasmid DNA extracted from *E. coli*

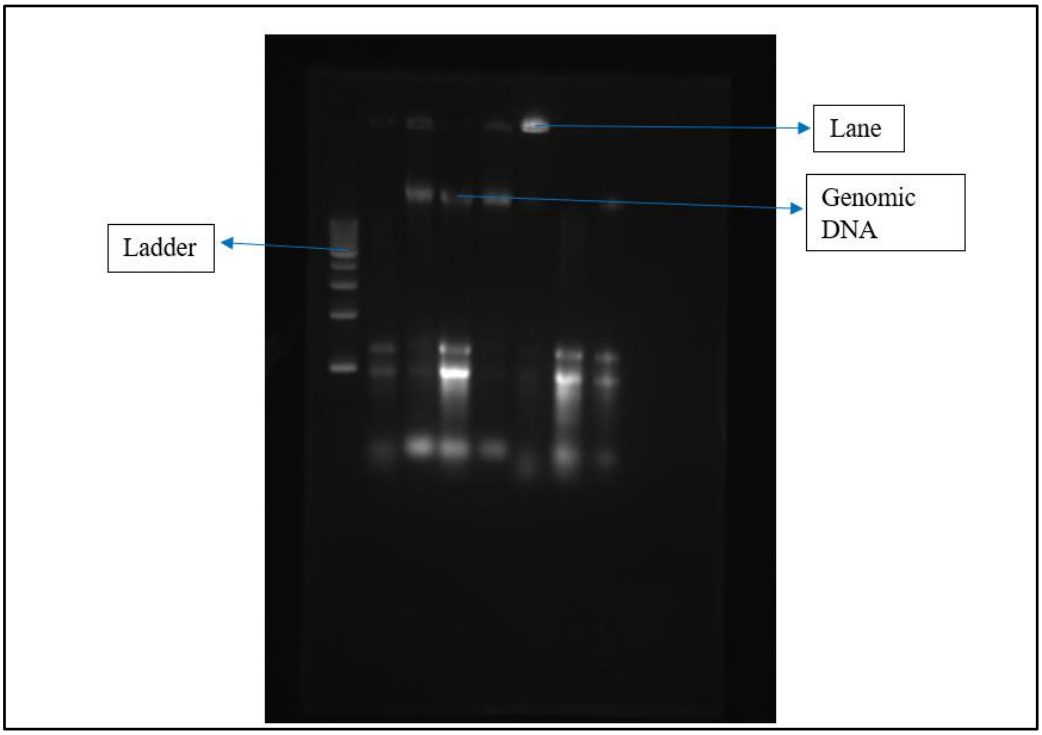
2. Isolation of Genomic DNA

Category	Description
Objective	To isolate genomic DNA from *Salmonella typhimurium* bacteria
Method	**CTAB Method –** 1. **Lysis:** Bacterial cells lysed using ionic detergent cetyltrimethylammonium bromide (CTAB), which forms an insoluble complex with nucleic acids in a low-salt environment. 2. **Extraction and Precipitation:** Under these conditions, polysaccharides, phenolic compounds and other contaminants remain in the supernatant and can be washed away. The DNA complex is solubilized by raising the salt concentration and precipitated with ethanol or isopropanol.
Materials	**Chemicals and Reagents**
	1. CTAB buffer = 2% CTAB (hexadecyltrimethylammonium bromide) + 100mM Tris-HCl [pH 8.0] + 20mM EDTA [pH 8.0] + 1.7M NaCl + 0.3% β-mercaptoethanol [add just before use] 2. Absolute alcohol 3. Chloroform 4. Iso-amyl alcohol 5. Sterile Distilled water
	Agarose Gel Preparation
	Gel: 0.6 g Agarose + 60 mL TAE buffer **Dyes:** EtBr (Ethidium Bromide), Bromophenol blue
	Instruments, Glassware and Miscellaneous
	1. Centrifuge 2. Electrophoresis unit 3. UV-trans illuminator 4. Microwave 5. Weighing balance 6. Power pack 7. Heating block/water bath at 37°C 8. 2 mL Eppendorf 9. Crushed ice 10. Eppendorf rack 11. Micropipette and Sterile microtips 12. Gloves
Results	DNA fragments from *Salmonella typhimurium* appear as distinct bands after staining with ethidium bromide.

Analysis/Discussion	1. CTAB method is a simple, cost-effective, and scalable approach for isolating genomic DNA from *Salmonella typhimurium.* 2. Purified DNA is suitable for downstream applications like PCR, sequencing, and genetic analysis. 3. CTAB method works with various samples and produces contaminant-free DNA, making it valuable for research, diagnostics, and epidemiological studies. This method facilitates the study of *Salmonella typhimurium's* genetic characteristics, virulence factors, and antibiotic resistance, ultimately aiding infectious disease management and public health initiatives.



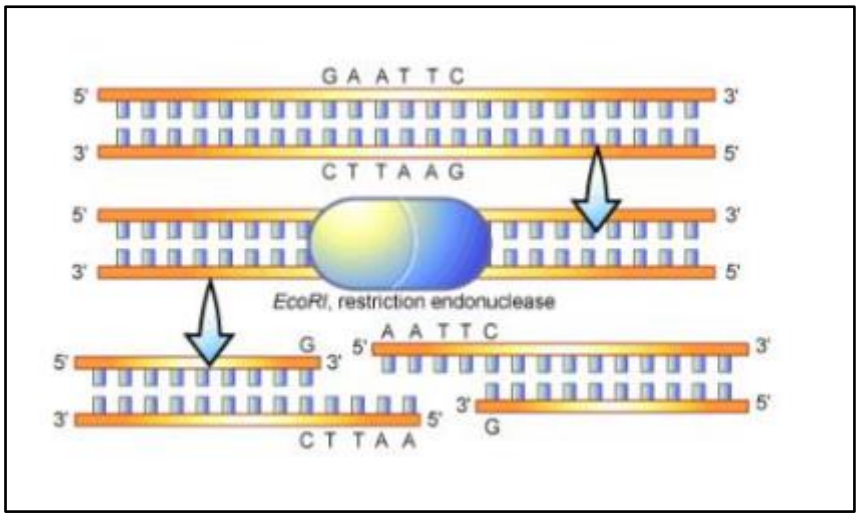
Method of Genomic DNA Isolation



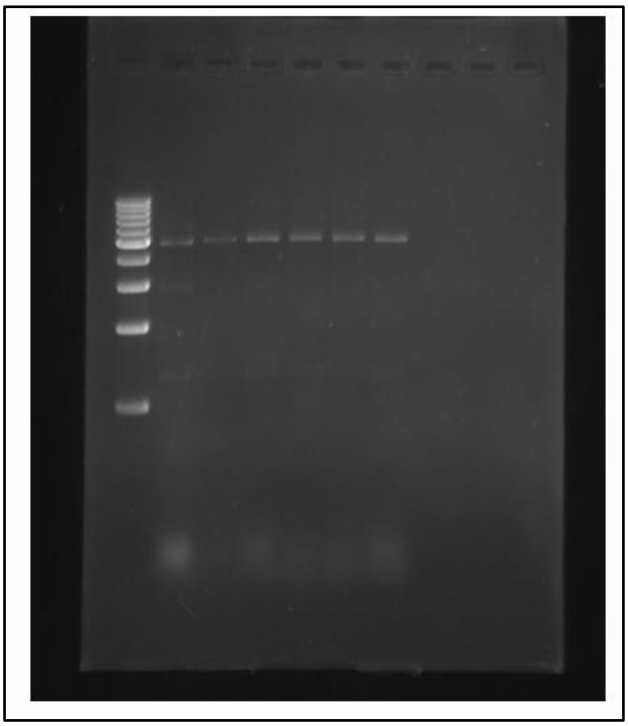
Gel image for Genomic DNA Isolation

3. Restriction Enzyme Digestion

Category	Description
Objective	To perform restriction enzyme digestion of the isolated plasmid.
Method	**Restriction Enzyme Digestion –** Fragmentation of DNA using restriction endonucleases.
Materials	**Chemicals and Reagents**
	1. Restriction enzyme (*Bam*HI) (1 µL) 2. Restriction enzyme (*Pst*I) (1 µL) 3. DNA sample (5 µL) 4. 10X assay buffer (1 µL) 5. Sterile distilled water (2 µL)
	Agarose Gel Preparation
	Gel: 0.6 g Agarose + 60 mL TAE buffer **Dyes:** EtBr (Ethidium Bromide), Bromophenol blue
	Instruments, Glassware and Miscellaneous
	1. Dry bath 2. Micropipettes 3. Micro tips 4. PCR Vials 5. Ice or frosty box 6. PCR vial racks 7. Vortex 8. Oven 9. Beaker 10. Weighing scale 11. Comb 12. Electrodes 13. Gel casting tray
Results	1. Restriction enzymes (BamHI and PstI) cut DNA at specific sequences. 2. Fragments are separated by size using gel electrophoresis. 3. Two fragments were observed – a. at ~4000-5000 base pairs b. at ~1000-1500 base pairs
Analysis/Discussion	1. Restriction enzymes, used by bacteria for defense, cut DNA at specific sites. 2. This allows researchers to analyze recombinant plasmids quickly and cheaply. 3. By looking at restriction fragments, scientists can determine presence/absence of inserts, their orientation, plasmid size, and even some sequence information – all at once for multiple samples.



Process of Restriction Enzyme Digestion

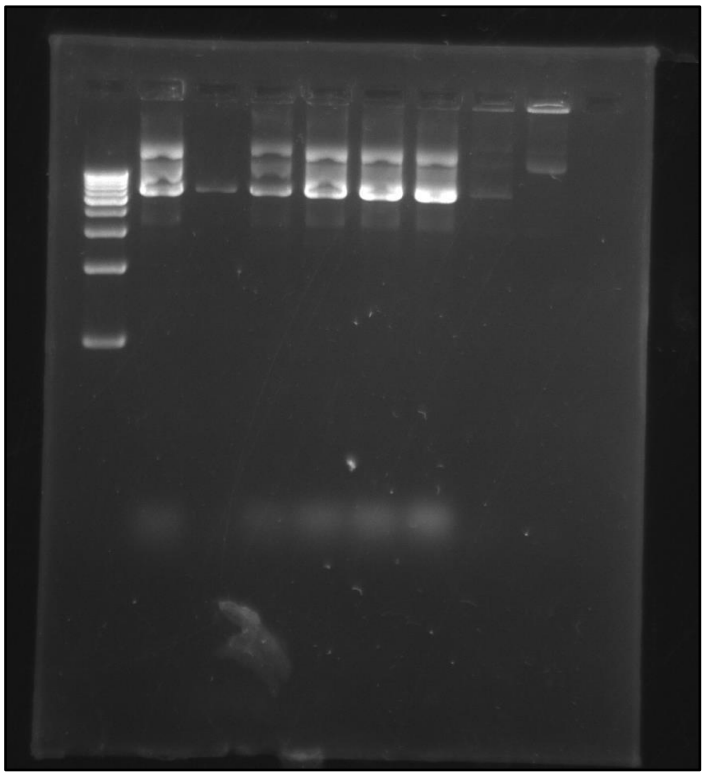


Evaluation of the Digestion by restriction enzyme was done and was evaluated on 1% Agarose gel and stained with 0.1µg/mL of Ethidium Bromide.

4. DNA Ligation

Category	Description
Objective	To perform DNA ligation using T4 DNA ligase enzyme.
Method	**DNA Ligation –** Joining of DNA fragments at their compatible ends (sticky and blunt ends) with covalent bonds using T4 DNA Ligase enzyme
Materials	**Chemicals and Reagents**
	10X Ligation Buffer 250 mM Tris-HCl, pH 7.8, 100 mM $MgCl_2$ 10 mM dithiothreitol T4 DNA Ligase 4 U/µL in 50% glycerol containing 10 mM Tris-HCl, pH 7.5 50 mM KCl 1 mM dithiothreitol 10 mM ATP Control DNA, pBR322 DNA HAE III Digest 0.5 µg/µL in 10 mM Tris-HCl pH 8.0, 1 mM EDTA 24% (w/v) PEG Solution, Water
	Agarose Gel Preparation
	Gel: 0.6 g Agarose + 60 mL TAE buffer **Dyes:** EtBr (Ethidium Bromide), Bromophenol blue
	Instruments, Glassware and Miscellaneous
	1. Micropipettes 2. Micro tips 3. Vortex 4. Oven 5. Beaker 6. Weighing scale 7. Comb 8. Electrodes 9. Gel casting tray

Results	1. Insert DNA (desired gene/fragment) is ligated into a compatible vector backbone. 2. Products are run on an agarose gel. 3. Applying electric current separates DNA fragments based on size and shape. 4. Bands on the gel show the different products of the ligation reaction. 5. Band analysis confirms ligation success and fragment size/quantity. 6. Ligation of 4000bp and 1400bp fragments resulted in a single 5400bp band on the gel, indicating successful ligation.
Analysis/Discussion	1. Ligation joins insert DNA to a plasmid backbone, creating a complete plasmid for further use. 2. Majority of ligations involve restriction enzyme-generated DNA fragments. 3. Ligation can connect DNA fragments to create longer sequences in PCR. 4. Gel electrophoresis is used to analyze the success of the ligation reaction.

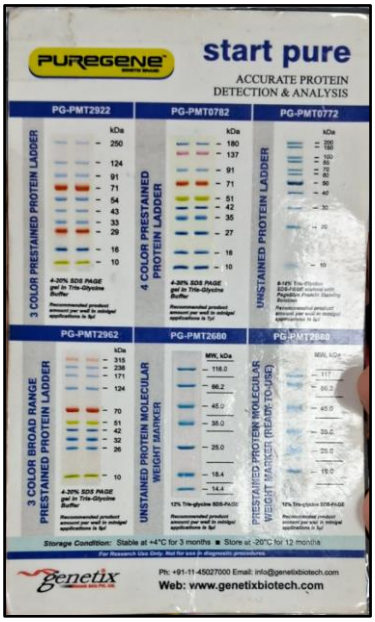


Ligation was done and was evaluated on 1% Agarose gel and stained with 0.1µg/mL of Ethidium Bromide.

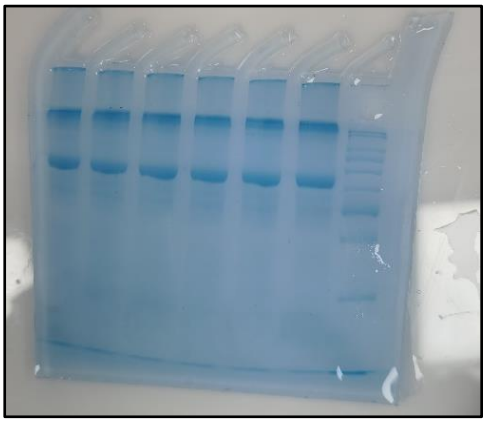
5. Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Category	Description
Objective	To separate proteins on the basis of their molecular weights by the technique SDS-PAGE.
Method	**SDS-PAGE –** 1. Denaturation of proteins and charge neutralization (uniform negative charge) of the proteins using anionic detergent sodium dodecyl sulphate (SDS) 2. Separation of proteins on the basis of molecular weight only
Materials	**Chemicals and Reagents for Agarose Gel Preparation**
	1. Resolving Gel: 1.5 M Tris pH: 8.8 + 30% Acrylamide + SDS + 10% APS + TEMED + Distilled Water (4mL) **2. Stacking Gel:** 0.5 M Tris pH: 8 + 30% Acrylamide + SDS + 10% APS + TEMED + Distilled Water (4mL) **3. Gel Loading Dye:** 0.2MpH 6.8 Tris Buffer + 10% SDS + 10mM Beta Mercaptoethanol + 20% glycerol v/v + 0.5% w/v Bromophenol Blue **4. Tracking Dye:** EtBr (Ethidium Bromide) **5. Staining Dye:** Coomassie brilliant blue R250 (250mg) + Glacial acetic acid (10ml) + Methanol (40ml) + Distilled water (50ml) **NOTE:** Glacial acetic acid, methanol, D/W in ratio 1:4:5 **6. Destaining Dye:** Glacial acetic acid (10ml) + Methanol (40ml) + Distilled water (50ml) **NOTE:** Glacial acetic acid, methanol, D/W in ratio 1:4:5
	Miscellaneous
	1. Comb (1mm) 2. Spacer (1mm) 3. Powerpack 4. Notch Plates
Results	1. Proteins were loaded into wells of a polyacrylamide gel. 2. Electrophoresis separated the proteins based on size (molecular weight). 3. Larger proteins migrated slower and appeared higher in the gel. 4. Smaller proteins migrated faster and appeared lower in the gel.

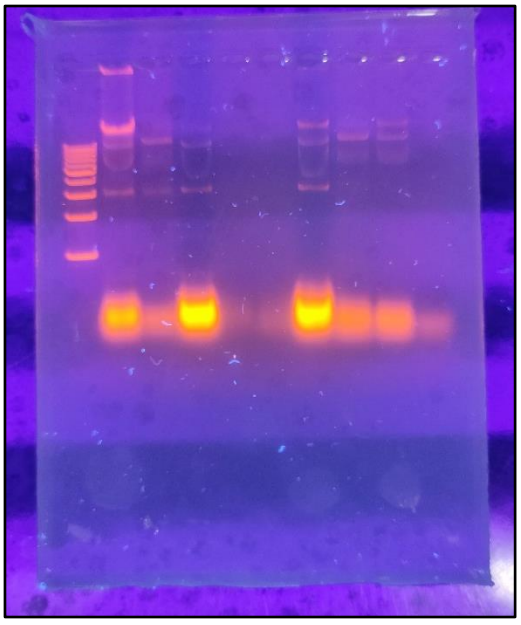
Analysis/Discussion	1. Protein samples were separated by SDS-PAGE electrophoresis based on molecular weight. 2. Staining and destaining dyes were used. 3. Molecules were separated by weight, with higher weights at the top and lower weights at the bottom. 4. SDS-PAGE allows researchers to easily, inexpensively, and accurately separate proteins based on polypeptide chain length.



Protein ladder chart for accurate protein detection and analysis



Destained Gel

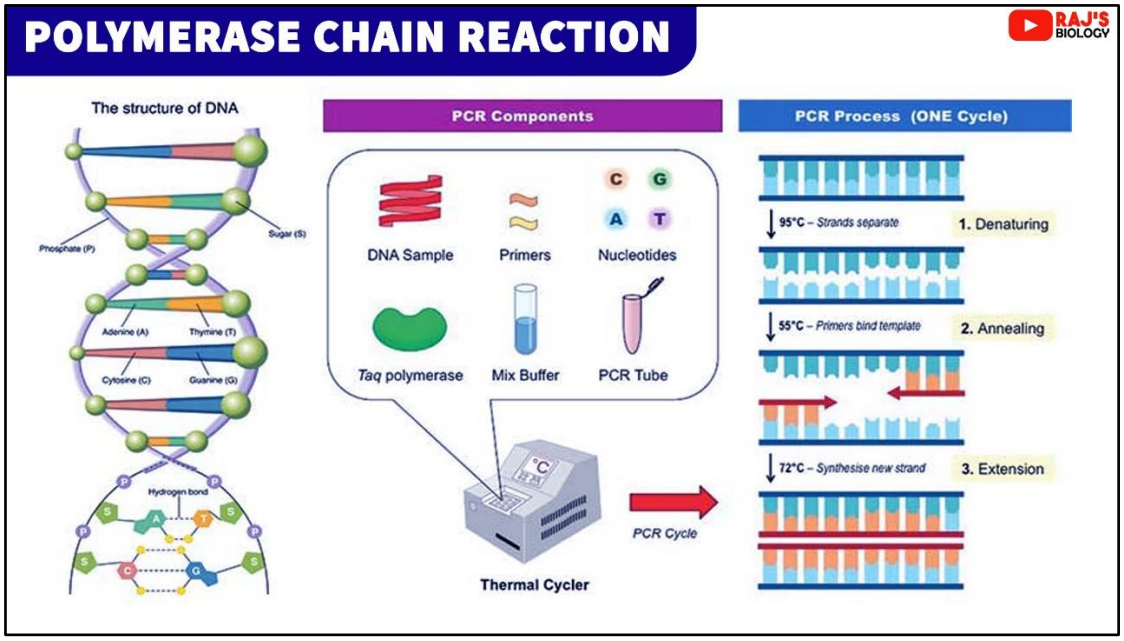


Gel analyzed under UV light

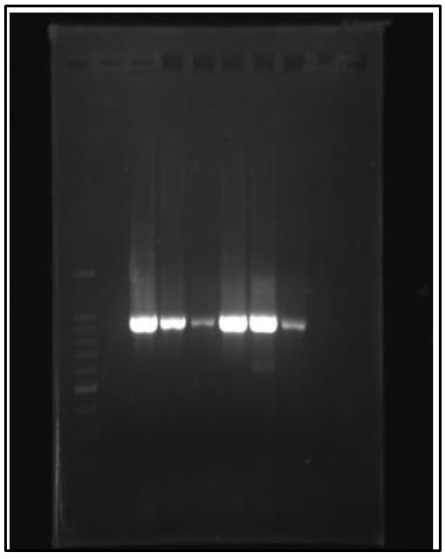
6. Polymerase Chain Reaction (PCR)

Category	Description
Objective	To amplify the given DNA sample by using specific primers in thermal cycler using PCR machine.
Method	**Enzymatic DNA Amplification using Polymerase Chain Reaction (PCR) –** 1. Initial Denaturation (at 95°C for 1 minute) 2. Denaturation (at 94°C for 30 seconds) 3. Annealing (at 55°C for 30 seconds) 4. Extension (at 72°C for 30 seconds) 5. Final Extension (at 72°C for 5 minutes)
Materials	**Chemicals and Reagents**
	1. Sample: 17S rRNA 2. dNTPs 3. 10X Taq polymerase assay buffer ($MgCl_2$) 4. Forward primers 5. Reverse primers 6. Taq DNA Polymerase 7. DNA Template 8. Distilled water
	Agarose Gel Preparation
	1. 1.5% Agarose gel 2. **Dyes:** EtBr (Ethidium Bromide), Bromophenol blue 3. DNA Ladder
	Miscellaneous
	1. Thermocycler 2. Electrophoresis equipment 3. UV-transilluminator 4. Microwave 5. Weighing balance 6. Power pack. 7. Gloves 8. Micropipettes and tips 9. PCR tubes 10. Cello tape
Results	1. Amplified band size ~900 base pairs 2. Band intensity suggests high sample quality/quantity. 3. No band indicates failed amplification.

Analysis/Discussion	1. Efficient amplification achieved based on band presence and size. 2. PCR is a rapid and accurate method for DNA replication. 3. Experiment success depends on factors like reagents, primers, and conditions. 4. PCR amplifies specific DNA fragments. 5. Applications include gene expression analysis, disease diagnosis, and genetic variation studies.



Process of Polymerase Chain Reaction (PCR)



PCR was performed on a gene of interest 17S rRNA and was evaluated on 1.5% Agarose gel and stained with 0.1µg/mL of Ethidium Bromide

7. Demonstration of DNA Sequencer – Summary

THEORY

Function – DNA sequencers are machines that read the order of genetic code (A, C, G, T) in a DNA sample. This information is crucial for understanding genes and genetic diseases.

Process – DNA sequencers automate the process of identifying the order (sequence) of the four bases (adenine, cytosine, guanine, thymine) in a DNA sample. They output this information as a text string called a "read".

Types –

1. Optical DNA sequencers: Analyze light signals emitted by fluorescent molecules attached to DNA building blocks (nucleotides).

2. Sanger sequencers (first generation): Pioneered by Frederick Sanger in 1987, these were essentially automated electrophoresis systems. They played a key role in the Human Genome Project.

Advancements –

Next-generation sequencing (NGS) technologies (since 2005) enable sequencing millions of base pairs of DNA much faster than Sanger sequencing.

Common Sequencing Methods –

1. Sanger Sequencing (Chain Termination Method)
2. Maxam and Gilbert Sequencing (Chemical Sequencing Method)

Category	Sanger Sequencing (Chain Termination Method)	Maxam and Gilbert Sequencing (Chemical Sequencing Method)
Method	Electrophoresis-based technique using dideoxynucleotides to terminate DNA replication during in vitro copying.	Chemical modification and subsequent cleavage of DNA backbone
Developed by	Frederick Sanger and colleagues (1977)	Allan Maxam and Walter Gilbert (1976-1977)
Historical significance	Dominant sequencing method for ~40 years (first commercialized in 1986 by Applied Biosystems).	First generation DNA sequencing method (alongside Sanger dideoxy) Major breakthrough in early DNA sequencing

Current use	Primarily for smaller-scale projects and validation of next-generation sequencing results.	1. DNA footprinting 2. Identifying DNA modifications 3. Structural DNA Analysis
Advantages	1. Reads longer DNA sequences (>500 nucleotides) compared to short-read sequencing technologies. 2. Exceptionally high accuracy (around 99.99%).	1. Can detect modifications such as methylated or damaged bases. 2. Applicable to different DNA types: including single-stranded DNA and RNA.
Disadvantages	1. Costly and time-consuming. 2. Prone to artifacts.	1. Ability to sequence limited read length of around 50-500 nucleotides 2. Use and difficult handling of Radioactive or fluorescent labelling
Applications	Public health initiatives (e.g., sequencing SARS-CoV-2 spike protein, norovirus outbreak surveillance).	1. DNA sequencing 2. Mutational analysis 3. Mapping protein-DNA interactions

Advantages of DNA Sequencing

1. **High throughput -** Sequences millions of fragments simultaneously.
2. **Accuracy -** Improved technology yields reliable data.
3. **Speed -** Generates results quickly for rapid analysis.
4. **Versatility -** Analyzes diverse DNA samples (human, microbes, etc.).
5. **Accessibility -** Affordable options allow wider research access.
6. **Advancements -** Revolutionized genomics with key discoveries.

Disadvantages of DNA Sequencing

1. **Cost -** High-throughput applications can be expensive.
2. **Read length limitations -** Can hinder assembly and variant identification.
3. **Errors -** Base-calling, PCR, and contamination can impact data accuracy.
4. **Sample preparation -** Time-consuming and prone to errors.
5. **Bioinformatics analysis -** Requires specialized tools and expertise.
6. **Data storage/management -** Large datasets require significant resources.

Applications of DNA Sequencing

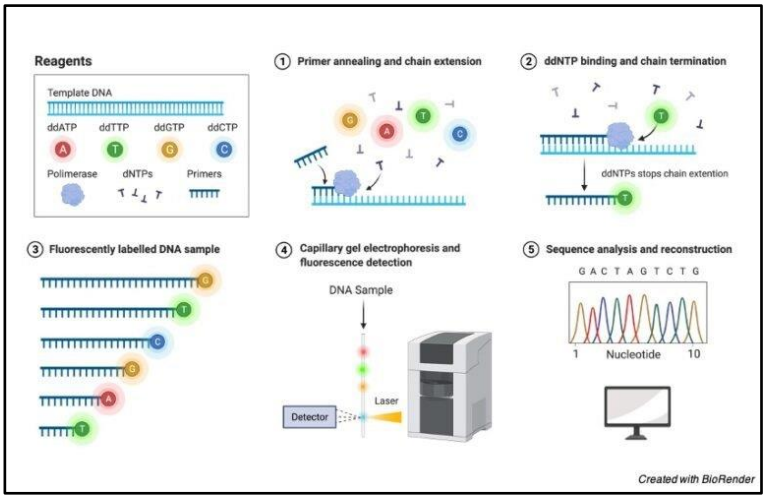
1. **Genomic analysis -** Understanding an organism's genetic makeup.
2. **Evolutionary studies -** Comparing DNA sequences across species.
3. **Disease diagnosis -** Identifying genetic links to specific diseases.
4. **Biotechnology/engineering -** Identifying genes for desired traits.

5. **Forensics -** Comparing DNA samples for suspect identification.
6. **Microbial diversity studies -** Analyzing microbial communities.
7. **Personalized medicine -** Tailoring treatments based on individual genetics.
8. **Agriculture -** Improving crops and food plants using microbial genomes.

PRINCIPLE

DNA sequencing determines the order of building blocks (nucleotides) in DNA. Two main methods emerged in 1977, but the chain termination method is more widely used today.
This method relies on creating single-stranded DNA copies with primers and then performing four separate reactions, each with a different termination molecule (dideoxynucleotide) added. These dideoxynucleotides halt DNA synthesis at random points, resulting in DNA fragments of various lengths.

By running these reactions alongside each other on a gel and analyzing the band patterns, the DNA sequence can be read. Newer variations, like cycle sequencing, use fluorescently labeled dideoxynucleotides for automated sequencing machines. This allows all four reactions to occur in one tube and be distinguished by color, simplifying the sequencing process.



Category	Description
Objective	To demonstrate the working of a DNA Sequencer.
Materials	1. **DNA sample** 2. **Library preparation kit -** contains enzymes and reagents needed to fragment and amplify the DNA, add adapters, and prepare the library for sequencing. 3. **Sequencing reagents -** nucleotides (A, C, G, T), sequencing polymerases, and fluorescent dyes. 4. **Sequencing instrument -** DNA sequencer that reads the DNA sequence and generates the data. 5. **Computer and software -** to process and analyze the raw sequencing data, including base calling, sequence alignment, and variant calling.
Steps of DNA Sequencing	**Sample preparation** DNA is extracted from the sample and purified to remove contaminants. ↓ **Library preparation** The DNA is fragmented into small pieces and adapters are added to each end to enable the DNA to be amplified and sequenced.

	↓ **Amplification** The DNA fragments are amplified using PCR (polymerase chain reaction) to create multiple copies of each fragment. ↓ **Sequencing** The amplified DNA is loaded onto the sequencer, which reads the DNA sequence. There are different sequencing technologies available, including Illumina, PacBio, and Oxford Nanopore, among others. ↓ **Base calling** After the sequencing is complete, the raw data is processed by a computer to identify the base calls (A, C, G, or T) for each read. ↓ **Assembly** The reads are then assembled into longer contiguous sequences (contigs) using specialized software. ↓ **Analysis** The assembled sequences are compared to reference genomes or other databases to identify genes, mutations, or other features of interest.
Conclusion	The DNA Sequencer practical offered a captivating glimpse into genomics and molecular biology, enabling the precise deciphering of genetic code through modern sequencing techniques. The practical highlighted the versatile applications of DNA sequencing in fields like genetics, medicine, and biotechnology.

8. Demonstration of Flow Cytometry – Summary

THEORY

Through the use of physical and chemical analysis, flow cytometry is a technique that may be used to analyze, count, and sort tiny particles in a fluid stream as they pass through a detection instrument. The origins of fluorescence-based flow cytometry may be found in Wolfgang Göhde's 1968 invention of the ICP 11 equipment at the University of Münster, Germany. This technology, originally called "Pulse Cytophotometry," was commercialized shortly after by Partec through Phywe AG in Göttingen, and it served as the model for contemporary flow cytometry techniques.

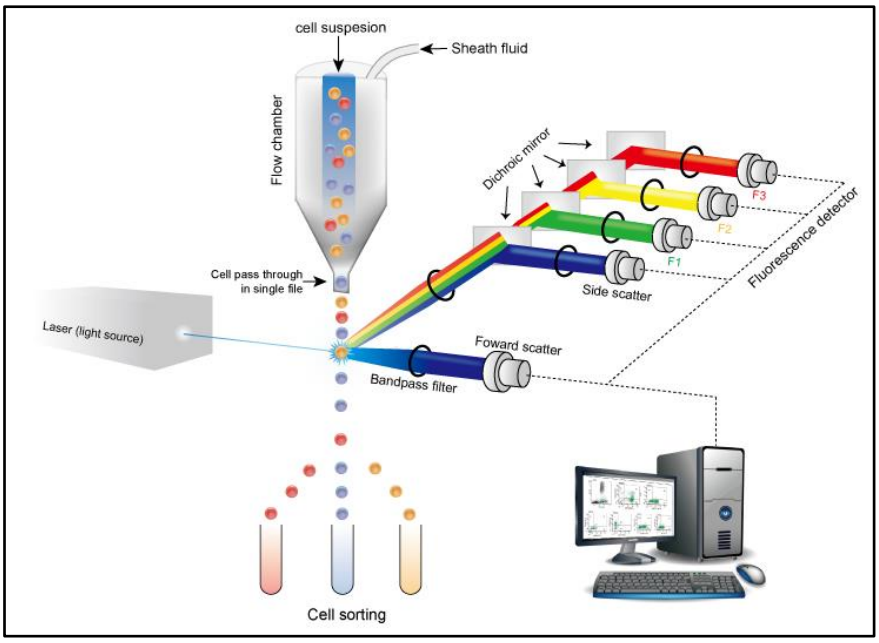
Feature	Advantages	Disadvantages	Applications
Throughput	Analyzes thousands of cells per second, facilitating efficient data acquisition.	---	Molecular biology research
Multiparametric analysis	Measures up to 30 parameters simultaneously, offering detailed insights into various cell characteristics.	---	Medical fields (transplantation, hematology, etc.)
Single-cell analysis	Examines individual cells	Requires fresh samples	Marine biology (plankton analysis)
Cell sorting	Isolates distinct cell populations	Cannot be used on fixed tissue	Protein engineering (variant selection)
Quantitative measurements	Quantifies cell populations and characteristics	Technical and analytical issues	
High sensitivity	Detects low-abundance cell populations	Expensive and sophisticated instruments	
Automation	Reduces human error and ensures consistency	Limited information on intracellular distributions	
Non-destructive analysis	Allows analysis of live cells	Slow for some applications	

PRINCIPLE

Flow cytometry utilizes lasers to analyze suspended particles like cells. A laser beam focuses on the particles, and detectors capture how the light interacts with them. Detectors measure both scattered light (forward and side) and emitted fluorescent light.

1. Forward scatter reveals particle size (cell volume).
2. Side scatter indicates internal complexity (shape, granules, membrane).

Some systems rely solely on light scatter for analysis, while others capture more detail by generating images of each particle's light interactions. This detailed analysis provides a wealth of information about the physical and chemical properties of individual particles within the sample.



Process of Flow Cytometry

Category	Description
Objective	To study the working and principle of Flow Cytometry.
Components of a Flow Cytometer	1. **Flow cell:** liquid stream (sheath fluid) carries and aligns the cells so that they pass single file through the light beam for sensing. 2. **Optical system:** commonly used are lamps (mercury, xenon); high power water-cooled lasers (argon, krypton, dye laser); low power air-cooled lasers (argon (488nm), red-HeNe (633mm), green-HeNe, HeCd (UV)); diode lasers (blue, green, red, violet) resulting in light signals. 3. **Detector and Analogue to Digital Conversion (ADC) system:** generating FSC and SSC as well as fluorescence signals from light into electrical signals that can be processed by a computer. 4. **Amplification system** 5. **Computational analysis of the signals.**

Types of Staining methods	Once the sample is prepared, cells are coated with fluorochrome-conjugated antibodies specific to surface markers on various cells. This can be achieved through direct, indirect, or intracellular staining. 1. **Direct Staining:** Cells are incubated with antibodies directly conjugated to a fluorophore. 2. **Indirect Staining:** In this method, cells are first incubated with antibodies directly conjugated to a fluorophore. Subsequently, a fluorophore-conjugated secondary antibody is introduced to detect the primary antibody. 3. **Intracellular Staining:** This procedure allows for the direct measurement of antigens within the cell cytoplasm or nucleus. To achieve this, cells are first made permeable, and then they are stained with antibodies in a permeabilization buffer. These staining methods enable the identification and characterization of specific cell populations based on surface or intracellular markers.

Fluorescence-Activated Cell Sorting (FACS) –

Fluorescence-activated cell sorting (FACS) is a powerful technique that isolates specific cell types from a mixture. It works by analyzing each cell's light scattering and fluorescence properties as it flows one at a time in a stream. Based on these characteristics, a charge is applied to the cell as it breaks off into a droplet. Electrostatic deflection then guides the charged droplet into a specific container, effectively sorting the cells based on their unique features. This method has valuable applications in research and clinical settings.

Antibody Staining in Flow Cytometry –

In flow cytometry, ensuring cells are in a single-cell suspension is crucial for accurate antibody staining. Clumped cells or those from solid organs require enzymatic or mechanical dissociation to achieve this. Mechanical filtration is then used to prevent instrument clogs, improving data quality. Following this, cells are incubated with antibodies in test tubes or plates before analysis with a flow cytometer.

Conclusion and Discussion –

Flow cytometry is a highly versatile and powerful analytical technique that enables researchers to gain deep insights into the composition and characteristics of complex cell populations within biological samples.

1. **Multi-parameter Analysis -** Flow cytometry allows simultaneous measurement of size, granularity, surface markers, and intracellular components of individual cells.
2. **Phenotypic Characterization -** Identifies and analyzes specific cell types based on surface and intracellular markers, useful for studying immune cells, stem cells, or cancer cells.
3. **Functional Analysis -** Measures cell functions like proliferation, apoptosis, cytokine production, and signaling, providing insights into cellular dynamics and states.

9. Demonstration of Real-Time Polymerase Chain Reaction (RT-PCR) – Summary

THEORY

Technique - Detects and quantifies specific nucleic acid sequences in real-time.
Method - Amplifies a target DNA sequence using primers and DNA polymerase.
Key Difference from PCR - Measures PCR products as they're being amplified.
Quantification - Uses fluorescent dyes or probes that bind to PCR products, emitting fluorescence proportional to the amount generated.

Process -

1. **Reverse transcription (RT) -** RNA converted to complementary DNA (cDNA).
2. **PCR Amplification -** Generates multiple copies of the target cDNA sequence through cycles of denaturation, annealing, and extension.

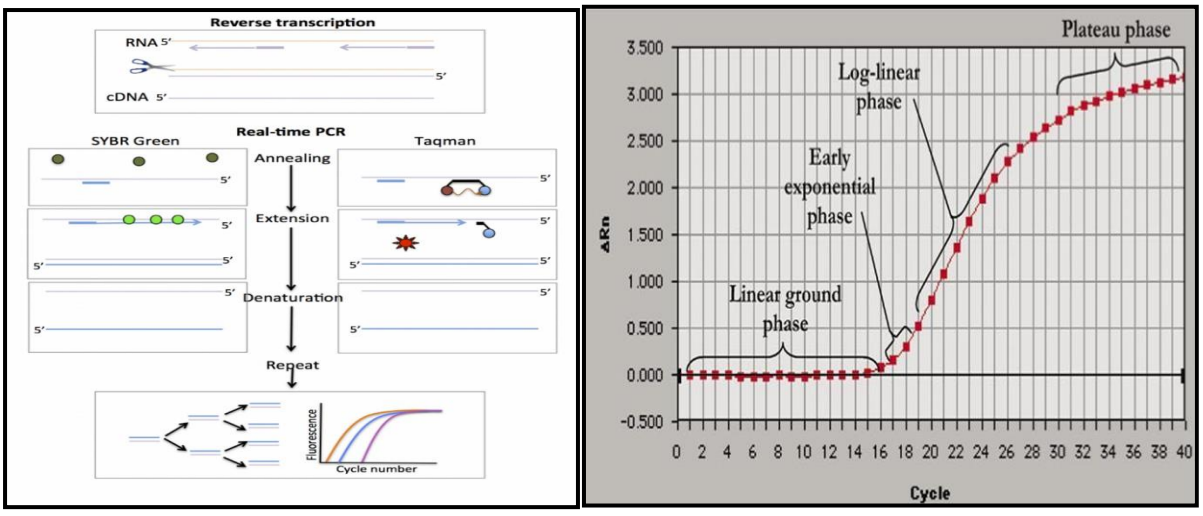
Detection Methods -

1. Fluorescent dyes intercalating into double-stranded DNA.
2. Sequence-specific probes hybridizing to the target DNA sequence. (e.g., Probe with reporter and quencher dyes)

Strengths - Powerful and sensitive, detects low amounts of DNA/RNA.

PCR Phases -

1. Linear ground phase (baseline fluorescence established).
2. Early exponential phase (fluorescence increases, Ct value defined).
3. Log-linear phase (optimal amplification, product doubles per cycle).
4. Plateau phase (reaction components limited, fluorescence not useful for data).



Real-time Polymerase Chain Reaction(RT-PCR) and its graphical representation to interpret the results

Applications –

1. **Gene Expression Analysis:** Quantifies gene activity in different tissues, cells, or experimental conditions.
2. **Cancer Research:** Detects cancer-specific mutations, aiding in diagnosis, prognosis, and treatment monitoring.
3. **Drug Research:** Studies drug metabolism, identifies drug targets, and assesses individual responses to medications.
4. **Agriculture:** Analyzes gene expression in plants for development, stress tolerance, and disease resistance.
5. **Food Safety:** Detects foodborne pathogens for ensuring food product safety.

PRINCIPLE

RT-PCR is a technique used to detect and amplify RNA molecules. It first converts RNA to DNA and then uses PCR to make many copies of the DNA, allowing researchers to easily analyze the RNA even if there's very little to begin with. This is useful in areas like gene expression studies, measuring viruses, and detecting RNA viruses.

Real-time PCR is a variation of RT-PCR that uses a special dye to track the amplification process in real time, allowing for quicker and more sensitive detection of RNA.

Category	Description
Objective	To understand the technique of Real-Time Polymerase Chain Reaction (RT-PCR).
Materials	1. **Thermal Cycler -** Controls precise temperature changes during PCR cycles for optimal amplification. 2. **Fluorescent Dye/Probe -** Binds to amplified DNA, emitting fluorescence for real-time monitoring (SYBR Green) or can be sequence-specific (probe-based). 3. **PCR Tubes/Plates -** Thin-walled tubes (polypropylene) or plates (polypropylene/polycarbonate) for efficient heat transfer and compatibility with automation. 4. **Optical Detection System -** Monitors and quantifies fluorescence emitted by dyes or probes during amplification. 5. **Computer & Software -** Analyzes fluorescence data, calculates cycle threshold (Ct) values, and quantifies target DNA based on signal intensity. 6. **Reagents -** Primers, dNTPs, Taq polymerase, and buffer solutions are essential for successful PCR and accurate nucleic acid detection.
Steps of RT-PCR	**DNA Extraction** Isolate DNA from the sample. ↓ **Primer Design** Create specific primers for the target DNA sequence.

	↓ **Amplification** 1. **Denaturation –** Separate double-stranded DNA into single strands at high temperature (95°C). 2. **Annealing –** Hybridize primers to complementary sequences on the target DNA at a specific temperature (based on primer Tm). 3. **Extension** – Extend primers using DNA polymerase at optimal temperature (70-72°C). ↓ **Real-time Monitoring** o Use fluorescent marker and thermal cycling to detect amplified DNA. o Fluorescence intensity increases with more DNA copies. o Detect signal exceeding a threshold level. ↓ **Data Analysis** Analyze data using software to quantify the original DNA sample.
Conclusion	Real-time PCR, also known as quantitative PCR (qPCR), is a powerful molecular biology technique that allows for the detection and quantification of specific nucleic acid sequences in real-time.

Real-time Polymerase Chain Reaction (RT-PCR)

CHAPTER 4 – SKILLS ACQUIRED

The key laboratory skills gained through the molecular biology training program include:

1. **Aseptic technique:** The program covers best practices for maintaining a sterile work environment and minimizing contamination when handling biological samples and reagents. This includes proper use of biosafety cabinets, sterile pipetting, and decontamination procedures.

2. **Pipetting:** Trainees learnt accurate and precise pipetting techniques using various types of pipettes, including how to select the appropriate pipette and properly calibrate and maintain the equipment.

3. **Data analysis:** The program trains students in analyzing data from molecular biology experiments, such as interpreting results from gel electrophoresis, PCR, and measurements of nucleic acid concentration.

4. **General laboratory skills:** The program covers fundamental skills like preparing solutions of known concentration, maintaining detailed laboratory notebooks, and following quality management practices to ensure reliable and reproducible results.

Overall, the molecular biology training program equips trainees with a comprehensive set of both technical and analytical skills required to work effectively in a modern molecular biology laboratory setting.

CHAPTER 5 – CONCLUSION

The hands-on molecular biology training program covered a comprehensive set of fundamental techniques that are essential for understanding and advancing the field of molecular biology. By mastering skills such as DNA isolation, plasmid isolation, PCR, restriction enzyme digestion, DNA quantification, ligation, agarose gel electrophoresis, and protein characterization by SDS-PAGE, trainees gained practical expertise in manipulating and analyzing the building blocks of life at the molecular level.

These techniques form the foundation for a wide range of applications in biotechnology, drug discovery, diagnostics, and personalized medicine. The ability to isolate, amplify, and characterize DNA, RNA, and proteins empowers researchers to unravel the complexities of biological systems, identify genetic markers, develop targeted therapies, and advance our fundamental understanding of molecular mechanisms underlying health and disease.

By equipping trainees with this versatile skillset, the hands-on training program has significantly contributed to strengthening the scientific community's capacity to tackle complex problems and drive innovation in the dynamic field of molecular biology. The acquired skills can be further applied to explore emerging areas such as genome editing, single-cell analysis, and systems biology, ultimately leading to groundbreaking discoveries and translational breakthroughs that improve human health and well-being.

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